

University of Nottingham
UK | CHINA | MALAYSIA

Post Exposure Prophylaxis for HIV in the sexual exposure scenario (PEPSE): Development of a novel nanoformulation

Carlos Sanders Velez

Msc

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy in Pharmacy

Index

Acknowledgements	iv
List of conference proceedings	vii
List of abbreviations	ix
List of tables and figures	xi
Abstract	1
Chapter 1: Introduction to the HIV pandemic and current gaps in prevention schemes	3
1.1.1 Current HIV/AIDS pandemic. Failure to reach 90-90-90 UNAIDS goals in 2020	3
1.1.2 Historical Evolution of the global pandemic	5
1.2.1 The human immunodeficiency virus	6
1.2.2 HIV pathogenesis	8
1.2.3 Establishing infection in the lymph nodes	12
1.3.1 Antiretroviral therapy and pharmacological prevention schemes	15
1.4.1 Pharmacological prevention schemes	21
1.5.1 The SARS-COV-2/COVID-19 pandemic and the negative effects on HIV treatment and diagnosis programmes	25
1.5.2 A case for an alternative PEPSE for low to moderate sexual risk of HIV acquisition	27
1.6.1 Selection of an antiretroviral agent for a Novel PEPSE	29
1.7.1 Localised antiretroviral formulations: Microbicides and future perspectives.	32
1.7.2 Vaginal tissue barriers for a localised delivery system	35
1.7.3 Vaginal antiretroviral delivery systems	36
1.7.4 Anal tissue as a target for antiretroviral delivery	38
1.7.5 Anal antiretroviral delivery systems	40
1.8.1 Nanoformulations in the HIV field	42
1.9.1 Project aim and hypotheses	42
Chapter 2: Unplanned unsafe sexual intercourse events, including condom failure, lack of condom availability and forgetting to use a condom, expose a high proportion of sexually active population to potential risk of HIV acquisition.	45
2.1.1 Interventions (pharmacological and non-pharmacological) designed to prevent HIV.	45
2.1.2 Condoms as a means of protection for HIV/Condom failure rates.	48
2.1.3 Exploring the views of the sexually active public.	49
2.1.4 State of the HIV pandemic in the UK and in Thailand	50
2.2 Methods	52
2.2.1 Ethical approval	52
2.2.2 Online Survey Studies	52
2.2.3 Statistical Analysis through logistic regression	54
2.3. Results	57
2.3.1 AUCROC Values of the model	57
2.3.2 Descriptive statistics	57

2.3.3 General UK Study descriptive statistics 58
2.3.4 UK population in unplanned low to moderate risk descriptive statistics 59
2.3.5 UK study logistic regression 61
2.3.6 General Thailand Study descriptive statistics 64
2.3.7 Thailand population in low to moderate risk descriptive statistics 66
2.3.8 Logistic regression for Thai study 67
2.4.1 Discussion 70
2.4.2 Unplanned events leading to lower to moderate risk of HIV acquisition through sexual intercourse 71
2.4.3 Size of a population that is unprotected with current pharmacological prevention measures for HIV. 73
2.4.4 Logistic regression UK population 73
2.4.5 Logistic regression Thailand population 75
2.4.6 Tackling an unprotected population and future research targets 77
2.5.1 Conclusions 79
Chapter 3: Development of a high drug content nano formulation for a localised delivery of antiretrovirals. 80

3.1.1 Role of nanomedicines as tools in the fight against HIV 80
3.1.2 Organic nano-carriers for drug delivery 84
3.1.3 Solvent diffusion method for the generation of nanoaggregates (nanoprecipitation) 85
3.1.4 Antiretroviral formulations using nanoprecipitation 88
3.1.5 Prodrug approach as a means of improving a compound's nanoformulation properties 89
3.1.6 Targeting Dolutegravir as a model compound for PEPSE 91
3.1.7 A modified nanoprecipitation technique for high drug loading 94
3.1.8 A novel formulation for PEPSE aim and hypotheses. 95
3.2. Materials and methods 98
3.2.1 Reagents 98
3.2.2 Synthesis of a lipophilic prodrug of Dolutegravir 99
3.2.3 HPLC system 100
3.2.4 HPLC sample preparation 101
3.2.5 Nanoprecipitation conditions 104
3.2.6 Nano coating conditions 105
3.2.7 Electron microscopy (Scanning electron microscopy/Transmission electron microscopy) 108
3.2.8 *In vitro* cytocompatibility and preliminary uptake 109
3.2.9 Statistical analysis and figure design 112
3.3 Results 112
3.3.1 DTG's lipophilic prodrug 112
3.3.2 Traditional nanoprecipitation formulations 115
3.3.3 MDTG nanocrystal precipitation 119
3.3.4 Polymeric coating of MDTG nanocrystals 121
3.3.5 In vitro cytocompatibility/preliminary cellular uptake 125

3.3.6 Stability of the polymeric coated formulations in strong ionic environments 130
3.3.7 MDTG Cleavage/Drug release from the formulation 137
3.4 Discussion 141
3.4.1 A modified DTG as a means of solving physiochemical shortcomings. 141
3.4.2 Nanoprecipitation as a means of formulation for a Dolutegravir Prodrug 142
3.4.3 Solid drug nanocrystals of MDTG 144
3.4.4 A polymeric nanocarrier as a means of stabilizing a nanocrystal emulsion 145
3.4.5 In vitro cytocompatibility and uptake in Caco-2 RAW 264.7 cell lines 146
3.4.6 Coated nanocrystals formulations in strong ionic environments 147
3.4.7 Parent drug cleavage from MDTG/Release from the formulation. 149
3.5.1 Conclusions 150
Chapter 4 "Role of a novel PEPSE formulation in the future of the HIV pandemic" 152
4.1.1 An unprotected target population for PEPSE 152
4.2.1 Coating an MDTG nanocrystal as a means of generating a PEPSE formulation. 158
4.3.1 Future work 168
4.4.1 Conclusions 171
4.5.1 COVID19 impact statement 172
Supplemental information 174
(UK and Thailand questionnaires. Logistic regression vs linear regression, Dynamic light scattering data)

Acknowledgements

I would like to express my gratitude towards CONACYT for funding this project. I would also like to express my gratitude towards my supervisors, Dr. Pavel Gershkovich, Professor Peter Fischer, Professor Cameron Alexander and Dr. Michael J. Stocks for allowing me the space and freedom needed to work, and for continued support and guidance from day one.

This work is dedicated to several family members, friends and colleagues that helped me survive my PhD ride with my mental health relatively intact.

To my two pillars, my two parents, Lourdes and Javier, whose unwavering support has made me who I am today. I have been so blessed to have them as parents, as I truly do not deserve them.

To my partner in crime, my fiancée Leah, whose uncanny ability to make me smile, when things get rough, keeps me going. Each day I thank God for allowing me to have her as a family member in this foreign country. This is over now, and for better or worse we can now plan our future.

Being a student in a foreign country has stressful situations that are often not foreseen. Early into my time in Nottingham a close uncle, Sergio passed away. I have missed our clever conversations about world politics, especially your ability to listen to other opinions. Nearing the end of my PhD, at the peak of the COVID19 pandemic in December 2020, my cousin Elvia and my uncle Alfredo passed away. Elvia's attitude towards life, even as she became paralysed in 2019, is something everyone should aspire to. I will remember her smiling, as the world needs more positive people like her.

Alfredo pretended to be Santa Claus when I was younger, and that memory will be with me forever. I hope this flawed work, can serve as a tribute for your truly exemplary lives.

This work is also dedicated to Vincenzo, Rob and Rowland, three colleagues and friends who had a significant impact in this work.

Vincenzo's unorthodox style towards teaching, along with his upbeat character allowed me to go into the world of nanoparticles without prior knowledge fearlessly. I can't thank you enough for your guidance through this journey. È molto gentile da parte tua.

Rob's is the one and only "cell whisperer" I know. With his well-known patience Rob taught me many things about cell culture as well as to how to be a better scientist. Besides the lab, Rob has an uncanny ability to always score points in a pub quiz, I look forward to a pub quiz with you and all the gang once all is over.

Rowland is a mathematical magician, whose simple approach made it easier for me to understand how to deal with numbers, and not be scared. I would never have thought that Maths was so easy, nor that I could understand logistic regressions.

To the Turners, Sarunne, Ross, and of course Coco Turner, the food stealing Labrador. I can't thank Sarunne and Ross enough for allowing me to be a lodger at their place during the darkest days of my PhD. Their friendship is something I will treasure always. Our cook-offs on the weekends made up for amazing memories. The memory of Coco knocking on my door to sleep on my bed or planning to do something mischievous is one of the best memories I will carry from those days.

To my Nottingham friends, too many to list, but I will try to thank you all. Yenju, Louis, Alice, Addie, Valentina (both Valentinas! Vale Cuzzocoli “Taresco” and Vale Hashad), Shan, Diego, Yarely, Carlos, Ana, Maria Barbadillo, Phoebe, Rachael, Napoleon and Dipak, thank you for your friendship. I know I missed many, but you all have a special place in my heart.

Thank you as well to the WetweetscienceMx and VIHve libre groups, as they rekindled my passion for science when I was doubting myself. Whatever happens in my life, I will always be grateful for having been invited to be a part of both groups.

Of course, special thanks to Miquel, Guillermo, Pavel Gonzalez, Male and the Birkenshaw crew. Checking up on me when I was clearly feeling down is something I appreciate deeply.

And finally, to my 4 marvellous MPharm students Alice, Christina, Desi and Keval, thank you for being so patient with me as I was learning how to mentor you in a better way.

Thank you all.

List of conference proceedings

Carlos Sanders-Velez, Pavel Gershkovich, Peter Fischer, Cameron Alexander, Vincenzo Taresco, Christophe Fromont. **Development of a Nanoformulation for the Post Exposure Prophylaxis for HIV**. PhysChem Forum 18 "Medicines of tomorrow: new therapeutic modalities" Conference 2019. Frankfurt, Germany. (Poster)

Carlos Sanders, Vincenzo Taresco, Sam Rosen, Andrew Kam-Tuck Yip, Michael J. Stocks, Peter M. Fischer, Cameron Alexander, Pavel Gershkovich. **A novel alternative "morning after" local administration approach for post-exposure prophylaxis of HIV: Developing the formulation and assessing the unmet need. "**22nd Bangkok International Symposium on HIV Medicine 2020". 2020. Bangkok, Thailand. (Poster)

Carlos Sanders-Velez, Vincenzo Taresco, Rob Cavanagh, Michael J. Stocks, Peter M. Fischer, Cameron Alexander, Pavel Gershkovich. **A novel alternative "morning after" local administration approach for post-exposure prophylaxis of HIV: Developing an alternative formulation.** Young Researchers in Macromolecules 2020 symposium. Online conference due to COVID19 restrictions. (Poster)

Carlos Sanders-Velez, Vincenzo Taresco, Rob Cavanagh, Michael J. Stocks, Peter M. Fischer, Cameron Alexander, Pavel Gershkovich. **A novel alternative "morning after" local administration approach for post-exposure prophylaxis of HIV: Developing an alternative formulation.** Midlands Polymer Conference 2020. Online conference due to COVID19 restrictions. (Poster)

C. Sanders, V. Taresco, R. Cavanagh, M. J. Stocks, P. M. Fischer, C. Alexander, P. Gershkovich. **A novel alternative "morning after" local administration approach for post-exposure prophylaxis of HIV:** Developing an alternative formulation. 2020 United Kingdom and Ireland Controlled Release Society symposium. Online conference due to COVID19 restrictions. (Poster can be accessed online through the link)

List of abbreviations

HIV: Human immunodeficiency virus

AIDS: Acquired immunodeficiency syndrome

UNAIDS: United Nations joint committee on HIV/AIDS

HAART: Highly active antiretroviral therapy

TaSP: Treatment as prevention

PrEP: Pre exposure prophylaxis

PEP: Post exposure prophylaxis

PEPSE: Post exposure prophylaxis in a sexual exposure

NRTI: Nucleoside reverse transcriptase inhibitors

NNRTI: Non-nucleoside reverse transcriptase inhibitors

PI: Protease inhibitors

INI: Integrase inhibitors

DTG: Dolutegravir

MSM: Men who have sex with men.

MC: Male circumcision

NATSAL: National Surveys of Sexual Attitudes and Lifestyles

MDTG: Dolutegravir Myristate

LogP: Partition coefficient

Wt%: Weight percentage

NMR: Nuclear Magnetic Resonance

HPLC UV: High pressure liquid chromatography with an ultraviolet absorbance detector

LC-MS: Liquid Chromatography–mass Spectrometry

DLS: Dynamic Light Scattering

SEM: Scanning electron microscopy

TEM: Transmission electron microscopy

FDA: United States Food and Drug Administration

CDC: United States Centers for Disease Control

PLGA: poly(lactic-co-glycolic acid)

PLA: Polylactic acid

PEG: Polyethylene glycol

mPEG-PLA: Methoxy poly(ethylene glycol)-poly(lactide)

mPEG-PLA-Cy5: Methoxy poly(ethylene glycol)-poly(lactide) linked with a Cy5 Blue dye

PEO: poly(ethylene oxide)

PPO: poly(propylene oxide)

P85: Pluronic 85

F127: Pluronic 127/Poloaxomer 407

PIP: Post exposure prophylaxis in pocket

List of tables and figures

Tables:

a) **Table 1.** Risks of HIV acquisition depending on the type of exposure

b) **Table 2.** Antiretroviral agents currently in use

c) **Table 3.** Predicted distribution inside the lymph node of different families of antiretrovirals.

d) **Table 4**. Predictors and their levels used in the logistic regression analysis of the data set.

e) **Table 5**. AUCROC values of the different models evaluated.

f) **Table 6.** Risky scenarios logistic regression data for the UK study.

g) **Table 7.** Logistic regression data from reporting an STD diagnosis and taking "No action" after a potential exposure in the UK study.

h) **Table 8.** Risky scenarios logistic regression data for the Thai study.

i) **Table 9.** Logistic regression data from reporting an STD diagnosis and taking "No action" after a potential exposure in the Thai Study.

j) **Table 10** Parameters that significantly alter drug content and/or size in a nanoaggregate formulated through nanoprecipitation while using acetone as the organic phase and HPLC water as the aqueous phase

k) **Table 11** Chromatographic gradient conditions for rat plasma data set.

l) **Table 12** Disclosed conditions tried in nanoprecipitation conditions.

m) **Table 13** Disclosed conditions tried in nanocoating conditions

n) **Table 14** Summary data for the MDTG prodrug with comparisons, where relevant, to the parent drug.

o) **Table 15** Summary table of DLS, Zeta potential measurements and biocompatibility tests of the formulations developed.

p) **Table 16** Generation of the "virtual" data set of MDTG residuals and the calculated half lives of the three data sets.

q) **Table 17** Ideal characteristics for a novel nanoformulated PEPSE.

Figures

I. **Figure 1.** Evolutionary pathway of HIV

II. **Figure 2.** HIV replication cycle and antiretroviral families

III. **Figure 3**. Schematic representation of HIV infective pathway showing relevant epithelium and transmission events.

IV. **Figure 4.** HIV forces histological changes after establishing infection.

V. **Figure 5.** Chemical structures of representative antiretroviral agents from four different families.

VI. **Figure 6.** Genetic Barrier plotted against viral potency when used in monotherapy in logistic scale.

VII. **Figure 7.** Female anatomical barriers for a microbicide.

VIII. **Figure 8.** Peyer's patches at the anal tissue.

IX. **Figure 9.** Objects in the nanoscale vs objects in the day-to-day life.

X. **Figure 10:** Examples of nano-carriers or nanoformulations and their microscopic structure/morphology, as defined by electron microscopy.

XI. **Figure 11.** Diagram depicting the nanoprecipitation process as described by Fessi et al.

XII. **Figure 12.** Commonly used functional groups to generate a prodrug with lipophilic properties

XIII. **Figure 13.** Chemical synthesis of Dolutegravir Myristate (MDTG).

XIV. **Figure 14.** Chemical structure of the polymers used.

XV. **Figure 15**. Diagram depicting a two-step solvent deposition method for coating nanoparticles, modified from Fessi et al. and Danai Styliari et al

XVI. **Figure 16.** H+ NMR data of the DTG Prodrug

XVII. **Figure 17.** C NMR data of the DTG prodrug.

XVIII. **Figure 18.** DLS size by intensity and wt% by HPLC UV of nanoprecipitation conditions tested

XIX. **Figure 19.** SEM micrographies for nanoaggregate formulations

XX. **Figure 20.** TEM images taken at 8 hours after precipitation of MDTG

XXI. **Figure 21.** Size by intensity DLS and wt% as measured by HPLC-UV of coated nanocrystals formulations.

XXII. **Figure 22.** TEM micrographies of representative coated nanocrystals formulations.

XXIII. **Figure 23.** Cytocompatibility data on Raw 264.7 and Caco-2 cell lines at 24 hours.

XXIV. **Figure 24.** Biocompatibility assessment of 50μM at 24 hours of the MPEG-PLA-Cy5 coated formulation.

XXV. **Figure 25.** Micrographs of particle internalization in Caco-2 intestinal cells and RAW 264.7 macrophage cells.

XXVI. **Figure 26.** Quantitative uptake of MPEG-PLA-Cy5

XXVII. **Figure 27.** Stability studies of the MPEG-PLAformulation in 150mM PBS, and 10mM HEPES buffer.

XXVIII. **Figure 28.** Stability studies of the P85 formulation in 150mM PBS, and 10mM HEPES buffer. A) Size by DLS of formulations in 10mM HEPES buffer.

XXIX. **Figure 29.** Size comparison by DLS of repeated DMEM stability studies of the P85 0.44mg coated formulation.

XXX. **Figure 30.** Drug cleavage from MDTG and drug release from P85 formulation.

XXXI. **Figure 31.** Chromatograms and calibration curves for both MDTG and DTG.

XXXII. **Figure 32.** Chemical structure of F127.

Abstract

Since the year 2012, 2 million newly diagnosed cases of human immunodeficiency virus (HIV) have been found every year. Prevention schemes based on condoms, and other pharmacological interventions such as post exposure prophylaxis in a sexual exposure scenario (PEPSE) have had positive impact in the pandemic. Under current PEPSE guidelines due to risk to benefit ratios, only high-risk unprotected sexual events (>1/1000) are prescribed antiviral prophylaxis. Such a situation leaves a population unprotected, whose size is unknown, that is in a low to moderate risk of HIV acquisition.

To assess the size and views of a population that is currently unprotected by current prevention schemes, two online survey studies were set up in Thailand and the UK (Chapter 2). Both questionnaires were designed to probe for unplanned low to moderate risk of sexual exposure to HIV. Both data sets were analysed through demographic statistics and logistic regression to assess for unsafe intercourse. The results suggest that the proportion of the population reporting unplanned unsafe sexual intercourse events is as high as 29.1% in the UK study and 15.8% in the study in Thailand. Such a high proportion of sexually active population presents a target for further alternative prevention schemes and a research priority.

One such alternative prevention scheme would be a localised (anal or vaginal delivery) nanoparticle drug delivery system, with high drug content, targeting local lymphatic nodes (Chapter 3). Dolutegravir (DTG) was chosen as the model antiretroviral agent due to its favourable side effect profile, high genetic barrier and high antiviral potency. However, the physiochemical properties of DTG such as its hydrophilicity are a

challenge for the entrapment of DTG under traditional nanoformualtions. A DTG prodrug, dolutegravir myristate (MDTG) was synthesized based on Sillman et al. to generate a high drug content formulation, a two-step solvent displacement experimental set up was designed. The first step was designed to formulate a MDTG nanocrystal emulsion, and the second step was designed to coat the nanocrystal with a minimal mass of a polymeric nanocarrier. The formulation developed through a coating method has a fast release of compound, as 100% of the payload gets released before 2 hours.

This work provides evidence that a substantial percentage of the sexually active population, may find themselves in an unplanned low to moderate risk scenario for sexual HIV acquisition. A potential solution for this population may lie in a localised nanoparticle delivery system to the vaginal or anal tissue (Chapter 4). In this work, proof of concept of coating an MDTG nanocrystal for this purpose was achieved. However, the drug release profile is not ideal for PEPSE purposes, thus future research is needed to perfect such a delivery system.

Chapter 1:
Introduction to the HIV pandemic and current gaps in prevention schemes

1.1.1 Current HIV/AIDS pandemic. Failure to reach 90-90-90 UNAIDS goals in 2020.

As of 2020 there are an estimated 38 million people living with the human immunodeficiency virus (HIV). With approximately 2 million newly diagnosed cases every year since 2012, a plateau has been reached within the incidence of new infections, and further actions must be taken to lower the burden of new cases [1]. The incidence of newly diagnosed cases is currently at an all-time low, and shows the positive impact that prevention schemes, such as condom campaigns, treatment as prevention (TasP), pre-exposure prophylaxis (PrEP), and post exposure prophylaxis (PEP) have had in the pandemic, however much is still needed to end the HIV epidemic [1-3]. Acquired immunodeficiency syndrome or AIDS occurs at the final stages of HIV disease. Deaths attributed to AIDS, have been on a steady decline each year since 1996, with most deaths being in the African continent [1].

The HIV pandemic affects different world regions differently. Currently most of the worldwide burden remains on eastern and southern African countries, where approximately >700 000 newly diagnosed cases are found every year [4]. Most of the newly diagnosed cases of HIV, an estimated 62%, belong to key populations and their sexual partners. Key populations for HIV prevention include gay men and other men who have sex with men (MSMs), sex workers and their clients, intravenous drug users,

prisoners, and transgendered people. While these populations represent small proportions of the sexually active general population, they are at an elevated risk of HIV acquisition [5]. Current estimates suggest that only 25.4 million people living with HIV (67%) are under treatment, meaning that approximately 12.6 million people can potentially transmit the virus to populations at risk [6]. Funding of early diagnosis, treatment, and new prevention strategies for high prevalent areas such as sub-Saharan Africa remains a high priority to end HIV [1, 5, 6].

To tackle new infections, the United Nations joint committee on HIV/AIDS (UNAIDS) developed ambitious targets in their 2013 meeting, the 90-90-90 goals for 2020. The 90-90-90 goals represent the following milestones: 90% of people living with HIV being aware of their status, 90% of those aware of their status receiving antiretroviral therapy, 90% of those receiving antiretroviral therapy achieving viral suppression [7].

In absolute numbers the 90-90-90 strategy means that 73% of the total number of people living with HIV would have achieved suppressed viral loads [7]. Several studies have provided data on how a virologically suppressed HIV positive individual will protect their partner by being on effective treatment [8-10]. Such evidence supports the consensus that the transmission risk under such circumstances is basically negligible [10]. Further evidence suggests that starting antiretroviral treatment as early as possible will provide better outcomes for the patient living with HIV and have a direct impact in lowering the HIV incidence [11, 12].

As 2019 ended, the 90-90-90 milestones were far from being reached, especially in high burden areas. Missing these targets prompted a new set of goals, proposed in the UNAIDS meeting in late 2019, with the goal of ending AIDS related deaths by 2030.

These are the more stringent: 95% of people living with HIV knowing of their status, 95% of people living with HIV under antiretroviral treatment, and 95% of people living with HIV achieving undetectable status [6].

1.1.2 Historical Evolution of the global pandemic.

The first recorded cases of HIV have been often diagnosed retrospectively. Currently there is a certain controversy on the first recorded case, which may have been a sailor in Manchester who in 1959 had a pathology resembling AIDS [13, 14]. However this case has been put into question, as subsequent PCR testing of this case has not managed to amplify HIV sequences [15]. Regardless of who the index patient may have been, there is extensive evidence of HIV genetic variants already present in Kinsasha, Congo by the 1960s, suggesting that the virus may have been present in isolated African communities earlier than what was originally thought [16].

It was not until the late 1970s and early 1980s when very initial reports of Kaposi's Sarcoma and Pnemocystis Jirovecci pneumonias were found in populations of MSMs [17]. In fact, retrospective studies have found antibodies to HIV in MSM sauna club users in Copenhagen by 1982, confirming an earlier start of the HIV pandemic [18].At the start of the pandemic, linking a causative agent to AIDS proved to be a very difficult task. One of the leading academic hypotheses as to the causative agent of AIDS being a retrovirus was put forward by Robert Galllo and Luc Montagnier's groups [19, 20].

Luc Montagnier's group in France managed to isolate HIV from a lymph node sample of a Caucasian patient with clinical signs and symptoms concordant with AIDS. This discovery along with the successful linking of sexual intercourse, contaminated blood

products and vertical transmission allowed for better understanding of the HIV pathogenesis, thus improving prevention strategies [21, 22].

1.2.1 The human immunodeficiency virus

HIV is a viral pathogen that under the Baltimore classification belongs to the VI group, meaning it is a double stranded positively sensed RNA virus with an intermediate step where the virus gets transcribed into DNA. Unlike other human pathogenic retroviruses, like the Human T-cell lymphotropic virus, HIV belongs to the lentivirus genus [23, 24].

Within the lentivirus genus HIV shares a significant genetic background with the simian immunodeficiency virus (SIV). SIV is a pathogen of relevance in Old World monkeys and in many ways, it can be seen as the evolutionary parent of HIV. As the human species began to differentiate from other primates through thousands of years of evolution, an innate immunological defence for SIV was developed. Thus, the SIV virus remained contained to other Old-World monkeys for centuries, until certain evolutionary crossovers allowed for the generation of a novel virus (**Figure 1**) [24, 25].

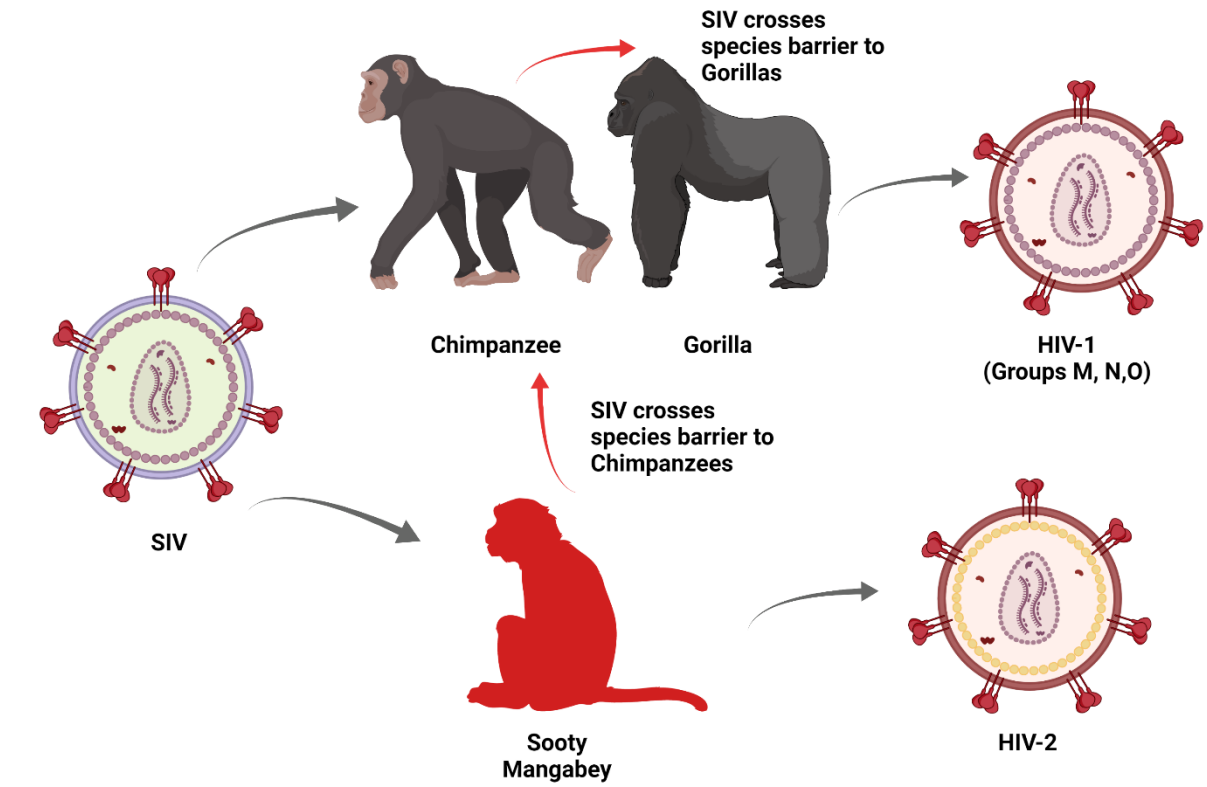


Figure 1. Evolutionary pathway of HIV. Viruses belonging to the lentiviral family, such as SIV and HIV, have a significant mutation rate per replication cycle. Such mutation rate has allowed SIV to eventually cross the species barrier in Old World monkeys and eventually allow for HIV to emerge as a novel virus. Current phylogenetic evidence suggests that SIV gave origin to HIV-1 (including the subgroups M, N, O) after cross species events from Sooty Mangabeys and Chimpanzees, and Chimpanzees to Gorillas. HIV-2 on the other hand is heavily linked to SIVs from Sooty Mangabeys. The two species of HIV have specific virological and clinical differences that will not be covered in this manuscript [23, 24, 26].

Current data suggests that HIV became a novel virus sometime around the 1900s, with two closely related but distinct species, HIV-1 and HIV-2. Both species of HIV had a different evolutionary course due to the different hosts they infected.

Evidence suggests that HIV-1 evolved from a SIV lineages found in chimpanzees and western gorillas. HIV-1 evolved after two major cross species events, from sooty mangabey to chimpanzee and from chimpanzee to gorilla, eventually finding its way to

a human host. HIV-1 and its variants have a worldwide distribution, being regarded as the more clinically aggressive of the two HIV species, as well as the culprit for the main epidemic [23, 24, 27].

HIV-2 had a different evolutionary path, as it is descended from lineages that originally affected Sooty Mangabeys. Due to the virological and evolutionary situations, HIV-2 is mostly restricted to sub-Saharan Africa. HIV-2 could be responsible of about 5% of all HIV infections, with an estimated 1 million people living worldwide with HIV-2. After analysing epidemiological, virological, and clinical data, evidence suggests that HIV-2 is not as pathogenic as HIV-1 [26, 28].

Both species can be acquired in through the same pathways: sexual intercourse, vertical transmission (perinatal) and blood-borne exposure. For the rest of this chapter and thesis, the abbreviation HIV will refer to HIV-1, as antiviral treatment (HIV-2 being mainly sensitive to protease inhibitors), virological features, antigenic and epidemiological implications vary significantly between species [23, 24, 26].

1.2.2 HIV pathogenesis.

Unlike other viral pathogens, HIV distinguishes itself by having a proviral DNA step and subsequent integration to the host's genome in its replication cycle (**Figure 2**). This DNA step is key in shielding the virus from the immune response as it culminates in the successful integration of the viral DNA to the host's genome, creating a reservoir for HIV in the infected TCD4+ cells [29].

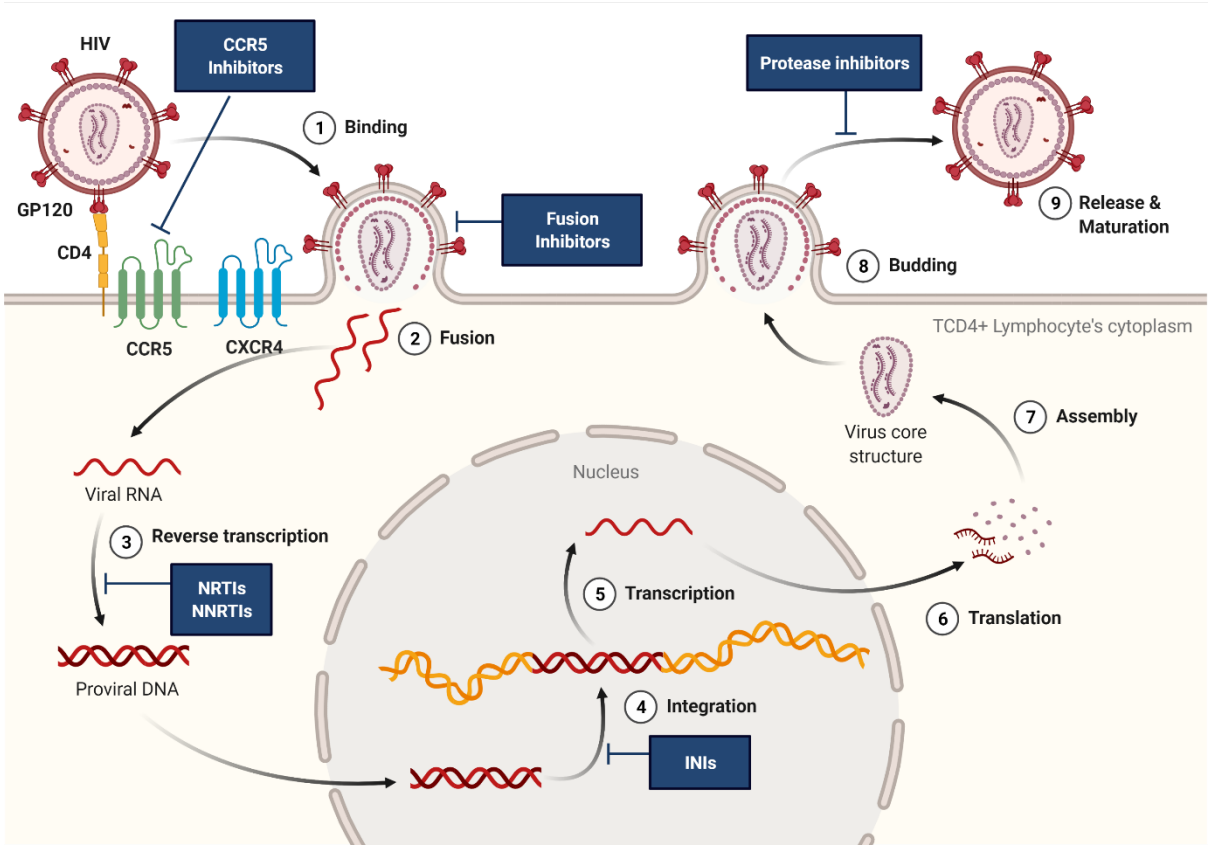


Figure 2. HIV replication cycle and antiretroviral families. HIV's Glycoproteins gp120/gp46 will bind to the CD4 receptor and use it as a main receptor (*1*). Fusion occurs and allows for HIV to unload the viral genome into the Lymphocyte's cytoplasm (*2*). Once in the cytoplasm HIV's reverse transcriptase will convert the viral RNA into a proviral DNA (*3*). The proviral DNA will get shuttled to the cell's nucleus. Once in the cell nucleus, the proviral DNA will be integrated into the cell's genome (*4*). Transcription and translation (*5,6*) of the proviral DNA allows for further assembly and budding with a protease mediated excision of the virion (*7,8,9*). Several antiretroviral families have been developed to combat HIV replication, with their mechanisms of action on the figure.

HIV can be transmitted in a cell-associated (such as a white blood cell in semen) or in cell-free form as a viral particle. To complicate matters further, HIV can express a particular tropism to a subset of receptors, a trait that can make some viral strains "fitter" than others to infect a particular host [23, 29]. These characteristics make HIV a difficult disease to model efficiently. Due to experimental difficulties, most of the data has been extrapolated from SIV disease models in macaques [30, 31]. Most people living with

HIV, have acquired the virus after the breakage of mucosal barriers with direct exposure to viral particles. It is important to note that not all infective routes confer the same risk (**Table 1**).

Table 1. Calculated risks of HIV acquisition depending based on exposure types [32-34]. The calculated risks represent how likely an exposure event is to result in HIV acquisition.

Type of exposure	**Risk of HIV acquisition per exposure event (Confidence interval 95%)**
Receptive anal intercourse	1.11% (0.042%-3%)
Insertive anal intercourse	0.06 % (0.006%-0.065%)
Receptive vaginal intercourse	0.1% (0.004%-0.32%)
Insertive vaginal intercourse	0.082% (0.011%-0.38%)
Receptive oral intercourse	0.02% (0%-0.04%)
Insertive oral intercourse	Close to 0%
Tainted Blood Transfusion	27-100%
Needlestick injury	Depending on type of injury: • Surgical needle injury is calculated around 2% • Intravenous drug user calculated around 2.4%.

Regardless of whether in cell-free or cell-associated form, HIV must overcome physical barriers such as epithelial and mucosal secretions to come in contact with a macrophage or a dendritic cell as a first step of infection (**Figure 3**). Unprotected sexual contact with an HIV carrier remains the main route of transmission [1].

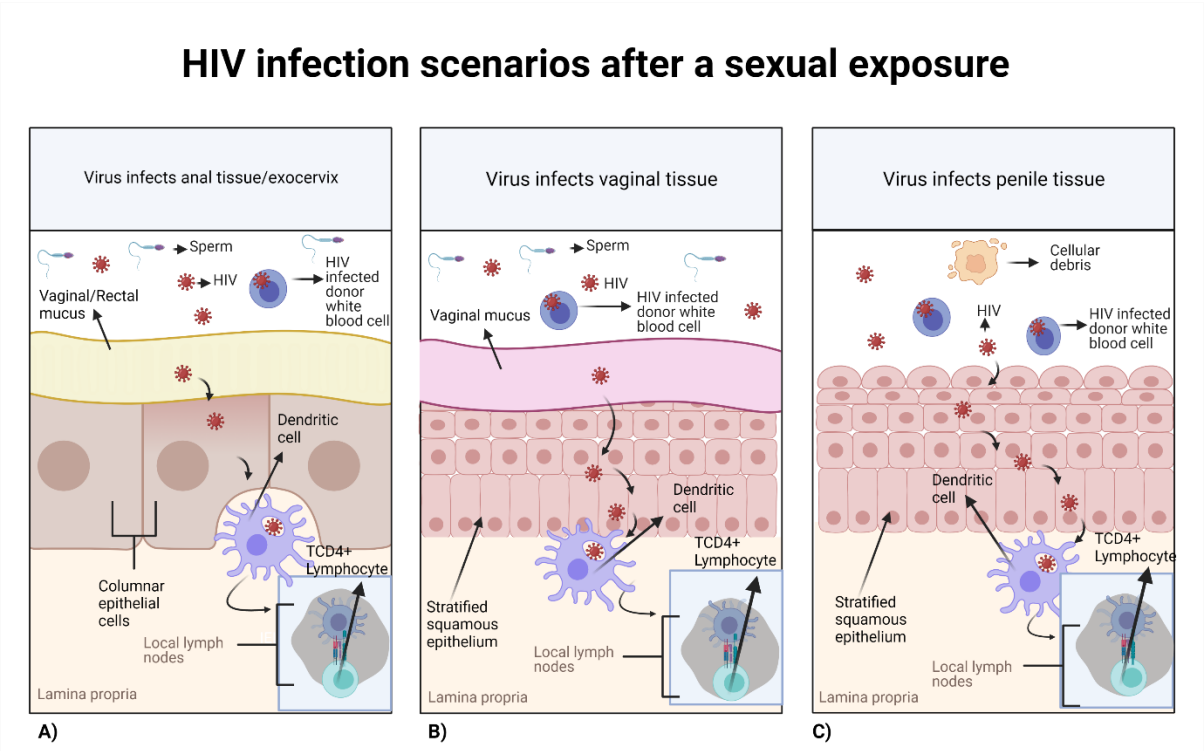


Figure 3. Schematic representation of HIV infective pathway showing relevant epithelium and transmission events. Risks for sexual HIV acquisition vary depending on many factors such as viral load (of the HIV+ partner), sexual act and/or use of barrier methods such as condoms. For females, risks can also vary depending on the time of exposure regarding the menstrual cycle. At the end of a menstrual cycle, the epithelia is more vulnerable to HIV, as often the epithelium layer is thinner and macrophages are found more often. **Panel A)** Depicts what would happen on a receptive scenario at the exocervix or anal tissue as both are comprised of primarily columnar epithelium. **Panel B)** Represents the receptive scenario at the vaginal tissue, which is comprised mainly of stratified squamous epithelium. **Panel C)** Represents an insertive scenario, with the stratified squamous epithelium found at the penile tissue. In the receptive scenario, HIV must traverse a mucous barrier found at the vaginal or anal tissues followed by the epithelial barrier, only to find a dendritic cell/macrophage and then be shuttled to a local lymph node. In the insertive scenario, fewer dendritic cells/macrophages are present, to shuttle HIV to local lymph nodes. Such histological differences also account for the difference in the calculated acquisition risks [23, 27].

The virus is then transported to the local lymph nodes, where it would eventually find a T CD4+ lymphocyte in the paracortex area of a lymph node. While HIV can, on some occasions, replicate in a macrophage or a dendritic cell expressing the chemokine

receptor CCR5, this event does not occur often, and thus the viral end point of the disease is the infection of a T CD4+ cell at a lymph node [35].

1.2.3 Establishing infection in the lymph nodes.

The lymph nodes are complex masses of specialized immune tissue. These bean shaped sacs, are found throughout the body, totalling approximately 600 nodules of 1 to 10 mm in size [36]. At the histological level, a lymph node is composed of a fibrous outer wall, connected to outside tissues by a set of venules, arterioles, and respective afferent and efferent lymphatic vessels [37]. By removing the outer fibrous wall, lymph nodes are divided into lobules, cortex, follicle, sinuses (that are derived from the afferent lymphatic vessel), paracortex and the hilus where the arterioles, venules and efferent lymphatic vessels lay (**Figure 4**) [38].

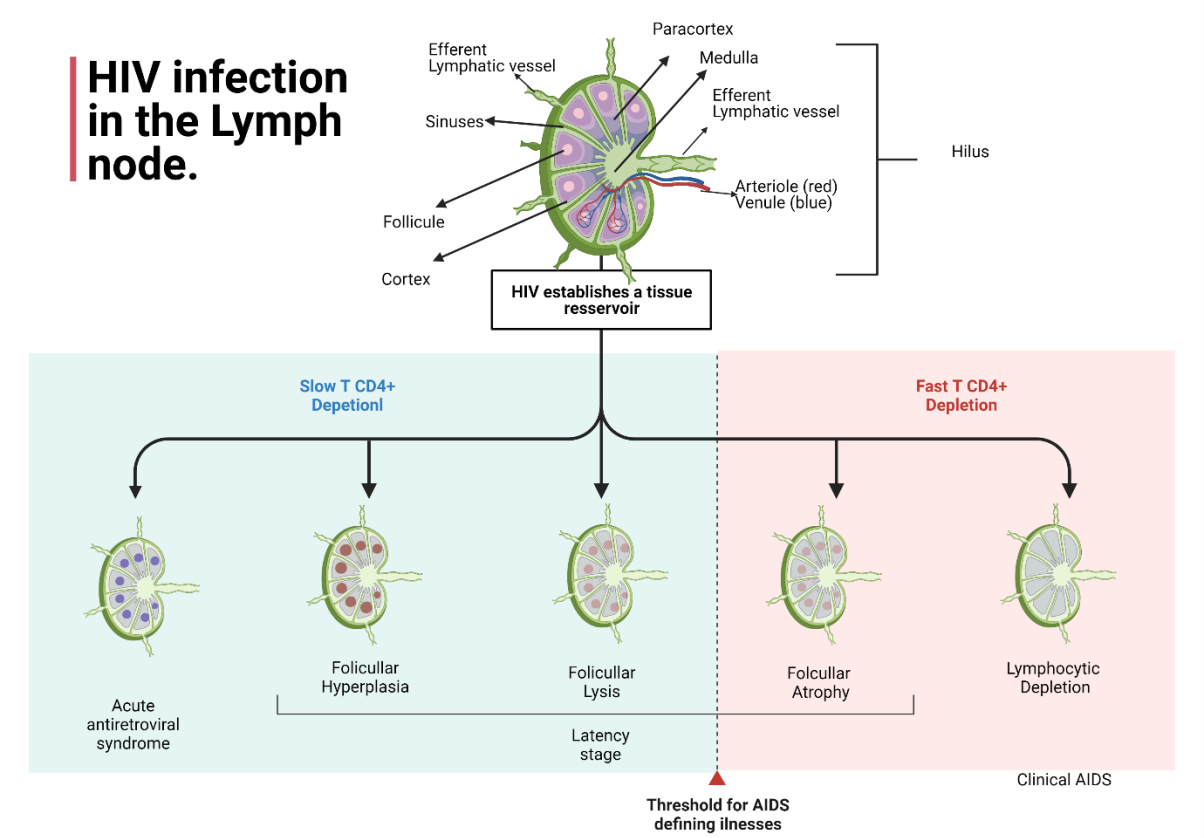


Figure 4. HIV forces histological changes after establishing infection. Once HIV has established infection in the lymph node, it will progressively damage the histological structure of tissue. Classical descriptions show that there are four histological stages of HIV related damage in lymphatic tissue: 1) Follicular hyperplasia 2) Follicular lysis 3) Follicular Atrophy 4) Lymphocytic depletion. After a threshold is reached, usually at the stage of follicular atrophy, clinical signs of AIDS are found on a patient [39-42].

The lymph, flowing into the node from the afferent vessel, travels through the subcapsular sinuses into the lobules, cortex, follicles, and paracortex. These regions, present distinct cellular populations that have important roles in the immune response. In the case of HIV, the paracortex and their important population of T CD4+ cells pose an important target for establishing infection [38, 42].

Early descriptions of AIDS showed that lymphadenopathy was a common sign within patients. At the histological level, morphological changes were found as the disease

progressed (**Figure 4**). This very early histological classification was based on the progressive destruction of the follicles and normal structure of the lymph node: 1) follicular hyperplasia 2) follicular lysis 3) follicular atrophy and 4) lymphocytic depletion [39, 41]. At the final stage AIDS defining diseases, such as Pneumocystis Jirovecci pneumonia or Kaposi's Sarcoma, were often identified [43].

Once reaching a local lymph node, HIV binds to two receptors to unload the viral RNA to the target naive T CD4+ lymphocyte. The main receptor being CD4, in most cases CCR5 and in rare occasions CXCR4, or a combination of both chemokine receptors (**Figure 2**) [35]. The secondary receptor that HIV uses is referred to as viral tropism, thus variants are referred to as CCR5, CXCR4 or CCR5/CXCR4 tropic viruses [44]. The viral tropism phenomenon does not have many clinical implications. It does however impact the therapeutical options that could be prescribed to a patient (such as the use of maraviroc as therapy) or the viral lineage that could infect a patient [44].

Once HIV binds its viral glycoproteins to CD4+ and to CCR5/CXCR4, it fuses to the lymphocyte's membrane and unloads the viral RNA, which gets reverse transcribed into a proviral DNA. The proviral DNA is then transported to the nucleus where it integrates the genome with the host's own DNA (**Figure 2**) [45]. When a T CD4+ cell has been infected with HIV, it is expected that a systemic dissemination would occur between the first 6-25 days after exposure [27, 42]. Of note is that a lymph node reservoir of HIV infected cells is established early on the infection, regardless of the use of antiretroviral therapy (**See section 1.3.1 for more details**) [37]

1.3.1 Antiretroviral therapy and pharmacological prevention schemes

During early stages of the pandemic, no therapeutic option was available for people living with HIV or AIDS. By the late 1980s, initial antiviral treatment emerged in the form of zidovudine, however due to side effects, it was reserved for the late stages of the disease [46]. Monotherapy proved to be far from the answer, as it did not prevent deaths from AIDS. HIV's replication process is bound to produce many mutations, culminating in a "quasispecies" phenomenon [47]. A "quasispecies" is a virological lineage that occurs spontaneously, directly related to the mutation rate from the reverse transcription [48]. Within one patient several quasispecies can emerge, usually dominated by the "fittest" HIV lineage. Monotherapy then allowed for HIV to eventually evade the pharmacological intervention, as selective positive evolutionary pressure for drug resistant HIV variants [49]. In order to account for this, by 1996, a triple antiviral scheme was developed, with different drugs (and different mechanisms of action) belonging to different families, with great success [50]. This scheme, currently known as highly [51] active antiretroviral therapy (HAART), has changed the overall morbidity and mortality of people living with HIV (**Table2**) [23, 52].

Table 2. Antiretroviral agents currently in use**.** Which are divided into five families NRTI, NNRTI, PI, Fusion inhibitors, CCR5 Antagonists and INIs. In parenthesis is the official abbreviation for each agent. Antiretrovirals of note are marked in black, red and green. Dolutegravir (DTG), in black, is a compound that due to the favourable adverse effects profile as well other antiviral factors has gained recently significant ground in HIV guidelines. Abacabir (ABC), in red, denotes an antiretroviral agent that requires testing for HLA-B*5701 as it has the potential to provoke fatal adverse effect in carriers. Enfurvitide (T-20, and Cabotegravir (CAB) in green, are the only two antiretroviral agents with a subcutaneous formulation [51].

NRTI	NNRTI	PI	Fusion Inhibitors	CCR5 Antagonists	INIs
Abacavir (ABC)	Efavirenz (EFV)	Atazanavir (ATV)	**Enfurvirtide (ENF,T-20)**	Maraviroc (MVC)	Raltegravir (RAL)
Didianosine (ddI)	Etravirine (ETR)	Darunavir (DRV)			**Dolutegravir (DTG)**
Emtricitabine (FTC)	Nevirapine (NVP)	Framprenavir (FPV)			Elvitegravir (EVG)
Lamivudine (3TC)	Rilpirivirne (RPV)	Indinavir (IDV)			Bictegravir (BIC)
Stavudine (d4T)		Lopinavir (LPV)			**Cabotegravir (CAB)**
Tenofovir (TDF)		Nelfinavir (NFV)			
Zidovudina (AZT o ZDV)		Ritonavir (RTV/r)			
		Saquinavir (SQR)			
		Tipranavir (TPV)			

Most of the antiretroviral formulations available (with the exceptions of the novel Cabotegravir/Rilpivirine and Enfurvitide) are based on oral formulations [40, 51]. While oral HAART has been successful in reducing the overall mortality and life expectancy of people living with HIV, patient compliance for the remainder of their lives remains an issue, as these oral formulations require at least daily dosing to achieve

viral suppression [40, 51, 53, 54]. Oral HAART is a significant measure in achieving viral suppression in blood, however, viral replication often bounces back to baseline levels if therapy is interrupted [51, 55, 56]. The first potential explanation to this phenomenon relates to viral sequences that are integrated to the DNA of a TCD4+ lymphocyte are reactivated by an unknown stimuli [51, 57, 58]. The second potential explanation refers to drug penetration to the lymphatic tissues is limited, therefore a reservoir due to low antiviral exposure is generated in the lymph nodes [40, 51].

Orally administered antiretrovirals have significant barriers to overcome in order to reach systemic circulation, and thus the lymphatic vessels [51]. The first difficulty to overcome is the solubility in gastrointestinal fluids and subsequent absorption [59]. Such an aggressive environment in the gastrointestinal tract can produce antiviral decomposition or incomplete dissolution of the drug prior to penetration of the cellular membranes and subsequent absorption [40, 59]. After absorption the potential for first-pass metabolism in the liver can potentially have an important effect in drug bioavailability, as it can lead to the formation of an inactive form of the drug. Once in systemic circulation, the antiviral can be in a protein bound or “free” drug presentation [51, 60]. “Free” drug is delivered to most tissues, including kidneys, which are organs that contribute significantly to the elimination of the antiretroviral agent [51]. Free drug has the tendency to have a short time in the lymph node, as it has the tendency to diffuse back to systemic circulation [40, 60]. As the ultimate target of HAART is the lymphatic tissue, small molecule antiretroviral agents are not of choice, as the majority is distributed through the bloodstream from the enterocyte [61]. Some physiochemical properties that favour lymphatic uptake are high molecular weights, lipophilicity (logP

>5), and a high solubility in long chain triglycerides (>50mg/ml) [40]. Most of the antiretrovirals listed in **Table 2** do not have those properties, as most NRTIs, NNRTIs and INIs have a hydrophilic nature (logP <1.0), with few of them passing 300 Da as molecular weight [40, 51]. Of note is that of all the parameters listed, only tipranavir presents these preferred properties, however it has since fallen out of use in clinical guidelines due to important side effects such as significant hepatic toxicity and intracranial haemorrhages. Due to these reasons tipranavir has been, as of 2014, relegated to be a third line therapy [62, 63]. Should current research continue the trend towards long-acting INIs, tipranavir will probably continue to fall further behind in guideline recommendations [62, 64-70].

Oral antiretrovirals can have the potential for accumulation in off-site tissues such as the liver and kidneys, often where major side effects are reported. This scenario has the potential of significantly limiting the proportion of drug that can cross the blood-lymph barrier and reach the target TCD4+ lymphocytes [51]. Currently only two antiretroviral formulations do not use an oral drug delivery. They are enfurvirtide and cabotegravir/rilvipirine. Enfuvirtide is a fusion inhibitor that has a subcutaneous administration. In enfurvitide's case, the reason for subcutaneous administration is due to a poor bioavailability that rules out oral delivery. Additionally, its short half-life (approximately 4 hours), requires twice daily injections to maintain levels required to inhibit HIV replication [71]. Enfurvitide has a high adverse reaction rate, particularly in the injection site, and thus is currently relegated to salvage therapy in most guidelines [40, 51, 71].

The novel formulation of cabotegravir/rilvipirine consists of a intramuscular delivery route [40]. Alternative routes, such as the subcutaneous or intramuscular routes can help avoid the first-pass metabolism, which often limits the possibility of a drug reaching the lymphatic system [40]. In the case of the cabotegravir/rilvipirine formulation, a significant advantage for both antiretrovirals was found when administered intramuscularly, as pharmacokinetic parameters such as T*max*, C*max*, and area under the curve were superior to their oral formulations [68-70].

HIV is a chronic condition that demands consistent treatment, usually in the form of one or two oral formulations in order to keep the viral load at low levels [40]. Paradoxically oral formulations of antiretroviral agents, despite being less intrusive than the subcutaneous or intramuscular route, present several issues that are particular to HIV therapy such as pill fatigue and discretion issues (due to HIV/AIDS stigma) [70, 72]. The cabotegravir/rivilpirine has another characteristic that makes it unique, it is a combined nanoformulation of 2 antiretroviral agents. Cabotegravir has specific characteristics that favour the generation of a long acting nanoformulation, such as a relatively hydrophobic logP (2.1) and a relatively high molecular weight (405 Da). Cabotegravir's nanoformulation is designed as a dissolution-controlled depot that uses polyethylene glycol 3500 and polysorbate 20 as stabilizing agents [68, 69, 73, 74].In the case of rilvipirine a similar case is found, as it has a logP of 4.5, and a molecular weight of 366 Da [70]. Rilvipirine is formulated as a nanosuspension stabilized by Pluronic 338 [69, 70, 73-75]. The combination of both antiretroviral agents in one single administration has allowed to prolong several important pharamacokinetic parameters notably the half-life, of both antiretroviral agents, allowing for a single IM dose per

month, instead of a daily pill [68-70, 73-75]. An additional advantage of nanoformulations, such as the cabotegravir/rilvipirine coformulation, is that the formulation can be tailored towards the selective uptake of specific cells, via size or the linking of a receptor agonist, thus opening a gateway for a targeted approach towards uptake in the lymphatic vessels [40, 68, 69].

Current HAART is composed of five families of antiretrovirals, with distinct mechanisms of action [40]. They can be divided into cell entry inhibitors, reverse transcriptase inhibitors, integration inhibitors and protease inhibitors. Cell entry inhibitors interfere with either the fusion of HIV to the cell (enfurvitide) or by blocking the coreceptor CCR5 (Maraviroc). Reverse transcriptase inhibitors: this broad family of antiretrovirals can be further divided into two subclasses, Nucleoside Reverse Transcriptase inhibitors (NRTI) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI). NNRTI and NRTI make up the largest families of all. Integrase Inhibitors (INI): this class of antivirals inhibits viral integration into the T cell's genome. Protease Inhibitors (PI): this class inhibits the viral protease cleavage, preventing budding of virions thus containing infection [23, 29]. Examples of antiretroviral agents can be found in **Figure 5.**

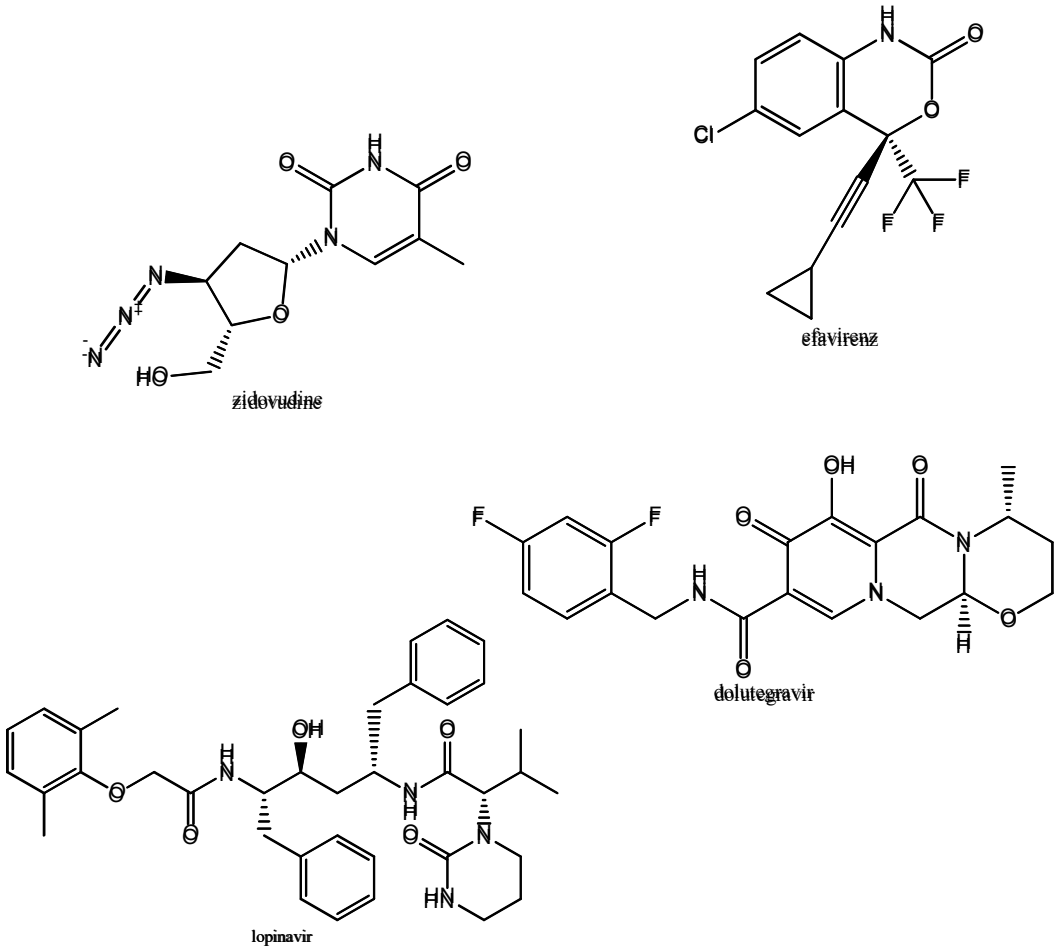


Figure 5. Chemical structures of representative antiretroviral agents from four different families. Zidovudine belongs to the nucleoside reverse transcriptase inhibitors family (NRTI). Efavirenz belongs to the non-nucleoside reverse transcriptase inhibitors family (NNRTI). Lopinavir belongs to the protease inhibitors (PI) family. Dolutegravir belongs to the integrase inhibitors (INI) family.

Experimental data on macaque models has found that despite antiretroviral treatment, the levels of SIV copies found in blood do not correlate with levels found within lymph nodes, as they are often higher than the ones found in blood [76-78]. Current research points to two potential reasons (or a combination of both) that allow for a creation of an HIV reservoir, despite antiretroviral therapy: a low-level viral replication at the lymph nodes or a clonal expansion of infected T-CD4+ cells [76, 79-84].

To have a high chance of success of aborting infection, in the context of a PEP any antiretroviral compound administered should target the paracortex area of a lymph node, as this is where most TCD4+ lymphocytes lie. Predictive models of the distribution of antiretroviral agents inside the lymph node are very revealing and could provide some insight into what agent to select for a novel formulation [40]. One such predictive model is based on Drugbank 2.0 (**Table 3**). Drugbank 2.0 is a project let by Wishart *et al.* that uses *in silico* algorithms with machine learning processes to predict different pharmacokinetic parameters, compound design and receptor docking as well as modelling drug metabolism, lymphatic distribution among other parameters [85].

Table 3. Predicted distribution inside the lymph node of different families of antiretrovirals based on Drugbank 2.0 [85]. Maraviroc is not mentioned, as by design it would only be a therapy of choice for CCR5 variants. Enfurvitide is not mentioned, as it is usually regarded as salvage therapy, and thus not in the first line of any guideline [40, 51].

Family of antiretrovirals	**Predicted distribution inside the lymph node**
PIs	Paracortex, Medulla, potentially the high endothelial venules (in the case of Darunavir)
NNRTI	Paracortex, medulla
NRTI	Subcapsular sinuses, cortex, high endothelial venules
INIs	Peripheral sinuses, cortex, high endothelial venules

It is of the utmost importance that any antiretroviral compound has a significant distribution across the high endothelial venules, as this can allow it to perfuse throughout T Cell rich zones, and thus abort an impending infection or contain an

established one [86]. The use of a nanoparticle formulation catered to lymphatic transport could allow for antiretroviral retention inside the lymph node, in similar fashion to what is done with antineoplastic agents [87].

Current research is undergoing to improve/simplify therapeutic schemes further, such as switching to dual therapy once virological control has been reached to minimize side effects [88]. However for prevention schemes, besides the use of condoms as prevention of further sexually transmitted cases of HIV, three pharmacological schemes are available for prevention: TasP, PrEP and PEP [23].

1.4.1 Pharmacological prevention schemes

TasP has evolved from the early days of antiretroviral therapy, as current guidelines agree that treating infected patients as soon as possible produces better patient outcomes and helps lower the transmission to potential partners. By lowering the viral load to an undetectable level, the transmission rates are almost negligible [8, 11, 89, 90]. Thus TasP is a crucial strategy for HIV prevention [1].

PrEP strategy is based on a daily oral regime of two antiretroviral agents, usually emtricitabine and tenofovir, which is prescribed to people who are sexually active and have certain high-risk characteristics. This strategy is mainly geared towards tackling new infections in men who have sex with men without condoms, sexual workers and certain behaviours (such as serodiscordant couples when the HIV positive individual has a detectable viral load). These populations have a significant higher risk than others of acquiring HIV. For this scheme to work as intended, testing for HIV infection prior to the administration of any antiviral agent is a key. Individuals under this scheme

should be regularly tested for HIV and other STDs. PrEP reduces the absolute risk of HIV acquisition, but it needs patient compliance to be effective. There are some concerns that PrEP may foster virological resistance, though when guidelines are followed, such events are extremely unlikely to happen [91, 92]. As the scale up of PrEP services continues, HIV resistance profiling should also be a priority [92].

PEP is a strategy based on the administration of antiretroviral agents in the immediate aftermath (with most guidelines specifying 72 hours) of a high-risk exposure, usually after an occupational exposure [93]. As with PrEP it is vital to establish the HIV status of the recipient before the administration of antiretroviral agents, as a PEP scheme may not be appropriate if the patient is already HIV positive. PEP is usually a 28-day prophylactic scheme, with medical and laboratory evaluations to be carried for six months, to ensure no HIV acquisition [94]. PEP is therefore intended as a "one time" intervention. In the case of PEP after a potential sexual exposure or PEPSE, several variables are to be considered, and thus it is not often prescribed [95-97] .

PEPSE is a strategy which is based on the prescription of antiretroviral agents for a month after a high-risk sexual exposure. Most data suggesting PEPSE as a successful intervention comes from occupational exposure reports rather than sexual exposure, as such clinical studies would be difficult to perform [93, 98]. Of note is that the overall prescription of these effective prevention schemes may have been interrupted by the ongoing SARS-CoV2 pandemic.

1.5.1 The SARS-COV-2/COVID-19 pandemic and the negative effects on HIV treatment and diagnosis programmes

In December 2019 a novel Coronavirus, SARS-COV2was described in China. Shortly after its appearance, SARS-COV-2, and its associated coronavirus disease 2019 (COVID19) generated an unprecedented demand on healthcare services worldwide [99, 100]. Just like HIV's own pandemic, the SARS-COV2generated a crisis that was felt unequally in nations. The full shockwaves of the COVID-19 pandemic will be felt for years, and in the case of accessing treatment and diagnosis for HIV, the ongoing situation can potentially disrupt international efforts to end AIDS and HIV for years to come[1, 6].

Early into the SARS-COV2pandemic, Hogan et al., modelled different scenarios as to elucidate how important the disruption to treatment and diagnosis of Tuberculosis, HIV, and other sexually transmitted diseases (STDs) could become. Their model used an effective replication number, or R0, of three, meaning that with this model one patient with COVID19 would transmit SARS-COV2to three more individuals [101].

Their model contemplated different scenarios: No action taken, mitigation (ie. reducing SARS-CoV-2's R0 by 45%), suppression-lift (ie. reducing SARS-CoV-2's R0 by 75% and lifting the restrictions by 6 months) and suppression (ie. reducing SARS-CoV-2's R0 by 75% and maintaining restrictions for over 1 year). Their calculations show a significant disruption in prevention schemes as PrEP schemes could have no new enrolments and no PrEP prescription renewals. Countries where condoms are supplied by the state, could potentially see a drop in condom usage by 50%. Of note is that these calculations did not consider the SARS-CoV2 variants that have proven to be more

transmissible than the original lineage [102]. In the case of HIV morbidity and mortality, the situation could be very dire, as countries with high HIV prevalence, could see a rise of new cases over the next five years by 10%, due to lack of testing and interruptions to antiretroviral therapy. These forecasts are particularly worrying, as addressing comorbidities, and securing antiretroviral therapy are priorities for HIV positive patients during the SARS-COV2pandemic [99, 101].

In a more recent study focusing on the MSM population in the United States by Mitchell et al., similar projections were obtained [103]. The SARS-CoV2 pandemic could provoke a rise in 5-10% in new HIV cases, as early diagnosis programmes, PrEP initiations and adherence would also be affected. This effect is also seen in different populations, as Booton et al, performed a study analysing MSMs from Chinese background. Booton et al, also found a significant rise in newly diagnosed cases (5%-10%), in line with published literature, in the year following the lifting of restrictions [104]. With these three models it can be concluded that a significant (10% - 25%) loss in the virally supressed HIV+ individuals could be a potential side effects of the suspension of normal healthcare services during the SARS-CoV2 pandemic [101, 103, 105].

As the current uncertain scenario that many countries are facing due to the SARS-CoV2 pandemic unfolds, measures should be in place to prevent these catastrophic numbers. Thus, studying behaviours of populations that are not traditionally covered by PrEP, PEP or TasP, should also be a priority in the years to come, as increases in the HIV newly diagnosed cases can be expected in the coming years [99, 101, 103, 105]. In this

context, exploring potential new PEPSE formulations can provide a new effective tool to prevent new HIV cases.

1.5.2 A case for an alternative PEPSE for low to moderate sexual risk of HIV acquisition

PEPSE has specific considerations as antiretroviral agents are often expensive and have significant adverse effects. Therefore, different guidelines suggest PEPSE to be prescribed only on the grounds of a high-risk event, which is many times not clearly defined. Such scenarios are often leaving the decision at the clinician's discretion [95, 106]. However, the 2016 PEPSE guidelines in the UK present a potential solution to this issue. Overall if the risk of HIV infection is assessed as >1/1000, PEPSE should be considered/prescribed. The risk of HIV transmission is quickly addressed by the following equation [95]:

$$Risk\ that\ Source\ is\ HIV\ positive \Big/ (based\ on\ local\ prevalence\ data) \times Risk\ of\ Sexual\ act\ performed \Big/ (based\ on\ estimates\ per\ act) = Risk\ of\ HIV\ infection$$

However, that formula presents a problem, as the local prevalence HIV data refers to the prevalence of the community where the potential source comes from. In other words, this formula should be adapted as much as it can be, as HIV prevalence can vary significantly, even in different cities from the same country. If we take the UK's example, the highest HIV prevalence is found in the Greater London metropolitan area [95]. The second part of the formula should be easier to identify, however different studies show different risk scenarios for sexual contact (**Table 1**). Effective transmission can vary depending on the type of sexual exposure viral load/strain of the

HIV infected partner, the use of condoms, male circumcision, and the potential use of antiretroviral agents on the infected party [95, 107, 108]. The two sexual activities confer the biggest risk are the unprotected receptive vaginal and receptive anal scenarios, with estimated risks of transmission of 0.1% and 1% respectively. As an additional barrier to HIV prevention after a sexual exposure, adherence to PEPSE can be poor even in high income settings, where access to healthcare is not an issue[109].

Guidelines for both PrEP and PEPSE inadvertently leave a gap of potentially exposed people to HIV that are currently unprotected by a pharmaceutical intervention. Not all sexually active individuals are members of key populations or find themselves in a high-risk scenario for HIV acquisition [95, 106]. The existence of such population has been acknowledged in UNAIDS documents, but its size and behaviours remain unknown [2]. In such scenarios, only medical follow-up is offered as the risk to benefit ratio is considered to be higher with the use of current PEPSE regimes than the potential risk of HIV acquisition [95, 106].

Most new HIV infections are related to unprotected sexual intercourse with a HIV positive individual, thus it is imperative to further study this population [1]. Further exploration of unprotected risk scenarios, including their frequency, in sexually active individuals should play a part in the design of future strategies for the prevention of new cases of sexually transmitted diseases and HIV. Scenarios with the risk of HIV acquisition that are often not considered in guidelines are condom failure rates (no condom, broken condom, inconsistent use of a condom), that are often linked with HIV acquisition [110]. This evidence suggests that a new formulation of PEPSE should also be part of the priorities to lower new HIV cases. Such a formulation could be

administered in a localised manner, to minimize systemic side effects [51]. Thus, the first question would be which antiretroviral to select.

1.6.1 Selection of an antiretroviral agent for a Novel PEPSE

Selecting an antiretroviral for a novel PEPSE from all the available options must consider several parameters, including side effects, "genetic barrier" of HIV to resistance, antiviral efficiency to lower viral loads and ideally lymph node distribution[40, 51]. Additionally, as reviewed in section 1.2.2, time is running out for such an intervention to be effective, as most guidelines suggest the prescription to be as soon as possible[106].

Unfortunately, many antiretroviral agents have very important side effects, such as hepatoxicity, central nervous system alterations or nephrotoxicity [23, 51]. In some cases, such as abacavir and patients with HLA-B*5701 a fatal hypersensitivity follows administration. Prevention of this side effect involves testing for that specific HLA, which in the context of PEPSE rules abacavir out [111].

Genetic barrier is a concept that applies mainly to HIV therapeutics. As touched on briefly in section 1.3.1, HIV has a high mutation rate per replication cycle, eventually causing an HIV quasispecies. This phenomenon allows for different virological lineages to coexist in one infected patient, with some lineage bearing mutations that confer certain resistance to antiviral agents. The genetic barrier of any antiretroviral is the calculation of how many specific mutations must a virological lineage undergo to be resistant to any antiretroviral agent. This is the reasoning behind the current antiretroviral schemes, as they are set up with at least two different compounds with at

least two different mechanisms of action. Monotherapy eventually leads to a selective pressure that favours genetic mutations with resistance to certain antiretrovirals [112].

Due to structural similarities, if resistance is obtained against certain antiviral agents, it may nullify the effect of other members of the same family. Such is the case of Efavirenz, which has a genetic barrier of 1, and whose mutation will confer resistance to all other NNRTI [113]. In some cases, the mutations will effectively alter the viral fitness to replicate, such an example is the mutations that can be induced with Emtricitabine use. The mutation M184V is associated with impaired viral fitness, and thus is one of the most "benign" lineages, and sometimes selected for in multidrug resistant HIV ([112, 114]. Selecting for the M184V mutation allows for the use of emtricitabine as salvage therapy in multidrug resistant HIV.

Ever since its inception the INI family of antiretrovirals has revolutionized HIV therapy. By preventing integration into the host's genome, the family has the potential to quickly abort infection. The first generation of INIs was comprised of raltegravir (RAL), which had significant adverse events, just like other antiretroviral agents, but had significant ability to lower the viral load and had a high genetic barrier threshold. Further targeting to HIV's integrase led to a new generation of INIs. Headlined by dolutegravir (DTG) these new INIs had a significantly improved side effects profile when compared to other antiretrovirals or RAL for that matter[66, 112, 115].

Early into DTGs inception, it was clear that it was a compound with high antiviral power, with an IC90 of 64 ng/ml in vitro [115]. Such good results were replicated in the subsequent Phase 3 trials, as it proved to have good antiviral properties and a favourable side effect profile when compared to RAL [116]. DTG presents an important advantage

in terms of virological resistance, as it is extremely unlikely that a wildtype virus presents resistance to DTG [117]. Evidence also suggests that it is very unlikely that DTG will select for resistant lineages, with some authors suggesting it may be impossible in clinical practice. By analysing these set of data, it has been proposed that DTG, or similar compounds can effectively be a potential "functional cure" for HIV [64, 66].

When plotting out Dolutegravir against other antiretroviral agents, it excels in lowering the viral load as well as having a high genetical barrier. When paired with such favourable side effects, it makes DTG an attractive model compound for a novel PEPSE (**Figure 6**). Additionally Dolutegravir's predicted distribution inside a lymph node allows for a potential saturation of the T-CD4+ rich zones, the paracortex area [40].

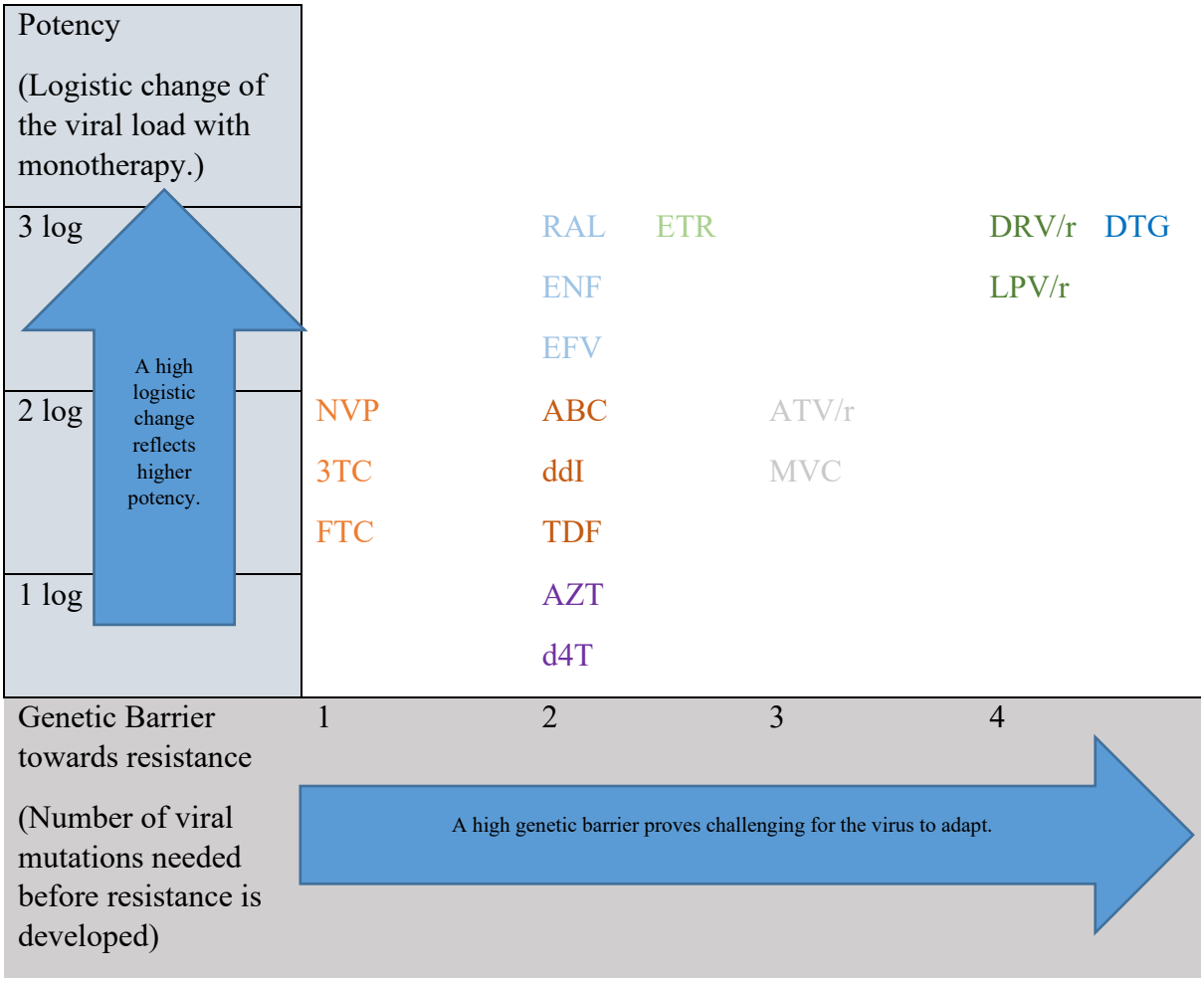


Figure 6: Genetic Barrier plotted against viral potency when used in monotherapy in logistic scale. Monotherapy has been slowly substituted by a combination of different antiretroviral agents to maximize lowering the viral load and minimizing the potential of variants that contain resistance genes. DTG is a compound that has a high viral potentcy and a high genetic barrier, additionally the predicted lymphatic distribution also make it an ideal compound for PEPSE [118, 119].

1.7.1 Localised antiretroviral formulations: Microbicides and future perspectives.

Most antiretroviral agents have an issue of penetration to rectal and vaginal mucosas, as tissue levels are lower than plasma levels [51, 78]. Both present challenging scenarios for the effective delivery of antiretrovirals as significant lack of predictability of penetration to vaginal and rectal mucosa remains a challenge. Most of these variations

can be accounted for, with physiological variations (particularly in the vaginal tissue) and the hepatic first-pass effect; however, they remain a challenge for future antiretroviral formulations.

Considering that the receptive partners often have a higher risk of HIV acquisition, recent attempts have been made for the development of localised antiretroviral formulations to be available for the public [120, 121].

These formulations, mainly researched as a subfamily of PrEP, have been under study for approximately 30 years [122]. Such formulations, known as microbicides, originated as vaginal gels that contained molecules, such as detergents that altered the local pH, but had no direct effect versus HIV or other STDs (**Figure 7**). As research evolved, different formulations containing antiretroviral agents were developed [121].

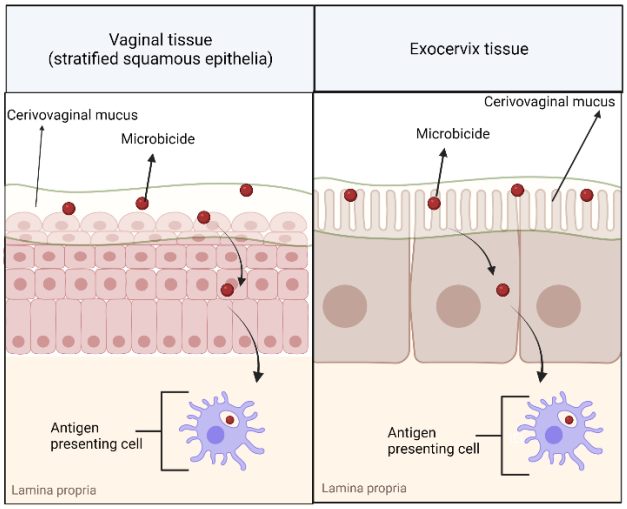


Figure 7 Female anatomical barriers for a microbicide. The female reproductive tract is a type II mucosae, therefore lacks a dedicated localised lymphatic tissue, and thus relies only on local antigen presenting cells for the identification of antigens [123]. The vaginal tissue has a symbiotic relationship with the local microbiota, often Lactobacilli, that secrete factors such as lactic acid. Such an acidic pH may be an issue for some compounds and

drug delivery systems[123, 124]. The cervicovaginal mucous presents an important barrier to overcome, as it traps highly hydrophobic systems. The stratified epithelium in vaginal tissue presents a challenging barrier in most of the vaginal tissue (except in the exocervix, where it is a columnar epithelium), as it would prevent any localised PEPSE formulation to be uptaken by the antigen presenting cells[124].

Vaginal microbiota play a critical role in female reproductive tract health. As such, nanoparticle-based drug delivery systems intended for vaginal applications must be designed with the preservation of healthy bacterial colonization in mind. The stratified squamous epithelium of the human vagina sheds continuously, thus enhancing the physical barrier to infection and providing a source of glycogen to promote growth of Lactobacilli. Lactobacilli compete with other microbes for nutrients, and secrete factors, such as lactic acid, that make the environment inhospitable to other bacteria and pathogens [42].

The rationale backing these formulations stemmed as a modified localised PrEP for females in high burden HIV scenarios. Therefore, vaginal formulations for HIV were the first to be targeted [125, 126]. These initial vaginal formulations were based on a "before and after" administration. Women in these studies were requested to dose the gel 12 hours before intercourse and have a second dose within 12 hours after intercourse, with no more than 2 doses on 24 hours (due to the lack of safety data) [125]. Such a strategy describes a localised PrEP scheme rather than a localised PEPSE formulation. In fact, many of the formulations to follow these illustrative studies would be based on similar prescriptions. As stated previously, even if these formulations were successful in preventing sexual acquisition of HIV, they would leave any individual in an unplanned lower to moderate risk of HIV acquisition without any pharmacological intervention.

1.7.2 Vaginal tissue barriers for a localised delivery system

As stated before, both rectal and vaginal mucosa pose important targets for the delivery of antiretroviral agents as a prevention scheme. However, they do have very distinct histological and physiological characteristics, as well as lymphatic node drainage (Figure) [121, 124, 127]. In the case of vaginal tissue, the menstrual cycle variations can significantly alter drug delivery. Additionally, the native microbiota poses another difficult barrier to overcome for adequate compound uptake.

The first barrier to overcome for any drug delivery system is the cervicovaginal mucus. The cervicovaginal mucus outside of the ovulation period is composed of enzymes, lipids and proteins, suspended in an aqueous media. With ovulation, the components of the cervical mucous become more diluted, however as its production is increased it poses a threat to drug absorption. The cervicovaginal mucus also has an immunological role, as it often traps pathogens and particulates of >500nm in size [128-132].

Due to the lack of mucous associated lymphatic tissue (MALT), the immune cells that are on site have the important duty to regulate a careful balance between the vaginal microbiota and potential threats. Such a role is difficult to elucidate, however current studies are undergoing to further the understanding of this microenvironment [133]. Compensating for a lack of lymphatic tissue, the squamous vaginal epithelia work as a physical barrier to infection. Thus, if there is any important disruption to the vaginal epithelia, infection risks to external pathogens are increased [123, 134-136].

The lack of MALT in the vaginal tissue, also implies that the dendritic cells or macrophages must travel to the local lymph nodes for the immune synapsis to occur

[134]. Another issue regarding the initiation of the immune response are the variations in sexual hormones through the menstrual cycle, as these hormones can alter immune responses [135, 137].

1.7.3 Vaginal antiretroviral delivery systems

Despite these difficulties, there have been several attempts for an antiretroviral formulation to be locally administered in vaginal tissue. Of all the microbicides trialled for vaginal tissue, two are of note: A gel loaded with 1% tenofovir and a dapivirine vaginal ring [121]. Both formulations are localised PrEP based schemes, as the tenofovir gel is based on a "before and after" dosing (described above) and the vaginal ring is to be inserted prior to sexual intercourse, intending to provide protection for a month (to be then replaced by a new vaginal ring) [125, 138]. As of this thesis being written, most of the localised antiretroviral formulations, either vaginal or anal, have a strong focus on PrEP, with no localised formulations intended for PEPSE.

The 1% tenofovir gel advanced to a phase II study, it stands alone amongst other microbicides as one that demonstrated a protective effect against sexual HIV transmission. This microbicide formulation consisted on a Tenofovir 1% gel comprised of 40mg of 9- [(R)-2- phosphonomethoxy)propyl]adenine monohydrate (PMPA) with edetate disodium, citric acid, glyceryn, methylparaben, propylparaben, hydroxyethycelluloce and purified water as excipients. The population studied, heterosexual females, from a high prevalence country, allowed for an early proof of concept for a PrEP microbicide [125].Unfortunately, such encouraging results have not been reproduced. A possible explanation to the lack of reproducibility of results points to low patient adherence to the treatment [139]. As with other antiretroviral preparations,

these formulations must have a high patient adherence to be effective, especially with the potential of resistant variants of HIV. With administration of the gel requiring constant application, such as before and after sexual intercourse, its research was not advanced to the next phase [140].

The second formulation, based on a silicone vaginal ring loaded with dapivirne, managed to get even further on clinical trials. A phase III placebo-controlled study, produced evidence that the ring allowed a reduction of HIV transmission by 30% [138, 141]. The protective effect was found to be greater with older age, thus suggesting that patient adherence was a significant factor in the effectiveness. In contrast to other PrEP formulations, the dapivirne ring proved to have a favourable profile as it had no major systemic side effects reported [142]. This vaginal ring presents a lot of opportunity for future localised PrEP or PEP formulations; however it is still pending a couple of large-scale studies, particularly with pregnant and lactating women before it is licensed for public use [121, 142].

Both formulations present a good proof of concept that an antiretroviral formulation with localised delivery can effectively be a solution for female populations in higher risk, as an alternative to other oral PrEP formulation schemes, although further studies are needed before they are licensed for public use [143]. However, as stated previously, there is a population who finds themselves in an unplanned lower to moderate risk scenario for HIV acquisition, to whom no intervention is currently offered.

1.7.4 Anal tissue as a target for antiretroviral delivery

As with vaginal tissue, the rectal mucosa poses an opportunity for the development of localised antiretroviral schemes, however similar to the vaginal case, most of formulation research has been focused on a rectal PrEP formulation. The rectal mucosa presents an entire different environment when compared to the vaginal tissue [127].

The rectum is the anatomical area that starts from the distal end of the sigmoidal colon and extends to the anal cavity. In contrast to other sites of the gastrointestinal system, it primarily acts as a means for the faeces to leave the body. Therefore, it is involved with minimal absorption of water and electrolytes from the cavity's contents [144, 145]. At the microscopic level, the rectum, unlike the colon, only harbours a residual fraction of the gastrointestinal microbiota, therefore little presence of bacterial enzymes are expected [146]. This poses a significant advantage to rectal delivery systems when compared to vaginal ones, as potential losses of drug due to bacterial enzymatic activity, are predicted to be low [147-149].

Histologically, the rectal mucosa is made up of a single layer of columnar cells that lack vili on their luminal surface for absorption. Intercalated within the epithelial cells, goblet cells are found secreting mucus. Unlike the cervicovaginal mucus, the purpose of the rectal mucus is to lubricate the passage of the faecal matter and has little changes overall [150-153].

Additionally rectal tissue does contain MALT, represented by Peyer's patches, as well as a bigger population of dendritic cells when compared to the vaginal tissue [153]. Peyer's patches are distinguished from the rest of the rectal epithelium, as they contain

a reduced number of intestinal vili, as well as a specialized type of cell, microfold cells or M cells [154]. M cells are vital cells in Peyer's patches, as their role is to uptake any potential pathogen and through transcytosis allow it to be internalized by local macrophages or dendritic cells (**Figure 8**) [155, 156]. These events lead to an adaptative immune response as the antigen is then transported to a local lymph node, where it will encounter a TCD4+ lymphocyte.

Both vagina and anus, due to their proximity, have similarities in the lymphatic drainage. The external areas of the perineum including female external genitalia and lower vaginal cavity, penis and scrotum as well as the lower parts of the anal canal are drained by the superficial inguinal lymph nodes [157, 158]. The superficial inguinal lymph nodes then drain their lymph to the lumbar aortic lymph nodes. Lymph will then be carried from the lumbar aortic lymph nodes through the para-aortic chain to the cisterna chyli, where lymph will find its way towards systemic circulation. Lymph from the cervix and upper anal cavity/lower rectum will be drained through the lumbar lymph nodes, following a similar pathway to find the cisterna chyli and then systemic circulation [158, 159].

While the influence in drug absorption of the lymphatic vessels around the rectum is still under investigation, current evidence suggests that it would allow for compounds to avoid the first pass effect, when such compound is delivered locally. Additionally, it may contribute significantly to the absorption of lipophilic compounds [153, 160]. Rectal formulations are ideal for compounds which have limited absorption in oral dosages, degrade easily in the gastrointestinal tract, or undergo a significant first pass effect [161]. Additionally, they pose a potential route of choice for compounds that have

a localised action in the rectal or distal colon, such as would be the case in a PEPSE scenario [151].

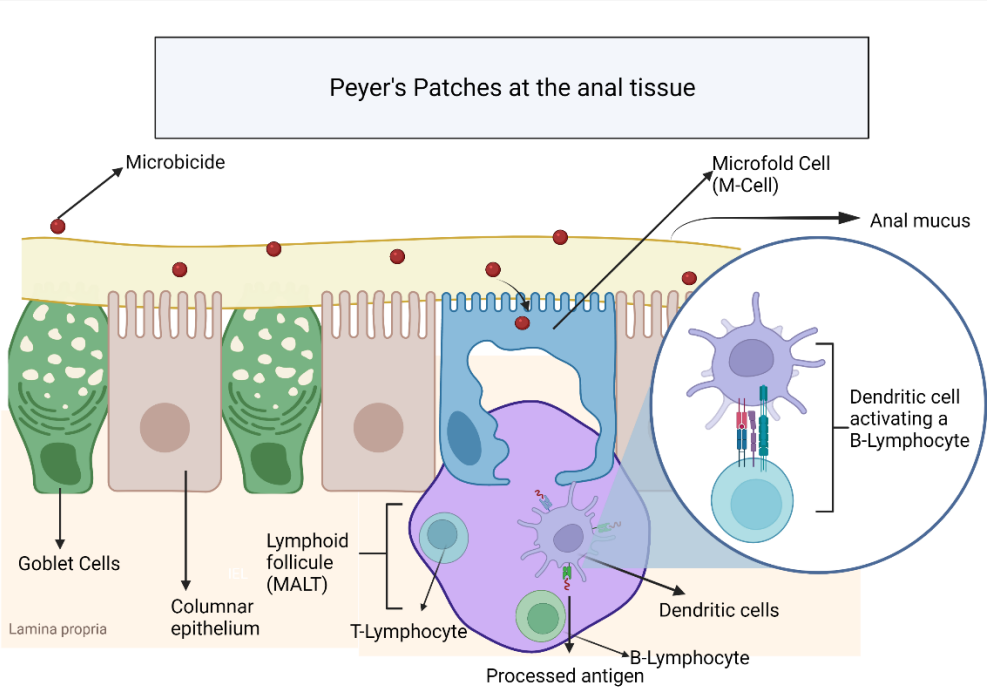


Figure 8. Peyer's patches at the anal tissue. The mucus found in the gastrointestinal tract has different characteristics than the cervicovaginal mucus, and it does not have significant variations. Overall, it is permissive to particulates that are negatively charged and less than 500 nm in size[162, 163].The gastrointestinal tract is comprised of a type I mucosae, with an associated lymphoid tissue. In the case of the anal tissue, the associated MALT is named Peyer's Patches[164]. Peyer's patches are characterized by microfold cells (M-Cell) whose purpose is to uptake any particulates and make them available to the local dendritic cells, which in turn may present any potential antigen to a local lymphocyte. If compared to type II mucosae, the process of lymphatic uptake and activation of any potential compound or antigen is extremely efficient [123, 154, 156]. Effective delivery of a localised PEPSE should take advantage of the Peyer's Patches for lymphatic uptake [123].

1.7.5 Anal antiretroviral delivery systems

Research into rectal antiretroviral delivery systems has had less examples than those found in the vaginal case. As of the writing of this manuscript only one rectal

microbicide has been advanced to a Phase II clinical trial. This formulation was based on a gel loaded with 1% tenofovir, similar in composition to the one used for vaginal purposes [165, 166]. The study of the formulation was based on two dosing schemes both PrEP oriented, thus not intended for a population that is in lower to moderate risk of sexual HIV acquisition. The first dosing scheme was daily application of the 1% tenofovir gel in the anal cavity. The second dosing scheme was based on a "before and after" receptive anal intercourse. This tenofovir 1% gel formulation targeted MSM and transgendered women, from 4 different countries. As with the vaginal formulation, a favourable safety profile was found, as there were no significant systemic side effects associated with the use of the 1% tenofovir gel. However unfortunately, no protective effect against sexual HIV acquisition has been found with this formulation.[121]. Due to risks associated with rectal intercourse, a local rectal drug delivery system could have a significant impact in lowering the HIV burden.

Targeting the lymphatic drainage, the evasion of the first pass mechanism as well as the saturation of the local lymph nodes by the antiretroviral of choice, poses a potential advantage when comparing rectal vs vaginal delivery. After careful consideration of this evidence, we have chosen to focus on a rectal delivery system for PEPSE. This mainly due to the fact that exposure to HIV in a rectal scenario leads to higher chances of infection compared to the vaginal route. It is our main hypothesis that such a delivery system would saturate the local lymph nodes and abort infection in the immediate aftermath of a risky exposure.

1.8.1 Nanoformulations in the HIV field

Saturation of the paracortex area of a lymph node is paramount for the success of a PEPSE formulation. As reviewed in section 1.3.1, current antiretroviral formulations often have important limitations in regards to the distribution of compounds to the lymph nodes, as often the concentrations found in plasma are significantly higher than the ones found in the lymph nodes [167]. As of the writing of this manuscript most research has focused on nanoformulations for treatment and PrEP [51]. Nanoparticle delivery platforms that have been tried previously have included polymeric nanoaggregates, solid lipid nanoaggregates, and nanoemulsions[51, 168]. Of them all a recent paper by Sillman et al, using Dolutegravir as the antiretroviral of choice, has showed very promising results via high pressure homogenisation of dolutegravir [169]. Sillman et al's work also involved a myristoylation of DTG, to produce a lypophillic compound a Dolutegravir Myristate (MDTG). This MDTG compound allowed for a significant increase in drug content or weight percentage (wt%) of the nanoformulation, when compared to the parent drug formulation (88% vs 66%). Of note is that after one intramuscular dose in Male Balb/cJ mice, DTG concentrations remained above the IC-90 (64ng/ml) for 8 weeks, and in non-human primates for 35 days [169]. However, this formulation requires a state-of-the-art high-pressure homogenizer, thus the scaling up of this formulation may prove difficult. Antiretroviral nanoparticle formulations are discussed more in depth in Chapter 3.

1.9.1 Project aim and hypotheses

The main project aim is to develop a nanoformulation for PEPSE in HIV to be administered locally in the rectum after an event of sexual exposure with a low to

moderate risk of HIV acquisition (risks of acquisition between 1/1000 and 1/10 000). This is in stark comparison to other pharmacologic prevention measures such as TasP, PrEP or PEP. Unlike conventional PEPSE, the proposed formulation is aimed at a sexual risk of HIV acquisition where current guidelines indicate that only medical follow-up is performed. This novel formulation would also need to be administered during the first 72 hours after intercourse, as this is the timeline that would abort impending HIV infection [170].

The secondary project aim is to estimate the size and views of a sexually active population, currently not covered by prevention schemes, such as TasP, PrEP or PEPSE, that could find themselves in a lower to moderate risk of HIV acquisition.

The main hypotheses of this project are:

A) There is a significant population that is currently uncovered by current pharmacological interventions such as TasP, PrEP and PEPSE. This population finds themselves in unplanned low to moderate risk scenarios for HIV acquisition through sexual route (i.e., risks of 1/1000 to 1/10000). The population that is currently unprotected and finds themselves in an unplanned low to moderate risk scenario is a population that is sexually active and mainly uses condoms as a means of prevention for HIV and other sexually transmitted diseases (STDs). The scenarios that lead them to an unplanned lower to moderate risk scenario are varied, but are likely linked to condom breakage, “wanting to use a condom but none available”, and “forgetting to use a condom”.

B) A nanoformulation with significant drug content could cover the needs for a population in a lower to moderate risk scenario in a sexual exposure for HIV, for which no prophylactic measure is currently prescribed. A significant drug content as well particle size control would allow for a higher chance of saturating the local lymph nodes with the compound. Such a formulation would be able to saturate the local lymph nodes, aborting HIV infection, and preventing systemic exposure to antiretroviral agents (limiting side effects).

Chapter 2

Unplanned unsafe sexual intercourse events, including condom failure, lack of condom availability and forgetting to use a condom, expose a high proportion of sexually active population to potential risk of HIV acquisition.

2.1.1 Interventions (pharmacological and non-pharmacological) designed to prevent HIV.

As reviewed on Chapter 1, despite global efforts to reduce the incidence of new cases of HIV since 2012 has remained consistent with approximately 2 million people newly diagnosed each year [1]. The bulk of new diagnoses are attributed to sexually transmitted HIV [1, 4, 26]. As 2020 ended, the UNAIDS 90-90-90 milestones had been missed, thus more stringent measures are needed if we are to reach the 2030 milestone of no new cases of HIV by 2030 [1, 6, 7].

Additionally, as reviewed in chapter one, the SARS-CoV2 pandemic has also derailed global efforts, interrupting key pharmacological such as TasP and PreP. Mathematical models predict upward trends in new HIV cases during the coming years, particularly in key populations such as men who have sex with men (MSM) [99, 101, 103-105]. Pharmacological interventions for HIV prevention in a sexual exposure are PrEP, TasP and post-exposure prophylaxis following sexual exposure (PEPSE). Further exploration of novel cost-effective and individually tailored PrEP approaches are emerging. PrEP

“on demand” is one such novel approach, which is tailored for individuals who can plan their sexual activity, are in substantial risk for acquiring HIV and, and due to diverse reasons prefer not to commit to a daily PrEP regime [171]. However, even PrEP “on demand” does not cover all individuals potentially exposed, as it is currently considered an option mainly for MSMs [172].

PEPSE is a strategy based on the administration of antiretroviral agents in the immediate aftermath (with most guidelines specifying 72 hours) of a high-risk sexual exposure. Such intervention was originally studied for occupational exposures and then expanded to high-risk sexual exposures [93, 94, 173]. PEPSE is therefore intended as a “one time” intervention with specific guidelines for its prescription [95-97]. A further experimental tool under investigation is post-exposure prophylaxis-in-pocket (PIP). PIP is an approach aimed towards individuals whose sexual activities present a high-risk scenario, however they have infrequent potential sexual exposures to HIV. [174, 175]. PIP users are given a full 28 day oral antiretroviral prescription and are instructed to initiate the treatment as soon as possible should a potential exposure happen and then required to initiate medical follow up [174]. PIP is undergoing further evaluations, and only used in experimental trials at the moment [175]. Both PrEP and PEPSE schemes often leave out individuals whose potential risk of acquisition lies between 1/1000 and 1/10 000, i.e., low to moderate sexual exposure risk scenarios [95, 96, 173].

Nonpharmacological interventions that have supporting evidence for HIV prevention in a sexual scenario are mainly barrier methods (such as condoms) and voluntary male circumcision (MC). MC has been evaluated in randomized controlled trials for a protective effect in the heterosexual transmission of HIV and other STDs. Evidence

suggests that there is a protective effect for insertive heterosexual intercourse [176-179]. These encouraging results have not been replicated for the MSM community, as a metanalysis provided evidence that there may be no association between MC and HIV protection within the MSM community [180]. Evaluating the published evidence both in heterosexual and anal intercourse suggests that the protective effect of MC is multifactorial [177]. Additionally, because of cultural or patient acceptability rates, it is not a procedure that would likely be universally accepted. Therefore, barrier methods remain the intervention of choice for HIV prevention [181, 182].

Barrier methods include spermicides, female condoms, and male condoms. Spermicides, usually based on the surfactant agent nonoxynol-9, are localised formulations that are designed to kill sperm. Regarding HIV protection, spermicides have had little evidence supporting a positive effect against HIV [183]. Additionally, common side effects, such as vaginal/rectal irritation have caused recommendations against their use for HIV prevention [184-186]. Such evidence suggests the male condom and the female condom are the barrier methods of choice for HIV prevention [182, 187].

Female condoms are casings that are made to cover the vagina and vulva, with a closed end. At the closed end, a ring is placed both to fix the female condom in place and to allow for insertion in the vagina [122, 187]. When assessing the protection factor versus HIV, it is assumed that the female condom and the male condom have similar protection rates, however, this is based on old models of the female condom, and thus further research may be needed. Unlike the male condom the female condom has a high cost, which eventually makes its availability lower than the male condom. Additionally,

cultural perceptions could make the female condom a less universal tool than the male condom, as it requires female genital manipulation for its correct insertion [188, 189].

Male condoms are sheaths that are meant to cover the penis from glans to the root of the penis. The ease of access to male condoms, high acceptability, relative low cost and ease of use remain among the top characteristics that make male condoms the intervention of choice to prevent HIV acquisition [190, 191]. For the rest of this thesis, male condoms will be referred to as condoms.

2.1.2 Condoms as a means of protection for HIV/Condom failure rate.

Condoms are also not perfect prevention tools as condom failure events and low usage rates alter the potential for HIV protection [192]. Condoms are currently the main focus for HIV prevention schemes, however their effectiveness in protecting from HIV is linked to multiple variables. Variables such as a high viral load in the HIV+ individual, genital ulcers, sexual act performed, and incorrect condom use are significant risk factors for HIV transmission [23, 90]. A Cochrane meta-analysis of 14 cohort studies found an estimated 80% risk reduction in HIV incidence among heterosexual individuals with a serodiscordant partner with penile-vaginal intercourse [182]. Evidence suggests that the effective condom protection from HIV in penile-anal intercourse is 70%, with no statistically significant difference between penile-anal and penile-vaginal protection rates [193]. Of note is that these protection estimates were computed assuming consistent condom use, as inconsistent use does not have the same protection rates.

In a study following serodiscordant MSM couples over long periods of time it was concluded that inconsistent condom use offers little to no HIV protection [192, 194]. Most of the studies regarding condom use, are based on self-reported information [192]. Self-reporting of HIV status can be of special concern, as the latency period in HIV infection allows for an unrecognized HIV+ status, and subsequent risk to the sexual partner [49].

In addition to the inconsistent use, condom failure events such as breakage or slippage, can result in the acquisition of HIV [192, 195]. As the condom breaks, there is a possibility of skin-to-skin contact with fluid exchange and thus facilitating HIV exposure, particularly if there was a violent intercourse [196]. Self-reporting of these events is, while not ideal (as not all condom break events are noticeable by the respondent), is an acceptable way to estimate for condom failure events in the sexually active population. Therefore, exploring the views and behaviour of sexually active population can allow for further understanding of a sub-population not covered by current pharmacological prevention schemes [192].

2.1.3 Exploring the views of the sexually active public.

Populational survey studies dedicated to sexual practices of a general population are not conducted often. One of the biggest examples of populational surveys is the National Surveys of Sexual Attitudes and Lifestyles (NATSAL) conducted in the UK. As of the writing of this manuscript, NATSAL has had three iterations: NATSAL-1 (1990), NATSAL-2 (2000) an NATSAL-3 (2010) with NATSAL-4 being currently conducted [197-199]. These populational studies give us significant insights into sexual behaviours of the sexually active population, as all the surveys enrolled over 10 000

participants in each edition. However, it is important to note that no NATSAL project has ever had a focus on HIV risk scenarios such as condom failure events. Studying these scenarios further would be of paramount importance, especially as the size of the pharmacologically uncovered population is unknown.

2.1.4 State of the HIV pandemic in the UK and in Thailand

The UK and Thailand were selected as target countries for studying the size and behaviour of a currently unprotected population by prophylactic schemes. These two countries have significant differences, both cultural and in the HIV related burden [1, 6]. Similar to many other high resource countries, the UK has an excellent track record of reducing new HIV cases with prevention and treatment programmes [200]. Like other Western countries, the UK has most of the current HIV burden in MSMs, as they represent approximately a 53% of the new diagnosed cases. However, the UK faces an issue of late HIV diagnosis (less than 350 T CD4+ lymphocytes at diagnosis), as infected individuals can effectively transmit the virus to a partner, for a significant period of time before diagnosis, varying from 3-5 years [200-202].

Thailand on the other hand has a substantial HIV burden, as the Thai population living with HIV accounts for a 9% of the Asia and the Pacific region's total prevalence [203]. Importantly, since 2010, Thailand has experienced a consistent decline in newly diagnosed cases. However there is still work to be done to reach the UNAIDS goals [1, 6]. Estimates for Thailand are incomplete for the 2020 UNAIDS data. At the moment it is difficult to estimate how many HIV positive Thai individuals are aware of their status. Thailand's incidence of HIV is particularly high in key populations, such as MSMs and sex workers (along with their clients), who account for approximately 50% of newly

diagnosed cases [1, 6]. Over 90% of Thailand's HIV new cases are related to unprotected sexual exposures, with young population (16-25 years old) being particularly vulnerable [1, 6].

In terms of diagnosis, the UK is considered by the WHO as a low prevalence region, while Thailand is considered as a high prevalence area [204, 205]. This translates to specific diagnostic algorithms employed in both countries. In the UK an HIV diagnosis is made after a positive rapid test (based on immunochromatography) and a confirmatory test, usually an fourth generation immunoassay that detects the p24 HIV antigen as well as IgM and IgG antibodies directed towards the p24 antigen followed by an RT-PCR for a viral load count [204]. The patient will then be referred for treatment.

In Thailand however there are two types of testing schemes, an ambulatory rapid testing scheme, and the confirmatory testing in a hospital setting, both being based on a three-test algorithm is in place. The rationale behind the ambulatory rapid testing schemes is to make HIV diagnosis without the need of specialised equipment, and therefore have an early referral for HAART, and thus prevent new cases. The ambulatory rapid testing algorithm includes three different immunochromatographic rapid tests. The first test to be used in a mobile is a highly sensitive rapid test, Alere Determine® HIV 1/2, that has a relatively low specificity, followed by DoubleCheck Gold® Ultra HIV 1/2 and SD Bioline® HIV 1/2 3.0, with the latter having the highest specificity of all the rapid tests used [205]. In a hospital setting, the three tests that are used, are a fourth generation immunoassay, followed by Alere Determine® HIV1/2, and then, if reactive to both, a particle agglutination test, Serodia® HIV 1/2. If an HIV diagnosis is made, then the

patient will be referred for an RT-PCR test for viral load count, and HAART therapy [205].

The main aim of this work is to test a hypothesis that a substantial proportion of the population that is exposed to low to moderate sexual risk scenarios for HIV infection in the UK and Thailand is not covered by current prevention measures.

2.2 Methods

2.2.1 Ethical approval

Two ethical approvals were obtained. The first ethical approval, has been obtained in the UK (109-1709) from Faculty of Medicine & Health Sciences Research Ethics Committee, School of Medicine Education Centre, Queens Medical Centre Nottingham University Hospitals. The second ethical approval, obtained in Thailand (TRC061) from Institutional Review Board, Faculty of Medicine, Chulangkorn University, Bangkok.

Participation for this project was voluntary with no economic gain for the participants. As shown in supplemental information, the questionnaire had a project description followed up by consent statements. No participant identifying data was collected or stored. Due to the nature of the questions, participants were allowed to not answer any question they did not feel comfortable with. Participants were allocated a study number to protect their identity further.

2.2.2 Online Survey Studies

Two separate survey studies were set within the Online Surveys platform (JISC, UK), focusing mainly on sexual behaviours and condom use [206]. The first study was set up

as a 14-question survey in the UK, recruiting 3565 participants between October 2017 and April 2020. Recruitment was performed with call for participants emails, and posts in student social media (such as Twitter and Facebook). Inclusion criteria for the study included being 18 years or older and agreeing to consent statements. The questionnaire was developed aiming to screen for low to moderate risk scenarios in a general population that uses condoms as HIV prevention. Events such as condom breakage, "wanting to use a condom but none is available" and "forgetting to use a condom", within a population that reports condom use would reflect a population that is currently unprotected by pharmacological interventions. This design would allow to determine the size and behaviour of a population currently unprotected by pharmacological interventions. Feedback for the initial UK questionnaire was provided by Professor Andrew Yip from the School of Sociology and Social Policy, University of Nottingham. The initial online set-up and programming of the questionnaire on the Online Surveys platform was performed by Sam Rosen from the School of Sociology and Social Policy, University of Nottingham.

The second survey study consisted of 18 questions in Thai language, recruiting between June 2020 and February 2021 a total of 508 participants in Thailand. Recruitment was performed via posts in social media, call for participants emails and promotional flyers in a sexual health clinic (the Tangerine Clinic) based in Bangkok. Inclusion criteria for the second study included being 15 years or older and agreeing to consent statements. The design of the questionnaire was very similar to the UK study, however as the Thai study was focused mainly on key populations for HIV prevention, due to participant recruitment, the questionnaire had extra questions. These questions referred to age of

the participant PrEP awareness, PrEP use and PEPSE use. Questions for Thailand were redesigned considering the UK experience. Feedback for the initial questionnaire was provided by Professor Andrew Yip from the School of Sociology and Social Policy, University of Nottingham, Dr. Reshmie Ramautarsing, Dr. Tanat Chinbunchorn, Dr. Nittaya Pungpapong, Sorawit Amatavete and Maytinee Numkerd, the latter being collaborators from the Institute of HIV Research and Innovation (IHRI) in Bangkok, Thailand. The full questionnaires for both studies can be found in the supplemental information for Chapter 2.

In both studies the demographic analysis of a population that is potentially unprotected by current PEPSE approaches was based on participants reporting sexual activity (during the last three years with at least 1 individual), self-reported condom use ("always", "seldom", "most of the time"), self-reported HIV-negative status, as well as a disclosure of condom failure scenarios that would fall within a low to moderate risk of HIV infection (such as condom breakage, "forgetting to use a condom" or "wanting to use a condom but none available"). In the case of the Thai study, an additional filter was set up related to the current use of PrEP. This was done because PrEP is a measure that provides HIV protection for high-risk populations.

2.2.3 Statistical analysis through a logistic regression

We used a multiple logistic regression model with a logit link function to analyse and understand the subpopulation that exhibits certain behaviours concerning condoms when carrying out sexual activities. Both data sets were submitted into the programming language R (version 4.0.5) for further analysis [207]. The code for the logistic regression analysis for both data sets was written by Dr Rowland Seymour. The

full code and data sets analysed can be accessed through this link: https://github.com/rowlandseymour/sexual_behaviours.

For the UK study analysis 68 participants were excluded due to ambiguous or no responses to questions regarding gender and sexual orientation. For the Thai study analysis 96 participants were excluded due to ambiguous or no responses to gender or sexual orientation questions. The question regarding PrEP awareness in the Thai survey was also excluded from the logit model, as 78 participants did not record an answer.

Various behaviours were analysed to observe the association, if any, with different predictors found in **Table 4**. Scenarios of particular interest in both UK and Thailand studies concerning a population potentially uncovered by current PEPSE interventions were: reporting a broken condom, "condom not being available", "forgetting to use a condom", STD/HIV diagnoses after a potential exposure and "taking no action after a potential exposure" (defined as neither seeking advice from a healthcare professional nor getting tested for HIV/STDs).

To fit the model, we began with a saturated model which contained all predictors. We also included interaction terms between sexuality and performing anal intercourse, and sexuality and receiving anal intercourse to avoid confounding factors. We then performed backwards elimination to identify the predictors that should be included in the model. Performing and receiving either vaginal or anal intercourse were eventually grouped into either participating in anal intercourse or participating in vaginal intercourse. In order to assess the fit for the model, we applied the Akaike Information Criterion (AIC) [208].

Table 4. Predictors and their levels used in the logistic regression analysis of the data set.

Predictors for the UK-based study	
Predictor	**Level**
Gender	Male Female Other
Sexuality	Heterosexual Gay Lesbian Bisexual Other
Condom Frequency	Always Most of the Time Seldom Never
Perform Vaginal Penetration	Yes No
Receive Vaginal Penetration	Yes No
Perform Anal Penetration	Yes No
Receive Anal Penetration	Yes No
Predictors for the Thai-Based study	
Predictor	**Level**
Gender	Male Female Other
Sexuality	Heterosexual Gay Lesbian Bisexual Other
Condom Frequency	Always Most of the Time Seldom Never
Perform Vaginal Penetration	Yes No
Receive Vaginal Penetration	Yes No
Perform Anal Penetration	Yes No
Receive Anal Penetration	Yes No
Prep User	Yes
HIV status	+ -
Age	15-99

In order to assess the accuracy of the model, the Area under the Receiver Operating Characteristic curve (AUCROC) was computed. We aim to achieve an AUCROC value as close to 1 as possible. Such value represents a model which correctly predicts behaviours or characteristics of each respondent. In contrast, an AUCROC value of 0.5 corresponds to a model which predicts behaviours uniformly at random.

2.3. Results

2.3.1 AUCROC Values of the model

All models analysed had an AUCROC higher than 0.650, however some models perform better in predicting participant behaviour than others. In the UK study the best performing model is reporting a "condom not being available", with an AUCROC of 0.722. In the case of Thailand, all models had an AUCROC higher than 0.720, except reporting a broken condom. The AUCROC values for the behaviours and condom failure events that were analysed are shown in **Table 5.**

Table 5. AUCROC values of the different models evaluated.

Behaviour predicted.	AUCROC for the UK based study	AUCROC for the Thai Based study
Reporting a broken Condom	0.694	0.659
Forgetting to wear a Condom	0.682	0.746
Reporting a Condom not being available	0.722	0.725
Reporting having been tested positive for an STD	0.665	0.748
Took "No action" after a potential exposure scenario	0.667	0.739

2.3.2 Descriptive statistics

2.3.3 General UK Study descriptive statistics

Within the 3565 participants recruited, a vast majority identified as female, 2512, (70.5%), followed by 1012 males (28.4%), 19 trans males (0.5%) and 19 other gender identity minorities (0.5%). A significant proportion of the population identifies as heterosexual, 2554 participants or 71.7%, followed by 540 identifying as bisexual (15.2%), 250 gay/homosexual/queer (7%), 54 as lesbian (1.5%), with the rest of the population (<5%) being composed of other sexual orientations. When reporting sexual activity in the last three years, 93.2% of the total population reported activity with at least one individual during the past three years. When asked about sexual activities performed, 69.1% reported receiving vaginal intercourse, 26.8% performing penetrative vaginal intercourse, 25.4% receiving anal intercourse, and 14.8% performing penetrative anal intercourse. Of note is that the question regarding sexual activity allowed for respondents to report more than one activity. Multiple answer questions such as this one, are reported as percentage of respondents who selected each answer. In other words, 100% would indicate that all of the question's respondents chose that option.

Participants were then asked about their condom use rates, with no clear trend being found within the study population. 693 participants (19.6%) reported always using condoms, 789 (22.4%) reported never using condoms, 983 (27.9%) reported using condoms most of the time and 1058 (30%) reported seldom condom use. Similar to the question regarding sexual activity performed, this question is a multiple answer question, reported as percentage of respondents. The most reported risky scenarios were

not regularly using condoms (27%) and consenting not to wear a condom (65.3%). Of note is that three relevant unplanned risky events were reported, “wanting to use a condom but none available” (16.4%), condom breakage (14%), and “forgetting to wear a condom” (11.9%).

In cases when such unprotected scenarios were reported, participants were asked to disclose whether or not they had “taken action” after a potentially risky situation. Participants were also allowed to report more than one answer in this question. Most of the participants (55.1%) did not get tested or seek for healthcare professional advice. 32.5% of the participants reported getting tested for HIV and other STDs but did not seek professional advice. Proportion of participants that reported diagnosis of an STD and was offered treatment was 5.1%, and 15.8% reported getting professional advice but not getting tested for HIV/STDs. When participants were asked if they would use an alternative PEPSE based on rectal or vaginal administration of antiretroviral agents most of the study population reported being interested in using such option (95.2%). When asked about their HIV status, most study participants 65.30% (2316) reported not being tested in the past year, 34.20% (1213) reported having been tested with a negative HIV result, and 0.30% (9) reported a positive HIV test.

2.3.4 UK population in unplanned low to moderate risk descriptive statistics

After applying the filters described in the methods section a significant population was revealed, a staggering 29% of the total study population (1035 participants), which find themselves in a low to moderate risk of HIV acquisition due to unplanned unprotected intercourse. Due to the high female participation rate, this currently unprotected population also had a significant female majority with 769 participants (74.3%),

followed by 260 (25.1%) male participants. This population also had a clearly defined majority of heterosexuals, with 760 (73.4%), followed by 165 (15.9%) bisexuals, 63 (6.1%) gay/homosexual/queer, with the rest being composed of other sexual orientations.

Due to the populational filters applied to define the population that is unprotected by current pharmacological measures, 100% of this population in risk reported sexual activity with at least one individual in the past 3 years. As for the nature of sexual activity, 73.5% percent of the participants in unplanned low to moderate risk reported receiving vaginal intercourse, 24.4% reported performing vaginal intercourse, 24.6% reported receiving anal intercourse and 14.6% reported performing anal intercourse.

Condom use rates in this population in risk were as follows: 510 (49.3%) of these participants reported using condoms most of the time, 137 (13.2%) reported always using condoms and 388 (37.5%) reported seldom condom use. When reporting the high-risk scenarios, 17.5% of this population in risk reported not regularly using condoms and 64.3% reported consenting not to wear a condom. When analysing the lower to moderate risk scenarios in this unprotected population, 43.4% reported condom breakage, 35.10% reported “forgetting to use a condom”, and 51.2% reported “wanting to use a condom but none available”. 48.8% of unprotected participants reported “taking no action” after a risky scenario, 7.2% reported being diagnosed with an STD and offered treatment, 21% reported getting professional advice, and 35.7% got tested for HIV/STDs. 96.6% of this population in low to moderate risk showed interest in alternative vaginally or rectally administered PEPSE if available to them should they find themselves in a situation where it was needed. When reporting HIV testing, 459

participants (44.4% of the population in risk following unplanned unsafe sexual intercourse) reported being tested in the past year with a negative result, while 570 participants (55.2% of this population) reported not being tested in the past year.

2.3.5 UK study logistic regression

Reporting a broken condom had several predictors that reached statistical significance. Data derived from our model, suggest that females are 54% (Odds Ratio or OR 1.54, $p<0.001$) more likely to report a broken condom than their male counterparts. Inconsistent or null use of condoms also showed significant results, as participants that never use condoms and participants that seldom use condoms were 77% (OR 0.232, $p<0.001$) and 28% (OR 0.718, $p<0.01$), respectively, less likely to report a broken condom than participants that always used condoms. As for sexual activities, participants that engage in anal sex (either receiving or performing) were 262% (OR 3.62, $p<0.001$) more likely to report a broken condom than participants who do not engage in anal sex. Reporting a condom not being available was associated with two predictors in the model, as gay/homosexual/queer participants were 116% (OR 2.16, $p<0.05$) more likely to report the situation than their heterosexual counterparts. Participants that reported using condoms most of the time, were 563% (OR 6.63, $p<0.001$) more likely to report a condom not being available than participants who always use condoms. Forgetting to wear a condom was a scenario that females were 54% (1.54%, $p<0.005$) more likely to report than males. Full results of these three scenarios are described in **Table 6**.

Reporting an STD diagnosis had several associations. Females were 76% (OR 1.76, $p<0.05$) more likely to report an STD diagnosis than males. Gay/homosexual/queer participants were 158% (OR 2.58, $p<0.05$) more likely to report an STD diagnosis than their heterosexual counterparts. Inconsistent use of condoms, most of the time and seldom, were 245% (OR 3.45, $p<0.001$) and 243% (OR 3.43, $p<0.001$), respectively, more likely to report an STD diagnosis than participants with consistent condom use. Participants engaging in anal sex were 54% (OR 1.54, $p<0.05$) more likely to report an STD diagnosis than participants who did not engage in anal sex. Taking "no action" after a potential exposure was a scenario that was also explored within the model. Females were 40% less likely than males (OR 0.608, $p<0.001$) to take no action after a potential exposure. Seldom use of condoms, use most of the time, and no use were 357% (OR 4.57, $p<0.001$), 253% (O 3.53, $p<0.001$), and 237% (OR 3.37, $p<0.001$), respectively, more likely than participants that always use condoms to take no action after a potential exposure. Vaginal sex was also a significant predictor, as participants who engaged in vaginal sex were 245% (OR3.53, $p< 0.001$) more likely than participants who do not participate in vaginal sex to take no action after a potential exposure. Full results of these two scenarios are shown in **Table 7**

Table 6. Risky scenarios logistic regression data for the UK study. Statistically significant data ($p<0.05$) highlighted in black.

Reporting a Broken Condom				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-3.24	0.231	-	-
Female	**0.429**	**0.125**	**1.54**	**<0.001**
Other (gender)	0.115	0.559	1.12	0.837
Never (condom)	**-1.46**	**0.559**	**0.232**	**< 0.001**
Most (condom)	**0.458**	**0.208**	**1.58**	**0.001**
Seldom (condom)	**-0.331**	**0.151**	**0.718**	**0.028**
Participate in vaginal sex	**0.315**	**0.110**	**1.370**	**0.004**
Participate in anal sex	**1.29**	**0.223**	**3.62**	**<0.001**

Condom not being available				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-3.576	0.302	-	-
Gay/Homosexual/Queer	**0.768**	**0.333**	**2.16**	**0.021**
Lesbian	-1.14	0.734	0.321	0.121
Bisexual	-0.088	0.134	0.916	0.514
Other (sexuality)	-0.123	0.252	0.884	0.624
Never (condom)	-0.195	0.239	0.823	0.415
Most (condom)	**1.89**	**0.184**	**6.63**	**< 0.001**

Forgetting to wear a condom				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-4.54	0.399	-	-
Female	**-0.432**	**0.142**	**1.54**	**0.002**
Other (gender)	-0.323	0.758	0.724	0.067
Gay	**1.06**	**0.411**	**2.87**	**0.010**
Lesbian	-0.406	0.532	0.666	0.448

Table 7. Logistic regression data from reporting an STD diagnosis and taking "No action" after a potential exposure in the UK study. Statistically significant data ($p<0.05$) highlighted in black.

Reporting an STD diagnosis				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-4.77	0.387	-	-
Female	**0.564**	**0.254**	**1.76**	**0.026**
Other (gender)	0.948	0.777	2.58	0.222
Gay/Homosexual/Queer	**0.951**	**0.355**	**2.588**	**0.007**
Lesbian	-14.2	451	7.15e-17	0.975
Bisexual	-0.136	0.254	0.872	0.591
Other (sexuality)	-0.378	0.520	0.686	0.469
Never (condom)	0.604	0.392	1.83	0.123
Most (condom)	**1.24**	**0.352**	**3.45**	**< 0.001**
Seldom (condom)	**1.21**	**0.354**	**3.43**	**< 0.001**
Participate in anal sex	**0.429**	**0.195**	**1.54**	**0.028**
Taking "no action" after a potential exposure				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-2.05	0.148	-	-
Female	**-0.500**	**0.087**	**0.608**	**< 0.001**
Other (gender)	-0.174	0.368	0.840	0.637
Never (condom)	**1.52**	**0.125**	**4.57**	**< 0.001**
Most (condom)	**1.26**	**0.120**	**3.53**	**< 0.001**
Seldom (condom)	**1.22**	**0.080**	**3.37**	**< 0.001**
Participate in anal sex	-0.120	0.079	0.887	0.129
Participate in vaginal sex	**1.24**	**0.131**	**3.45**	**< 0.001**

2.3.6 General Thailand Study descriptive statistics

From the total 508 participants recruited in the survey conducted in Thailand, 286 identified as male (56.4%), 162 (32%) identified as women, 15 (3%) identified as trans male, 17 (3.4%) identified as trans female, with the rest identifying as another gender minority. Regarding sexual orientation, approximately half, 254 (50.2%), identified as gay/homosexual/queer, followed by 186 (36.8%) heterosexuals, 38 (7.5%) bisexuals and the rest being composed of other sexual orientations. Thai participants also reported a considerable rate of sexual activity in the past 3 years, as 426 (84%) reported sexual activity while 78 (15.4%) did not. Types of sexual activities performed included 38.2% reporting performing anal intercourse, 48% reporting receiving anal intercourse, 15.4% performing vaginal intercourse and 25.2% receiving vaginal intercourse.

Condom use rates were high, as 198 (39.8%) reported always using condoms, 118 (23.7%) reporting using condoms most of the time, 106 (21.3%) seldom condom use and 75 (15.1%) never use condoms during intercourse. High risk scenarios for HIV acquisition such as “not regularly using condoms” or “consented not to wear a condom”, were reported by 12.9% and 33.7% respectively. Unplanned low to moderate risk scenarios, such as condom breakage, “wanting to use a condom but none available”, “forgetting to wear a condom” were reported by 15.1%, 8.4% and 6.9% respectively in the study in Thailand.

42.5% of the participants in the Thai study, reported “taking no action”, 20.5% reported getting tested for HIV/STDs, 14.7% reported getting diagnosed with an STD and being offered treatment, and 30.2% sought advice from a healthcare professional. The Thai study population also reported interest in using an alternative vaginally or rectally administered PEPSE should they find themselves in a low to moderate risk scenario, as 87.8% referred acceptance to the idea. Regarding HIV testing in the last year, 266 (52.9%) reported being tested negative, 49 (9.7%) reported being tested positive, and 186 (37%) reported not being tested in the last year.

The Thailand questionnaire had additional questions regarding age, PrEP awareness, PrEP and PEPSE use. Regarding PrEP, most participants in Thailand (83.4%) were aware of its use as an HIV prevention tool, however only 10.7% of participants in Thailand are current PrEP users. Regarding PEPSE, 83 (16.4%) participants indicated that they have used it in the past, and 54 (10.7%) reported having never heard of PEPSE as a prevention tool. A majority (56%) of the participants were between the ages of 15-30.

2.3.7 Thailand population in low to moderate risk descriptive statistics

The filters designed for identifying a population in low to moderate risk and not covered by current PEPSE approaches in Thailand yielded 80 participants or 15.7% of the total populational sample. This population was comprised by 53 (67.1%) males, 13 (16.5%) females, 5 (6.3%) trans male, 3 (3.8%) trans female with the rest being other gender minorities. Forty-nine (61.3%) identified as gay/homosexual/queer, 24 (30%) as heterosexuals, 5 (6.3%) as bisexuals and the rest as other sexual orientation minorities. Due to the filters applied all participants in this population in risk reported sexual activity in the past 3 years. Sexual activities performed were tilted towards anal intercourse, as 55% reported receiving anal intercourse and 51.2% reported performing anal intercourse. Regarding vaginal intercourse, performing vaginal intercourse was reported by 17.5% and receiving vaginal intercourse was reported by 16.3%.

Condom use rates within the population in Thailand in low to moderate risk intercourse had 40 (50%) participants reporting always using condoms, 21 (26.3%) reporting using condoms most of the time and 19 (23.8%) seldom using condoms during sexual activities. High risk scenarios for HIV acquisition such as consenting not to wear a condom and not regularly using condoms were reported by 25% and 5% respectively. Unplanned low to moderate risk scenarios, including condom breakage, “wanting to use a condom but none available”, and “forgetting to wear a condom” were reported by 56.3%, 32.5% and 27.5% of this population in risk, respectively. Similarly to the total study population in Thailand, 43.2% of participants in low to moderate risk of HIV acquisition reported “taking no action” after a risky scenario, 20.3% got tested for HIV/STDs, 12.2% were diagnosed with an STD and offered treatment, and 33.8%

reached out for healthcare professional advice. The population in Thailand in unplanned low to moderate risk had a significant acceptance rate of an alternative vaginally or rectally administered PEPSE should they find themselves in a "risky" situation (instead of current de facto "no intervention" situation), as 97.5% would agree to using such an approach. In this low to moderate risk population group, 48 (60%) reported being tested negative in the last year, while 30 (37.5%) reported not being tested in the last year.

Regarding the extra questions found in the questionnaire applied in Thailand, 86.3% of participants in unplanned low to moderate risk were aware of PrEP as a prevention tool. As PrEP users by definition are not in a low to moderate risk scenario for HIV acquisition, thus they are not included in this population. Of note is that 18 (22.5%) participants in unplanned low to moderate risk had taken PEPSE previously and that 6 (7.5%) were not aware of PEPSE as a prevention tool. The population in Thailand in low to moderate risk due to unplanned unsafe sexual intercourse was overwhelmingly within a young adult range, as 70% of them were between the ages of 15-30.

2.3.8 Logistic regression for Thai study

Participants in the study in Thailand that engage in anal sex were 320% (OR 4.20, $p<0.05$) more likely to report a condom not being available than participants who do not engage in anal sex.

The data contain no evidence to suggest females were less likely than males to report "forgetting to wear a condom". Other gender minorities were approximately 88% (OR 0.128, $p<0.05$) less likely than males to report forgetting to use a condom. As the age of a participant increased, they were 7% (OR 0.932, $p<0.05$) less likely to report

"forgetting to use a condom". Interestingly, self-reported HIV+ participants were 158% (OR 2.58, $p<0.05$) more likely to "report forgetting to use a condom" than HIV- participants.

Females were 87% (OR 0.225, $p<0.05$) less likely than males to report an STD diagnosis after a potential exposure. Self-reported HIV+ participants were 607% (OR 7.07, $p<0.001$) more likely than HIV- individuals to report an STD diagnosis. Not being a PrEP user was 70% (OR 0.316, $p<0.05$) less likely than PrEP users to report an STD diagnosis.

Similarly, participants who are not PrEP users were 1114% (OR 12.14, $p<0.001$) more likely to take no action after a potential exposure than PrEP users. Never using condoms, using condoms most of the time, seldom use of condoms were, respectively, 236% (OR 3.36, $p<0.005$), 147% (OR 2.47, $p<0.005$), 172% (OR 2.72, $p<0.005$) less likely than participants that always use condoms to take "no action" after a potential exposure. Full results are described in **Tables 8** and **9**.

Table 8. Risky scenarios logistic regression data for the Thai study. Statistically significant data ($p<0.05$) highlighted in black.

Reporting a Broken Condom				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	0.104	0.0459	-	-
Engage in Anal intercourse	0.096	0.050	1.93	0.055
Female	-0.068	0.057	0.935	0.236
Other (gender)	0.082	0.056	1.09	0.146
Condom not being available				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-2.068	0.982	-	-
Participate in anal sex	**1.44**	**0.547**	**4.20**	**0.009**
Age	-0.049	0.028	0.952	0.079
Forgetting to wear a condom				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-0.059	0.769	-	-
Female	-0.816	0.509	0.442	0.109
Other (gender)	**-2.06**	**1.04**	**0.128**	**0.047**
Age	**-0.070**	**0.026**	**0.932**	**0.008**
HIV+	**0.985**	**0.461**	**2.58**	**0.040**

Table 9. Logistic regression data from reporting an STD diagnosis and taking "No action" after a potential exposure in the Thai Study. Statistically significant data ($p<0.05$) highlighted in black.

Reporting an STD diagnosis				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-1.24	0.335	-	-
Female	**-1.49**	**0.759**	**0.225**	**0.05**
Other (gender)	-0.015	0.0473	0.864	0.757
HIV+	**1.96**	**0.386**	**7.07**	**<0.001**
Not a PrEP user	**-1.15**	**0.412**	**0.316**	**0.005**
Taking "no action" after a potential exposure				
Predictor	**Estimate**	**Std. Error**	**Odds Ratio**	**p-value**
Intercept	-3.11	0.878	-	-
Never (condom)	**1.21**	**0.416**	**3.36**	**0.004**
Most (condom)	**0.902**	**0.295**	**2.47**	**0.002**
Seldom (condom)	**0.999**	**0.296**	**2.72**	**< 0.001**
Participate in vaginal sex	**0.951**	**0.238**	**2.59**	**< 0.001**
Age	**-0.031**	**0.015**	**0.969**	**0.036**
Not a PrEP user	**2.50**	**0.741**	**12.14**	**<0.001**

2.4.1 Discussion

Within both surveys conducted in the UK and Thailand, there was an extremely high proportion of sexually active individuals whose behaviours would place them in an unplanned low to moderate risk exposure to HIV. In the UK study, this population was a staggering 29% of participants, while in the Thailand study it was composed of 15.7% of the participants. We defined the population in unplanned low to moderate risk, usually not covered by current PEPSE approaches as those who meet all the following characteristics: participants that had sexual activity in the past three years with at least one individual, self-reported condom use (always, most of the time, seldom), reported unplanned low to moderate risk scenarios (condom breakage, or "forgetting to use condom", or "wanting to use a condom but none available"), and were not HIV positive nor PrEP users (in the case of Thailand). These scenarios are scenarios that are not covered in traditional PEPSE nor TasP or PreP as the risk of HIV acquisition is lower than 1/1000. This population is reflective of a group who is currently exposed to sexual HIV acquisition, but often receive only medical follow up after a risk scenario [94, 95].

The results from the surveys in the UK and Thailand show some similarities. Condom failure rates were reported more frequently than what the authors expected in both studies, as reported condom failure rates are often less than 6% [192, 209, 210]. Investigating condom breakages is particularly important, as previous reports have shown an association with having an STD diagnosis and reporting multiple condom breakages in the past 12 months, particularly in young men [211].

Due to the cultural differences and some differences in participants' recruitment pattern, it would be inappropriate to directly compare the results of the logistic regression

analysis of the results obtained in two countries to each other. The UK study mainly recruited UK based university students, while the survey in Thailand mainly recruited participants in a sexual health clinic based in Bangkok (the Tangerine Clinic), with a majority of them being key populations in risk of HIV acquisition by the sexual route.

2.4.2 Unplanned events leading to lower to moderate risk of HIV acquisition through sexual intercourse

Even with correct use, condoms can be permissive to HIV and other viral particles, as evidence suggests that 2.6% of condoms can allow for viral particle crossings, without any visible breakages [212]. Condom breakage rates seem to fluctuate around 5%, but the rate depends on the population studied and the time when the study was conducted [209, 213-215]. Recent figures from the United States Centers for Disease Control estimate that approximately 6.5% of women aged 15-44 years old have experienced a condom breakage or a slippage at withdrawal. In the CDC data set, rates of condom failure increased significantly when accounting for condom usage only in a fraction of the duration of the sexual intercourse, reaching as high as 26% of women aged 15 to 44 population [216]. Condom failure rates, such as the ones displayed in our data set, are particularly worrisome, and would require further study and intervention. The results of the surveys in both countries in this work indicate higher condom breakage rates than previous reports, with approximately 15% of the study population reporting this event. Previous reports have shown that individuals with a history of condom failure events, such as condom breakages, are more likely to experience them in the future [211, 217]. Future research on how frequent these condom breakages are within the same individual

are of the utmost importance for the design of better condom models and condom educational policies.

Inconsistent condom use in the form of "forgetting to use a condom" or "wanting to use a condom but none available" is also a problem to address. Evidence suggests that depending on the partnership type (casual, commercial or stable/primary partnership) different condom usage rates are present [218, 219]. Stable partnerships have a tendency to have lower condom use rates than other types of partnerships. This is a limitation of our data, as the participants did not disclose the type of partnership they were involved in when unprotected intercourse event occurred.

The second important limitation of our study is that the use of condom resistance tactics were not investigated in our study. Condom resistance tactics such as risk reassurance (such as suggesting to the partner being STD "free"), personal factors (such as "reduced sensitivity" or fear of male impotence) can lead to any individual to report "forgetting to use a condom" or "wanting to use a condom but none available"[220, 221]. An additional factor that was not studied in this work was the use of alcohol, which has been linked previously to lack of condom use due to an erroneous risk assessment when inebriated [222, 223]. Further examination of the potential association of alcohol and/or other drugs with these two scenarios, as well as condom failure events, is needed to design integral campaigns catered on safe sex.

2.4.3 Size of a population that is unprotected with current pharmacological prevention measures for HIV.

The UK study recruited mainly students, which can reflect on the sexual behaviours of a general young population. On the other hand the study in Thailand mostly recruited participants within key populations at risk at a specialized clinic. The difference between sizes of the unprotected population found in this work can be potentially partially accounted for with the availability of PrEP as a prevention tool for key populations. The size of the population in low to moderate risk of HIV acquisition in both studies suggests that a significant number of people could find themselves without any pharmacological tool to prevent HIV acquisition following potential unplanned exposure. Thus, targeting this population and investigating the behaviours further would allow for the design of better programmes for HIV prevention [98]. Further research into additional pharmacological interventions for this population can allow to reduce the rate of new HIV cases further, and thus allowing us to get closer to the 2030 UNAIDS goals [1, 6].

2.4.4 Logistic regression UK population

Identifying as female had a significant association with reporting a broken condom, as they were 54% more likely to report a condom breakage than male counterparts. The study was skewed towards higher female recruitment, however the association of females and reporting a broken condom is of importance. One hypothesis would be that females in the UK have extensive sexual education and thus are more likely to notice when/if a condom breaks during intercourse.

Not surprisingly, participants who reported low rates of condom usage (never, seldom) were less likely to report a broken condom than consistent users (always/most of the time). Anal intercourse was associated with reporting a broken condom, suggesting that despite using condoms substantial proportion of MSMs can still be exposed to HIV. Considering that receptive anal intercourse is the most efficient way for HIV to be acquired within sexual activities, broken condoms in anal sexual intercourse should also be explored further [23].

Reporting a condom not being available was associated with identifying as gay/homosexual/queer. A similar association, with condomless sex, was previously reported in NATSAL-3 study [224]. Given that NATSAL-3 and our study are populations that are UK based, and thus with similar cultural background, it exposes a point to address in future STD/HIV prevention campaigns. Females were also associated with reporting a condom not being available, as well as reporting "forgetting to wear a condom". Both effects could be linked with self-awareness of potential unwanted pregnancies. Within the UK survey study, there was an association between using condoms most of the time and reporting a condom not being available. The association can be explained by a potential self-awareness of condom use within participants.

Females in this study were 76% more likely to report an STD diagnosis than males. In the NATSAL-3 study, females were more likely to attend Sexual Health Clinics than males [225]. Considering both pieces of evidence suggests that males should be especially targeted for further campaigns focusing on the detection, treatment and prevention of HIV and other STDs. Participants identifying as gay/homosexual/queer

and participants engaging in anal intercourse were also more likely, 158% and 54% respectively, to report an STD diagnosis than heterosexuals and participants who did not engage in anal intercourse, respectively. UK government guidelines suggest that MSMs should get tested for HIV every year or every three months if there is a casual relationship. Such campaign has contributed to a situation where approximately 42% of MSMs are attending specialized sexual health clinics for HIV and other STD diagnosis and treatment [226]. Our data provides evidence that despite the efforts and relative success in the UK, further HIV/STD prevention schemes focused on MSMs and people who engage in anal intercourse are needed.

Females were less likely than males to "take no action" in our model, suggesting that further sexual education campaigns should target males in the UK. Such results are reaffirming the high attendance of females to sexual health clinics in the UK, and the association with reporting an STD diagnosis [225]. Interestingly, participating in vaginal sex was associated with taking no action after a potential exposure. These two predictors appear contradictory, however in our model receiving and performing vaginal penetration (ie. participating in vaginal intercourse) were grouped together for analysis. Therefore, this effect can potentially be explained by males not taking action after a potential exposure, which is in line with published literature and our own data [225]. Further research is needed to confirm this trend, and design campaigns to increase male attendance to sexual health clinics if the trend is confirmed.

2.4.5 Logistic regression Thailand population

Engaging in anal intercourse was associated significantly in our model with reporting a condom not being available. Survey data from MSMs in Thailand suggest that

approximately 80% use condoms during sexual activities, thus this effect can be related to condom self-awareness [203]. Unprotected anal intercourse is a high-risk sexual scenario, and thus studying the condom use associated with it allows for targeted prevention strategies.

Gender minorities (trans male, trans female, etc) were associated with being less likely than males to "forget using a condom". However, the sample size for this group is small and further research is needed to confirm if this trend indeed exists. In our logistic regression model, the older a participant is, the less likely they were to report "forgetting to use a condom". Such an effect can be explained by the HIV condom awareness programmes that the government of Thailand has implemented since 1991 [227]. Self-reported HIV+ participants were more likely to report "forgetting to wear a condom". However, our study did not gather information on whether or not they were being treated, and potentially being virologically suppressed.

Females in Thailand were less likely to report an STD diagnosis than their male counterparts. This is an opposite trend when compared to the UK study. A potential reason for this difference can be found in previous reports of stigmatization of female sexual behaviours, and potentially STD diagnosis found in females in Thailand [228-230]. Further prevention strategies, based on non-stigmatization of sexual activity in females in Thailand are needed, if this trend is proven to be correct.

Participants who reported being HIV positive were significantly associated with reporting an STD diagnosis. Similarly, being a PrEP user was associated with reporting an event of STD diagnosis. Both results reflect upon the population bias when recruiting the study participants, with many of the participants being recruited at a specialized

sexual health clinic. PrEP and HIV treatment programs often involve periodical screenings for STDs, thus securing prompt diagnosis and treatment [94].

Inconsistent condom use (never using condoms, seldom use of condoms, using condoms most of the time) was associated with taking no action after potential exposure when compared to always using a condom. Similar survey studies set in Thailand have found this trend, thus it is imperative to make sure that the Thai public is better informed about safe sexual practices [203, 215, 227, 229-231].

2.4.6 Tackling an unprotected population and future research targets

In these studies, we present evidence that a population not usually targeted by pharmacological prevention approaches has a significant size within the sexually active population in two different countries. This population consists of people engaging at least once in the last 3 years in unplanned unsafe sexual intercourse (condom failure, forgetting to wear condom or condom not being available). Current PrEP and PEPSE guidelines do not usually cover this population, but they do acknowledge that this unprotected population exists. However not enough research has been conducted on the size of this population, as well as their sexual behaviours and views [1, 2, 6]. With the size of such population being so significant (29% of UK survey responders and 15.7% of Thailand survey responders), there is an opportunity for the development of a targeted intervention for his population to reduce further the rate of new HIV cases. Targeting this population with alternative prevention approaches presents a potential new research priority in order to reach the new 95-95-95 goals to be met by 2030, set by the UNAIDS [101, 103-105].

Considering current PEPSE guidelines and risk/benefit considerations worldwide, it is clear that in many cases the population in question is offered only follow up, or in the worst-case scenario no professional advice is sought. Thus, the risk of HIV or STD acquisition remains. In the case of HIV, such unplanned unsafe sexual intercourse events are a race against the clock, as evidence suggests that PEPSE can only be effective in a 72-hour window [94-96, 98]. Such a restrictive timeline implies several barriers for PEPSE to be prescribed and administered quickly. As of today, sexual health clinic attendance, PEPSE awareness/availability, and risk to benefit case specific considerations are substantial barriers for the effective prescription of PEPSE.

A potential solution for this unprotected population would be to target local lymphatic nodes via the administration of a localised PEPSE formulation. The target cell of HIV infection, the TCD4+ lymphocyte, is found in the paracortex area of a lymph node [37]. Once HIV has integrated its DNA to a host TCD4+ lymphocyte, the infection has been established [37]. As reviewed in Chapter 1, current antiretroviral formulations often achieve lower concentrations in lymphatic tissues than in systemic circulation, thus potentially having a role in the generation of a viral reservoir in the lymph nodes via low level replication of HIV [167]. Two distinct strategies for targeting the lymph nodes can lie in the nanoformulation of antiretroviral agents as a means of delivery and the use of a lipophilic prodrug [40, 51, 86]. As of the writing of this manuscript, research has focused on nanoformulations for treatment and PrEP, and not on the potential development of a PEPSE localised agent [51]. A localised PEPSE could cover the unmet need for this population, maximizing exposure of antiretroviral agents to the lymph nodes, while limiting exposure to systemic side effects.

2.5.1 Conclusions

In this work we have provided evidence of a very high proportion of a sexually active population (15-29%) in a risk for HIV infection by unplanned unsafe sexual intercourse, including condom failure, forgetting to wear condom and condom not being available. This population is largely unprotected by current pharmacological prevention schemes. Such a big population presents a target for further alternative prevention schemes and a potential research priority.

Chapter 3:

Development of a high drug content nanoformulation for a localised delivery of antiretrovirals.

3.1.1 Role of nanomedicines as tools in the fight against HIV

Antiretroviral compounds often have important side effects, thus prescribing them to healthy individuals that have been potentially exposed to HIV needs to be justified [51, 94, 173]. Current research is leaning towards high specificity and selectivity towards drugs and their biological targets, working almost like a "magic bullet" that specifically targets the diseased cell or microbe in question [232]. The concept of a "magic bullet" was originally popularized by Nobel prize winner Paul Ehrlich in the XIX century [233]. The process of developing such a targeted therapy involves several disciplines, such as molecular biology, bioinformatics, and medicinal chemistry, all disciplines that were outside of Ehrlich's scope, but that now are available for researchers. Due to significant side effects, early HIV therapy, in the form of zidovudine, was initially reserved for patients in later stages of the disease (<350 TCD+ Lymphocytes) [27, 234]. As newer and more targeted approaches were developed, therapy became more accessible and with a more favourable side effects profile. Such advances led to a shift in treatment protocols with highly active antiretroviral therapy (HAART) and the advent of treatment as prevention (TasP) [3, 8]. While many advances have been made in safety profiles of antiretroviral agents, they still have significant side effects. In the case of dolutegravir (DTG) the total incidence of adverse events is high, with approximately

90% of users experiencing an unwanted effect [235]. DTG's most common reported side effects are headaches, nausea, weight gain and diarrhoea. When comparing DTG based treatment schemes versus other antiretroviral regimes in five different studies (SAILING, SPRING-1, SPRING-2, SINGLE, FLAMINGO) the proportion of individuals who withdrew in the DTG branch due to side effects was low (approximately 2% in each study) [116, 236-239]. DTG's severe side effects are often reported early in the initiation of treatment, in other words in the first doses ever taken (such as what would happen in a post exposure prophylaxis in a sexual scenario or PEPSE). Such side effects include, psychiatric effects (major depression syndrome), neurologic symptoms (insomnia), rheumatological complaints and significant gastrointestinal complaints [240, 241]. Further research into DTG, or any antiretroviral for that matter, being paired with a nanocarrier or formulated as a nanoparticle, can allow for a safer side effect profile and improve significantly the patient adherence [51, 232, 242-244].

A nanoparticle is generic term that refers to a nanoscopic object with nanoscale dimension, being between 1-1000 nanometers in size (**Figure 9**) [245, 246]. A nanoparticle may show therapeutic properties by itself (such as antibacterial activity), or act as a nano-carrier for a compound [245]. Current research has allowed to deliver active compounds with nano-carriers that are able to navigate through complex biological systems [247]. One such example, is Doxil® a doxorubicin formulation based on PEGylated nano-liposomes as nano-carriers. Due to the design, Doxil® exhibits prolonged systemic circulation as it evades the reticulo-endothelial system,

passively targeting tumors in the process. Doxil® was approved by the FDA in 1995 [248].

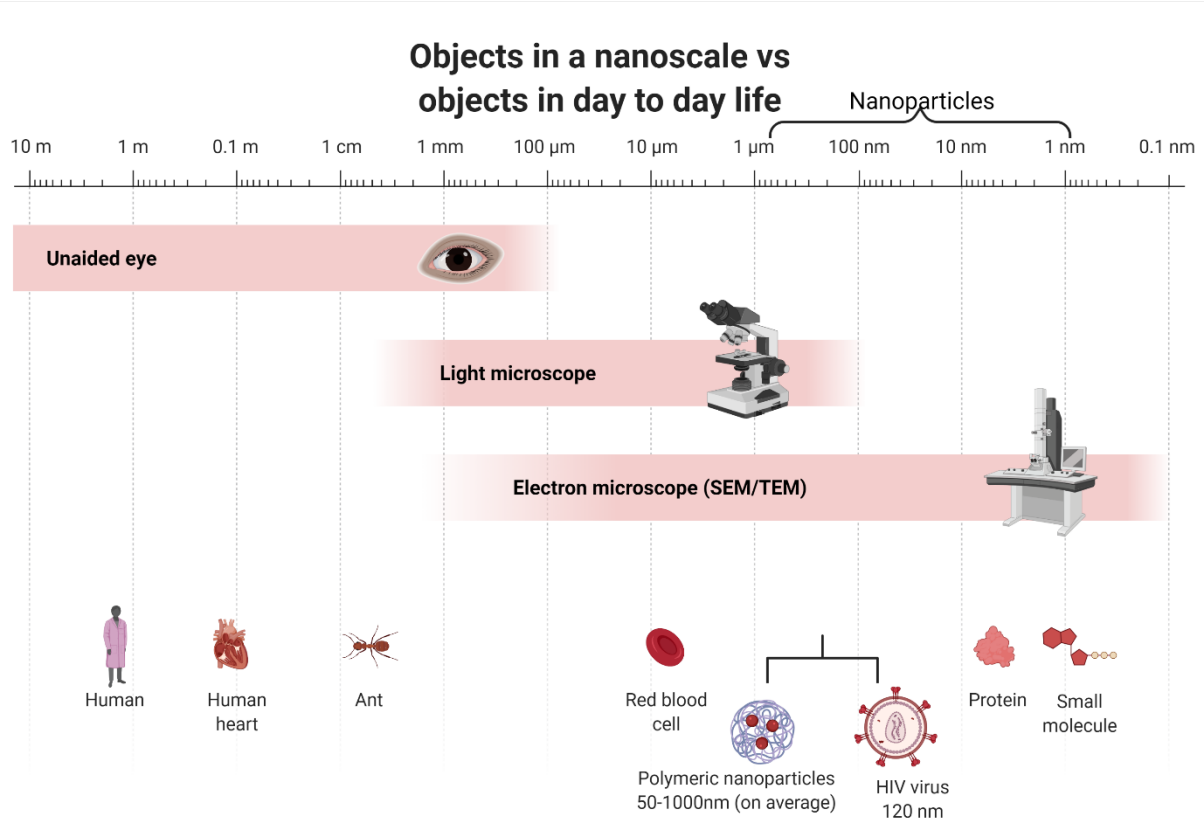


Figure 9. Objects in the nanoscale vs objects in the day-to-day life. The HIV virus is measured at 120 nm by electron microscopy, and nanoformulations are usually in the 50-1000nm range, this leaves them out of the range of what the unaided eye can see.

Nano-carriers can be divided into two mayor categories, inorganic nanoparticles/nano-carriers (such as gold or silica nanoparticles) and organic nano-carriers (polymeric nanoparticles, liposomes, micelles) [242]. Inorganic nanoparticles are synthesized from copper, gold, silver, iron oxide, zinc oxide and other semiconductors such as silicon (**Figure 10**) [245]. While growing evidence suggests that these inorganic nanoparticles are not toxic materials for humans, their antiviral effect is not well understood and still

is undergoing further research [249, 250]. Within the HIV field, limited evidence exists for inorganic nanoparticles as potential therapy [251]. Organic nanocarriers are, at the time of this thesis being written, major research areas for drug delivery, with examples being found in current antiretroviral research. As of late 2020, a long acting nanoformulation containing cabotegravir and rilpivirine has showed exciting results in phase III studies, as it is better tolerated as a bimonthly intramuscular injection than current oral based regimes[68]. This formulation is under study as a PrEP option in the HPTN 083 trial comparing it with the current regime. Preliminary results of this formulation, also seem to outperform the existing oral PrEP formulation, based on emtricitabine and tenofovir [69]. These encouraging results, highlight the potential for organic nano-carriers as drug delivery vessels.

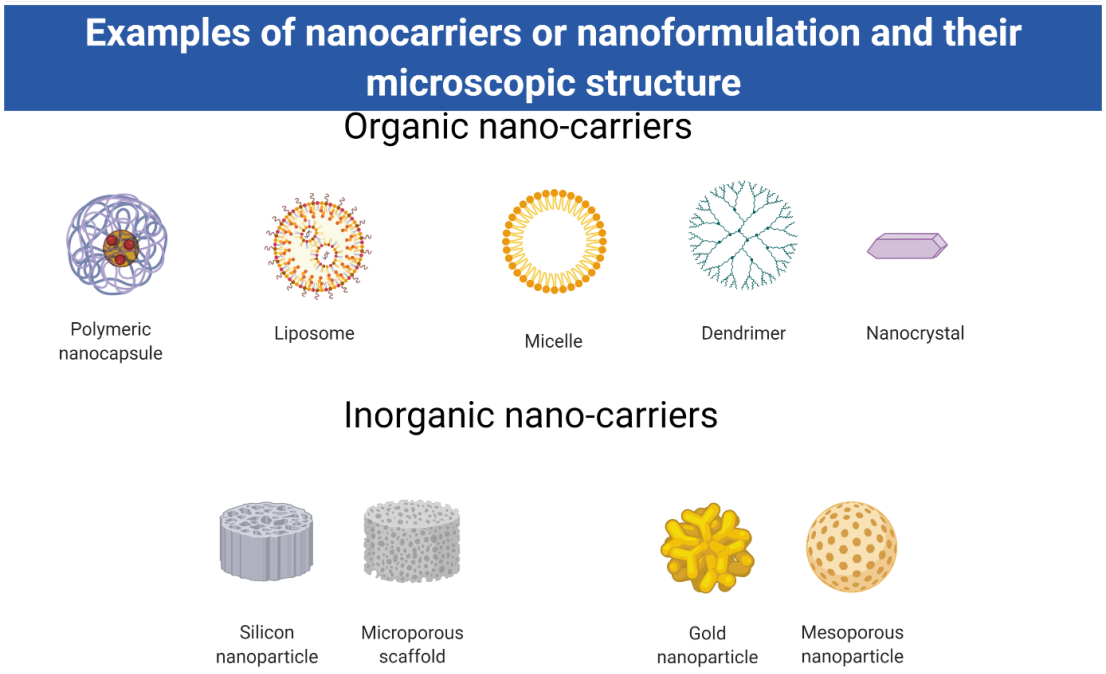


Figure 10. Examples of nano-carriers or nanoformulations and their microscopic structure/morphology, as defined by electron microscopy.

3.1.2 Organic nano-carriers for drug delivery

Most organic nanoparticles are based on the successful entrapment of an active compound inside a nano-carrier [252]. There are several carriers that have been previously used to achieve formulations on the nanoscale (**Figure 10**). The field of organic nanoparticles is wide, with various techniques, each with advantages and disadvantages[252, 253]. Some of these formulations have had important examples within the HIV field. Carriers that have had more experience with the entrapment of antiretroviral agents are liposomes, solid lipid nanoparticles, dendrimers, micelles, and polymeric nano-capsules (see below) [51].

Liposomes are spherical vesicles made up of nontoxic phospholipids. They have shown promise for drug delivery in HIV prophylaxis [254]. However, their main disadvantages are high production cost and often being physiochemically unstable [254]. The potential destabilisation) of the liposomes in aqueous environments and subsequent early leakage of the compound are important drawbacks for this drug delivery technique. In the context of PEPSE, those are important drawbacks to liposomes, as early leakage of the antiretroviral agent may prevent it from reaching the desired target in the lymph nodes [255, 256]. Solid lipid nanoparticles are similar to liposomes, however they have a couple of key differences: 1) Their structure is maintained by lipids that are solid at room and body temperature. 2) Liposomes tend to have a contiguous bilayer, while solid lipid nanoparticles do not [257, 258]. Previous studies have shown high drug loading of antiretroviral compounds (efavirenz) in solid lipid nanoparticles, however the particles had a low zeta potential and thus have a potential to aggregate [259]. Dendrimers are branched polymers that form branches with significant number of functional groups on

their surface. Within, the HIV field there has been emerging evidence of their use as a potential entry inhibitor (blocking the CXCR4 and CCR5 coreceptors) in *in vitro* cultures [260, 261]. However, their use remains limited in animal models mainly due to toxicity and poor control of drug release [262]. Toxicity in dendrimers appears to be linked to peripheral moieties such as guanidine, carboxylate, amine or phosphonate. In regards to drug release, one example of poor control of drug release would be with covalently bound dendrimer-drug complexes, as they experience immediate release from the complex after exposure to biological fluids.

Polymeric nano-capsules that are formulated through a solvent-displacement are commonly used in most research settings. Solvent-displacement, also known as nanoprecipitation, has been extensively studied with FDA approved polymers for human use such as poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA) among others, and thus presents a potential formulation technique for a novel PEPSE [252, 253, 263].

3.1.3 Solvent diffusion method for the generation of nanoaggregates (nanoprecipitation)

Nanoprecipitation was originally described in 1989, as a means of encapsulating indomethacin in a PLA nano capsule [264]. Nanoprecipitation has several characteristics which make it a good target for compound entrapment, as it is a simple technique no need of high energy input, has high reproducibility rate and allows for a narrow size distribution [265]. It has the drawback that it requires a lipophilic compound or a compound poorly soluble in water to work as originally described [266]. An additional disadvantage is that the drug content of the formulations made through this

technique usually do not cross the 10% wt% barrier due to the polymer to drug ratios used [252].

Nanoprecipitation works by the mixing of two miscible phases, an organic/oil phase and an aqueous phase. Under a traditional set up, the organic phase contains a polymer and the compound of interest, while the aqueous phase is usually HPLC grade water [263]. The aqueous phase is kept under magnetic stirring as the organic phase is added dropwise. The mixing of phases, with supersaturation of polymer and compound, is the driving force in the formation of nanoaggregates [263, 267]. As the organic phase droplet hits the aqueous phase the difference between a high surface tension liquid (aqueous phase) and a low surface tension liquid (organic phase) forms thermal inequalities that lead to interfacial turbulence. Such turbulences lead to the formation of eddies of organic phase at the interface of both liquids (**Figure 11**). The process provokes organic phase diffusion from regions of low surface tension, which causes gradual precipitation of the polymeric nanocarrier on the organic surface and forms nanoaggreates [252, 253, 263]. A nanoagreggate is a generic term for a kinetically trapped polymeric aggregate, usually with hydrophilic segments at the surface and lipophilic segments at the interior. In other words, it is a vesicular system, where a compound is held in usually an inner lipophilic core surrounded by a polymeric shell [252, 268].

Nanoprecipitation allows the user to control certain aspects of the formulation by the adjustments of certain parameters. Overall nanoprecipitation parameters could be divided into two major categories, ones that have an impact on size and drug content and ones that have impact on size only. While acetone is usually the organic phase,

certain organic phases such as Acetonitrile (ACN) have shown increases in particle size and drug content, particularly in the case of Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) and paclitaxel [252, 253, 263, 269].

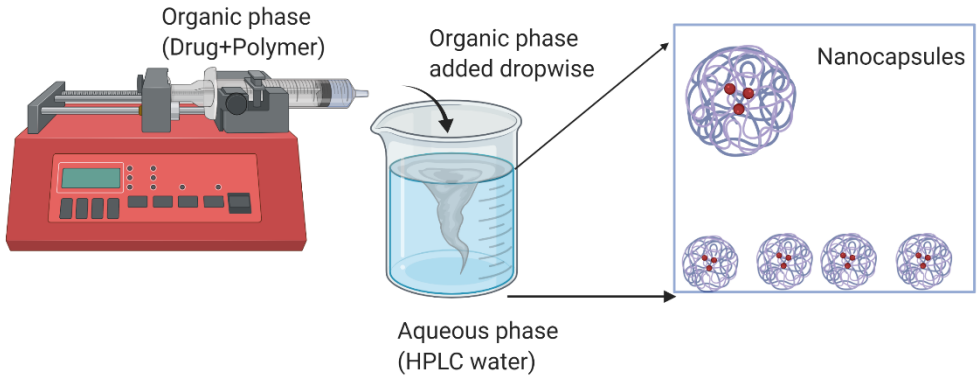


Figure 11. Diagram depicting the nanoprecipitation process as described by Fessi et al. [264]. The process is based on the correct mixing of two miscible phases. In the traditional set up, the organic phase will be a solution of acetone with a known concentration of a polymeric carrier and a compound of interest. As the organic phase is added dropwise to an aqueous phase that is kept under magnetic stirring, thermal inequalities will lead to the formation of vortices in the interphase. The liquid with a high surface tension, the aqueous phase, will pull strongly on a surrounding liquid with a lower surface tension, the organic phase. The organic phase will diffuse from regions of lower surface tension, with a gradual precipitation forming nanoaggregates. Under the original set up described by Fessi *et al*. no stabilizing agent such as a surfactant is used[264]. The addition of a surfactant for stabilizing purposes of a formulation has been described elsewhere [252, 253, 263, 270].

Table 10. Experimental parameters in a nanoprecipitation and their influence on the formulation's characteristics [252, 253, 263, 264].

Parameter	Effect of decreasing the parameter	Effect of increasing the parameter
Organic Phase to Aqueous phase ratio	Less drug content per weight of formulation [252, 253, 264] Smaller nanoaggregates [253]	More drug content per weight of formulation [252, 253, 263, 264] Bigger nanoaggregates[253]
Polymer mass	Potential increase in encapsulation efficiency [253] Bigger nanoaggregates[253]	Smaller nanoaggregates [253]
Drug mass	No clear effect reported [253]	No clear effect reported [253]
Organic addition rate	Smaller nanoaggregates[253]	Bigger nanoaggregates[253]

3.1.4 Antiretroviral formulations using nanoprecipitation

Nanoaggregates by nanoprecipitation have been previously studied as potential therapeutic or prophylactic options for HIV [51].One first barrier for the successful formulation of any antiretroviral agent is the lipophilicity of the compound. Current guidelines indicate treatment (or prophylaxis) with two distinct families, NRTI and INIs. However, these two therapeutic families do not have a tendency being lipophilic (i.e. having a high logP) [95].

Previous nano capsule research has focused on nevirapine, efavirenz, saquinavir, tenofovir and emtricitabine, however certain drawbacks of those compounds must be stated [168, 249, 271]. Nevirapine and efavirenz are two drugs that have a low genetic barrier (less than 2 mutations required for resistance) and share a common resistance mutation with other NNRTIs, thus misuse of these formulations in a prophylactic scenario would allow for selective pressure towards multidrug resistant HIV [112]. Saquinavir is not a compound that has a tendency of lowering the viral load quickly, additionally it selects for resistance to other PIs [272]. Nevirapine, efavirenz and saquinavir all share a common characteristic, as they are all on the lipophilic side of the

partition coefficient scale (logP >2) , thus their encapsulation does appear suitable for the technique [37].

Tenofovir and emtricitabine are two drugs that are currently used in most PrEP guidelines and remain a backbone for treatment in most settings [273]. The reason of this is both a favourable side effects profile. Additionally, both compounds, particularly emtricitabine, apply positive selective pressure for HIV to have the MV184 mutation, which is linked to lower viral fitness and thus more benign clinical features should resistance to both agents occur [114]. However, both compounds are hydrophilic (logP < -1), thus alternative approaches for their formulation under nanoprecipitation had to be made. In the case of emtricitabine, the formulation was generated by a water in oil in water emulsion, that did not achieve a significant drug content, as the total drug content was approximately 5% wt% [168]. Tenofovir's nanoformulation was generated through high energy method of emulsification through sonication, and it required a lipophilic prodrug approach [274].

3.1.5 Prodrug approach as a means of improving a compound's nanoformulation properties

The main goal of any prodrug strategy would be to target delivery across any physiological barrier and to allow for better compound encapsulation [263, 275, 276]. Antiretroviral prodrugs by themselves do not usually possess antiretroviral activity, however they should be able to release biologically active compounds under a relevant biological fluid [277]. Therefore, the success of any antiretroviral prodrug approach relies on the successful cleavage of the parent drug and the unactive chemical group to produce a desired effect [276, 277]. The chemical moiety chosen to modify a parent

drug is of utmost importance, as prodrugs should not be linked to cellular toxicity. Chemical linkers for the synthesis of a prodrug can be varied, including ester, carbamate and amide bonds that can be hydrolysed by carboxylesterases (**Figure 12**) [275]. As of this thesis being written, only three antiretroviral agents have prodrugs approved by the FDA, fosamprenavir, tenofovir alafenamide and tenofovir disoproxil fumarate, with no prodrug approved for the INI family [278, 279].

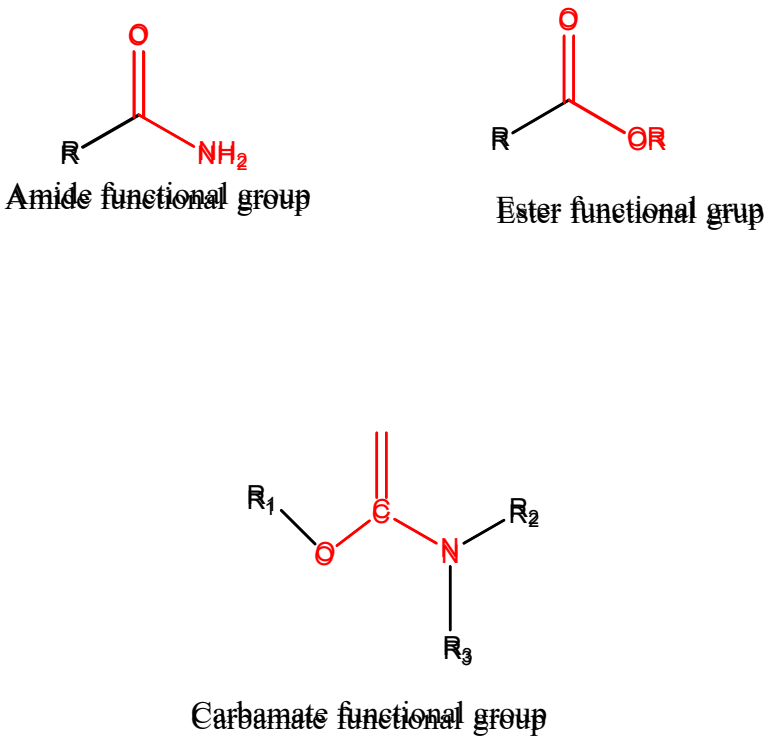


Figure 12. Commonly used functional groups to generate a prodrug with lipophilic properties. Functional groups are labelled in red. All of these chemical linkers are vulnerable to carboxylesterases in biological fluids [275].

As stated previously on Chapter 1 current therapy and prophylaxis relies heavily on the INI and NRTI families, however both families often display a hydrophilic nature [37]. Two recent examples, emtricitabine and lamivudine, from the NRTI family suggest that a prodrug approach can be a possible solution to improve HIV therapy. Agarwal's

approach for emtricitabine prodrugs involved the addition of fatty acids and evaluating with in vitro cell models that mimicked cell free and cell associated HIV. From all their model prodrugs, the myristoylated prodrug of emtricitabine displayed a significant improvement on intracellular delivery of emtricitabine, as well as better inhibitory activity against multidrug resistant HIV lineages [280]. Such encouraging results with myristoylated NRTI (abacavir and lamivudine) were also encountered by Gendelman's research group [281, 282]. However, unlike the emtricitabine prodrug work, these two prodrug projects involved nanoformulations produced under high pressure homogenization that generated a nanocrystal form of the antiretroviral compound that was later encapsulated with pluronic F127 (F127). Both formulations were able to achieve high intracellular concentrations, particularly in macrophages. Additionally, in rodent models, both formulations achieved relevant concentrations after a single intramuscular dosage. Similarly, to abacavir and lamivudine, DTG (as well as many other INIs) has a well-defined hydrophilic nature, which poses a barrier for most nano-formulation approaches [40].

3.1.6 Targeting Dolutegravir as a model compound for PEPSE

DTG was first synthesized as a collaboration proyect between Shinogi and GlaxoSmithKline [283]. To the naked eye, DTG appears as a white to yellow powder. At the physiochemical level, DTG has no formal charge, a molecular weight of 419 da, a logP of 2.2 [284]. Like other INIs DTG has documented activity versus HIV's integrase. Within each HIV viral particle, approximately 100 integrase molecules are packed. The primary role of this enzyme is to insert the proviral DNA into the genome of an infected cell. Integration is a vital step for HIV replication, as the virus requires

full integration to a chromosome for transcription of viral proteins. The integrase enzyme is responsible for 2 main reactions, “processing the 3’ ends” and a strand transfer. Following reverse transcription the proviral DNA is trimmed at the 3’ ends, such process allows for a reactive hydroxyl end [285]. The second reaction, the strand transfer, inserts the two active 3’-hydroxil proviral DNA ends into an acceptor host DNA strand. The process leaves a two base 5’ end that gets released, and a five base gap at each junction between acceptor strand and proviral strand. The gaps then get repaired by cellular enzymes. DTG inhibits the strand transfer of the 3’ reactive hydroxyl ends [115]. Unlike other INIs, DTG presents beneficial properties that favour its use as a first line therapy backbone[65]. As a summary, DTG presents a higher genetic barrier to HIV resistance, does not share any resistance mutations with other INIs and does not need the administration of a booster such as ritonavir or cobicistat [115, 286].

The PK profile of DTG is very similar between healthy and people living with HIV. The approved formulation of DTG consists of a film-coated tablet, that allows for rapid absorption in the gastric tract, co-administration with high fat foods can slow the rate absorption [287]. In people living with HIV, the apparent volume of distribution ranges between 17 and 20 L [287]. Bioavailability of DTG has not been fully established despite continuing studies. Once absorbed, the majority of DTG (>99%) is bound to plasma proteins , based on in-vitro data [115, 287]. DTG is characterized by exercising a rapid decline in HIV-1 RNA, as by day 11 of a continuous oral 50mg DTG regime, the reduction can be estimated in 2.5$\log_{10}$ of HIV RNA viral copies/ml. In adults of over 35 kg, an oral dose of 50mg of DTG has a C_{max} of 4.93µg/ml (2.66-9.08), a Area under

the curve 0-24hours of 54µg*h/ml (24.4-118) and a C24 of 814ng/ml (142-3310) [287]. (Excretion of DTG is done mainly by faeces and urine. An oral dose of 50mg of DTG has an approximate half-life of 14 hours [287]. A more accessible version of DTG, dolutegravir sodium, has been developed as a bioequivalent generic drug, and is available in several countries, such as Thailand. No significant pharmacokinetic profile differences are observed between a DTG based formulation or a Dolutegravir sodium formulation [288]. As of today, only oral formulations have been approved by regulatory bodies[40].

As mentioned previously, Gendelman's group has also been responsible for a formulation based on DTG using very similar principles to their work with abacavir and lamivudine [281, 282]. In their work, DTG was modified with a myristic acid thus generating an ester prodrug. This ester prodrug, a myristoylated DTG or MDTG had significantly different properties to DTG. Using a high-pressure homogenizer technique and F127 as a stabilizing agent, they nano-formulated MDTG with a high encapsulation efficiency reaching 82.2% (+/- 4.4) wt%. Similarly, to the other two formulations developed, a single intramuscular dose of the nanoformulated dolutegravir prodrug to murine models, produced concentrations significantly above the IC-90 (64ng/ml) over 8 weeks [115, 169].

The MDTG compound is a success story among nanoformulations and antiretroviral prodrugs. Preliminary data of this antiretroviral prodrug shows that approximately 80% of the parent drug is cleaved from the prodrug in rat plasma by one hour [169].The formulation was generated via a "top-down" coated nanocrystal method. This methodology requires a high-pressure homogenization, which allows for MDTG to be

coated with a surfactant, in this case F127, via a high energy "milling" of drug particles to a specific size [289, 290]. To generate such a formulation, specific ratios of drug and surfactant are needed. In this case F127 was first dissolved in 10mM HEPES buffer ph7.8, to generate a 0.1%w/v solution. Once this solution was prepared, MDTG was added at a 10:1 drug to polymer ratio to create a pre-suspension solution. The pre-suspension solution was subject to approximately 20 000 psi, until a suspension of approximately 250-350nm in size was formed, confirmed by DLS[169].

However, the formulation has the caveat of having a difficult scale-up process as the Avestin Emulsiflex C-3 model can only use up to 3000ml of raw material per batch. Additionally, this equipment is cost prohibitive for many research environments. On the other hand, the physiochemical properties of MDTG allow for formulation techniques such as nanoprecipitation, which are more resource friendly.

3.1.7 A nanocrystal technique for high drug loading

Gendelman's group has focused on nanoformulations based on a coatable nanocrystal generated with high pressure homogenization for antiretroviral delivery [169, 281, 282]. Nanocrystals are solid drug nanoparticles that have become attractive recently due to the high drug loading possibility they present [289]. As a drawback, they need an excipient for their successful delivery, as they have a tendency to be unstable. Materials used previously as stabilizers have been polymeric agents (such as pluronic agents) or even metallic salts such as calcium carbonate [289, 291]. Nanocrystals can be produced by mainly by two types of methodologies: A) Top-down methods, that are based on the size reduction to a nanoscale of the compound of interest. An example of a top-down method is high pressure homogenization, such as the one used by Gendelman's group

[169, 281, 282, 292]. B) The bottom-up method is based on controlled precipitation of a compound on an antisolvent and the subsequent evaporation of a solvent [289, 292]. Unlike the top-down method, the bottom-up method, does not require high energy outputs, and thus probably more accessible to more research scenarios. As with other nanoformulation techniques, the bottom-up method ideally requires a lipophilic compound to have its full potential [293].

While the bottom-up technique is similar in many ways to nanoprecipitation (as both techniques involve supersaturation of the solution and solvent evaporation), nanocrystals are not usually produced through a modified nanoprecipitation. The possibility of generating a nanocrystal through a modified nanoprecipitation has been explored recently with encouraging results, particularly for brain drug delivery [294, 295]. Such new approaches are based on the idea of coating a solid drug nanoparticle of a compound of interest with a small amount of a polymeric nanocarrier, thus having a significant drug content not achievable by other low energy settings. Nanoprecipitation and the modified two step coating procedure offer low energy and easily scalable options for the development of MDTG based PEPSE nanoformulations.

3.1.8 A novel formulation for PEPSE aim and hypotheses.

The overall aim of this work is to develop a nanoparticle formulation for PEPSE for HIV, which could be administered locally into the rectum or vagina after an event of unprotected intercourse with a moderate to lower risk. For the nanoformulation branch of the project several secondary hypotheses are considered.

In this work, nanoprecipitation conditions will be tested for the potential of generating a nanoformulation with a high drug content, >20 wt%, with different FDA approved polymeric carriers, such as mPEG-PLA and PLGA [296, 297]. It will also explore the role that triblock Pluronic 85 (P85), PLGA and $mPEG_{5000}$-PLA_{100} (for the rest of this manuscript, $mPEG_{5000}$-LA_{100} will be referred to as mPEG-PLA) have as potential coating agents for a DTG prodrug [168, 271, 298, 299].

The gastrointestinal mucus provides a significant barrier that any rectal drug delivery system must cross. The gastrointestinal mucus has a tendency producing aggregates in lypophillic polymeric carriers, such as polystyrene [300]. Therefore, polymers with a relative hydrophilic balance were preferred, such as mPEG-PLA, PLGA and P85[298, 301]. The three polymers selected have been considered non-toxic by relevant advisory boards such as the FDA [298, 301]. PLGA has been used, either alone or in a mixture, with pluronic 68, as a drug delivery system for antiretroviral agents (lamivudine, zidovudine, emtricitabine), for PrEP intramuscular formulations with encouraging results [168, 271, 302]. mPEG-PLA formulations have been described as having a sustained and controlled release of the drug payload. One such example is a intramuscular paclitaxel formulation that delivers 95% of the compound over two weeks [269]. If this release profile is shared with an antiretroviral formulation, there would be clear benefits for HIV prevention. In the case of P85, there has been growing evidence that it can stimulate the T-Cell clonal expansion, while being used as a gene delivery adjuvant [303]. Such a characteristic can be exploited, as the TCD4+ lymphocy are the final target for HIV infection. Recent data regarding P85's use as a vaccine

excipient has found a potential toll-like receptor agonistic action, at least in rodent models, such action could potentially favour T CD4+ cellular uptake. [303-305].

Therefore, the secondary hypothesis are as follows:

A) Traditional nanoprecipitation conditions with a DTG prodrug will not allow for a formulation with a significant drug content.

B) Due the molecular characteristics found in DTG's prodrug, it could be possible to form a nanocrystal with a modified solvent displacement method **(Figure 13, Figure 15, Table 14**) [295].MDTG's structure will have a 14 carbon chain attached to the 7'OH end thus it will behave like an amphiphilic polymer. Once this nanocrystal emulsion has been produced, it could be coated with a modified two step nanoprecipitation [294]. This would allow for a minimal polymeric carrier use, thus maximizing the drug content per formulation.

C) Nanoformulations developed through this two-step nanoprecipiation method will have favourable biocompatibility in target cell lines (Caco-2, Raw 264.7 and DC2.4).

D) Uptake of the coated nanoformulations developed will be higher in antigen presenting cell lines, such as Raw 264.7 and DC2.4.

E) Due to the potential instability of the nanocrystal emulsion, the polymeric coating would allow for long term stability in complex ionic environments, such as HEPES, PBS or cell culture media (DMEM).

F) As per the results found with Sillman et al, the cleavage of DTG from the prodrug approach would be in a short time frame (<2 hours), as the ester bond is

vulnerable to enzymatic activity of carboxylesterases found in lymph or lymph surrogates such as rat plasma [169].

G) Drug release from the nano-coated formulation needs to be fast (<72 hours after sexual exposure) and sustained (antiretroviral levels must be higher than the IC-90, which is 64ng/ml, for at least a week) for the formulation to be a preventive tool [23].

3.2. Materials and methods

3.2.1 Reagents

Dolutegravir sodium was purchased from Chemshuttle (China). Drug and immunization free, male Sprague Dawley rat plasma was purchased from Sera Laboratories International (West Sussex, UK). PLGA (4000-12000 Kda), Myristoyl Chloride, P85, Benzo[a]pyrene, Triton X-100, Ammonium Formate, and anhydrous N,N-Dimethylformamide (DMF) were purchased from Sigma Aldrich (San Louis, Missouri, United States). PrestoBlue™ Cell Viability Reagent was purchased from Thermo Fisher scientific (Waltham, Massachusetts, USA). LDH-Glo™ Cytotoxicity Assay was purchased from Promega (Southampton, UK). PD-10 Sephadex columns were purchased from GE Healthcare (Little Chalfont, United Kingdom). mPEG-PLA and the labelled Cy5 blue mPEG-PLA or mPEG-PLA-Cy5 were a generous gift from Dr. Vincenzo Taresco, with the full characteristics being published elsewhere (**Figure 14**) [306]. The mPEG-PLA used was synthesized by Dr. Vincenzo Taresco and its full data is published elsewhere [306]. Briefly, a PEGylated chain initiator and D,L-lactide were weighted out in a glass vial that was dried overnight at 100°C and capped. Dry DCM was added to the vial mixture and allowed to evaporate at room temperature. 1,8-

Diazabicyclo[5.4.0]undec-7-ene (DBU) was then added to the mixture, in order to catalyze the ring-opening polymerization. After 15 min, the polymerization reaction was stopped by adding the solution dropwise to cold hexane. The resulting polymer was purified via precipitation in diethyl-ether and hexane, and then dried under vacuum. mPEG-PLA had a composition ratio of PEG:LA molecular percentage by H NMR of 1:102. mPEG-PLA had a calculated molecular weight by NMR of 20 000 daltons and 24 000 daltons by GPC. The mPEG-PLA used had a hydrophilic wt% of 26%. Unless explicitly stated, all solvents used were HPLC grade or higher**.**

3.2.2 Synthesis of a lipophilic prodrug of Dolutegravir

The hydrophobic prodrug of DTG, was synthesized through the myristoylation of the parent 7'OH group. The methodology was modified from Sillman et al [169]. In short, DTG was suspended in anhydrous DMF and cooled to 0°C under nitrogen. As the DTG used was already deprotonated, and in salt form, no deprotonating agent was used. Therefore, four equivalents of myristoyl chloride were reacted with DTG for 24 hours (**Figure 13**). The resultant product was purified by a silica gel column chromatography, using a gradient mobile phase of dichloromethane and ethyl acetate. Initial mobile phase consisted of 100% dichloromethane, and it slowly introduced ethyl acetate in 10% total mobile phase increases. The gradient was maintained until both solvents reached 50% of the total mobile phase. Once purified, the fractions containing the prodrug, according to thin-layer chromatography spotting techniques, were dried with a rotary evaporator. The compound was analysed through LC-MS, proton nuclear magnetic resonance (1 H+ NMR) and carbon nuclear magnetic resonance (13 C NMR) (**Figures 16-17**).

A Bruker NMR spectrometer operating at 400 MHz (1H) and 101 MHz (13C) was used to perform NMR analysis on the purified MDTG in deuterated chloroform. Chemical shifts were assigned in parts per million (ppm). Mestrenova 14.2.1 was used for analysing the spectra. Chemical shifts are reported as parts per million (ppm) relative to tetramethylsilane (internal standard) to the nearest 0.01 ppm and coupling constants (J)to the nearest 0.1 Hz. Mass spectrometry analysis was performed by Dr Christophe Fromont.

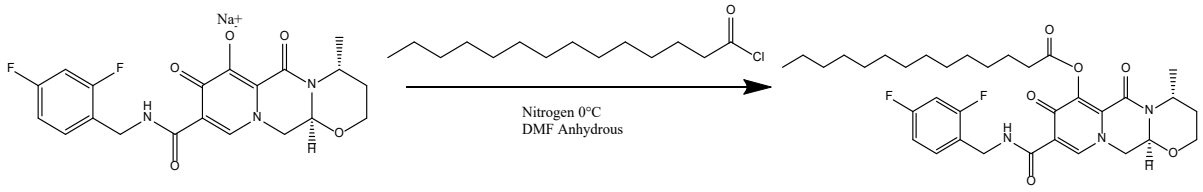


Figure 13. Chemical synthesis of Dolutegravir Myristate (MDTG). Dolutegravir sodium was suspended in anhydrous DMF and cooled to 0 °C under nitrogen. The suspension was then immediately reacted with 4 equivalents myristoyl chloride for 24 h. Modified from Sillman et al. [169].

3.2.3 Nanoprecipitation conditions

MDTG loaded nanocarriers were formulated via a solvent displacement method using different polymers [264]. All nanoprecipitation conditions used 4ml of HPLC grade water as the aqueous phase under magnetic stirring, 20mg of polymer mass, and 2.75 mg of MDTG dissolved in the organic phase. In all cases the organic phase was added at 0.667 ml/min. The full disclosure of conditions can be found in **Table 12.** Nano capsule size and polydispersity index (PDI) were measured by DLS intensity via a Malvern Nano-ZS (Worcestershire, UK). DLS conditions were as follows: Sample was filtered with a 0.45 μm cellulose acetate syringe filter, diluted 1/10 in HPLC grade water,

equilibrated at 25°C for 2 minutes, and read at 173°. Each sample had three technical repeats. Structures of polymeric carriers used can be found in **Figure 14**.

Table 12. Disclosed conditions tried in nanoprecipitation conditions. All groups had a N of 6 samples.

Polymer	Organic Phase	Ratio of Organic to Aqueous phase
mPEG-PLA	Acetone	1:2
PLGA	Acetone	1:2
PLGA	Acetone	1:3
PLGA	Acetone	1:4
PLGA	Acetone	1:6
PLGA	Acetonitrile	1:6

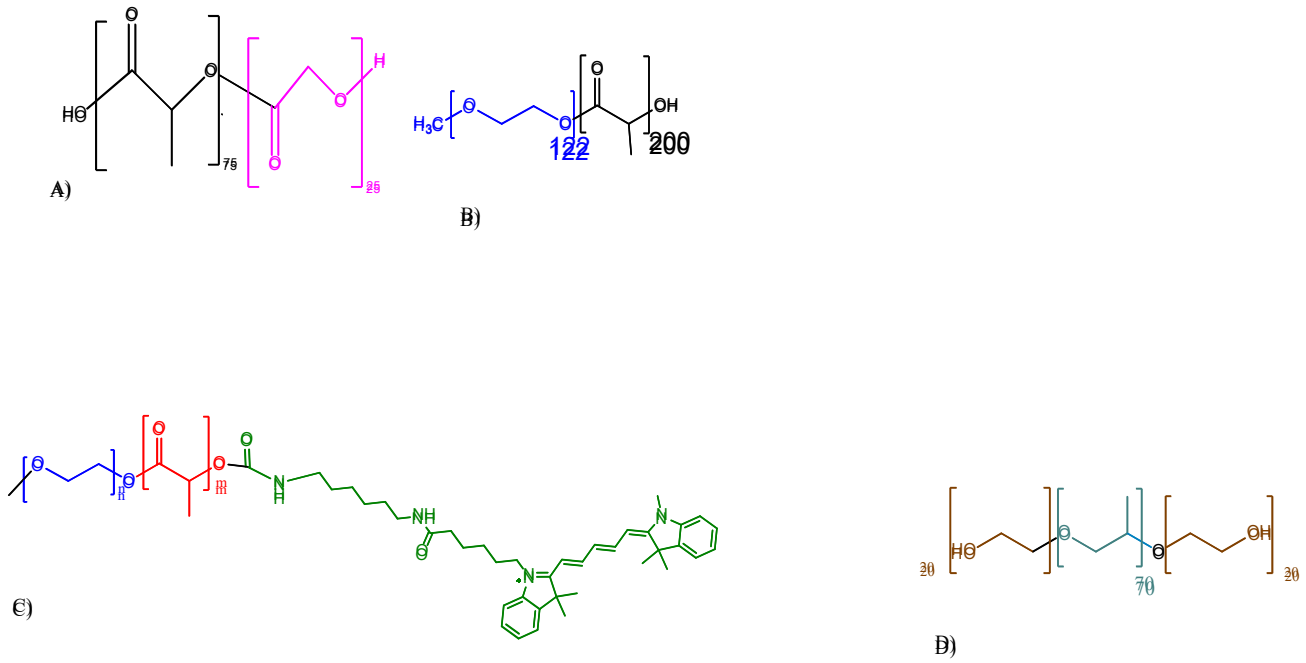
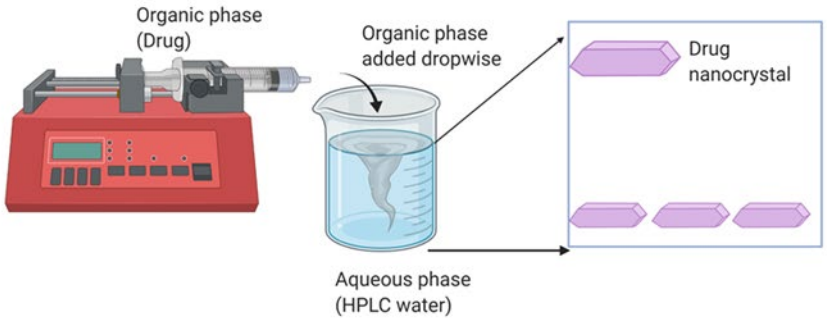


Figure 14. Chemical structure of the polymers used. A) PLGA. This commercial PLGA had 75 units of PLA and 25 units of Glycolic acid. B) MPEG-PLA. The MPEG-PLA polymer used had 200 units of PLA and 122 units of Poly (ethylene glycol) or PEG. C) MPEG-PLA-CY5. D) Pluronic P85. P85 has 70 units of PPO being flanked by 20 units of PEO on each side. P85 was only used for nanocoating conditions. Different functional groups are labelled with different colours.

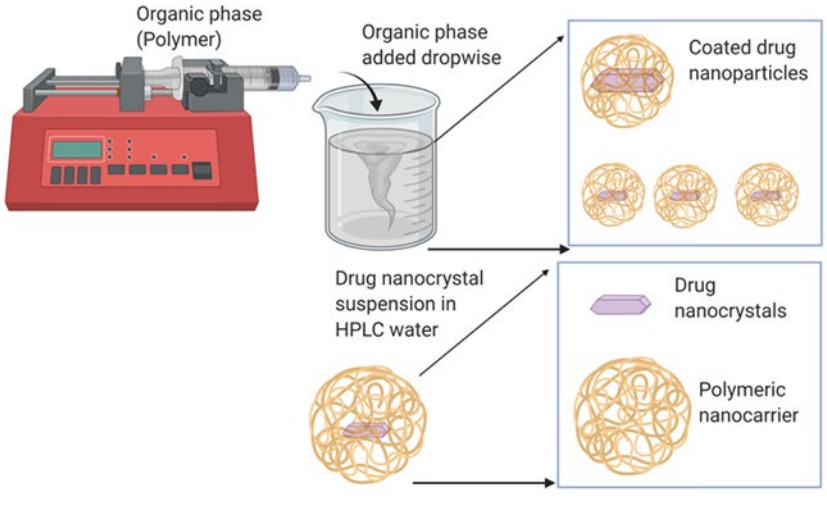
3.2.4 Nano coating conditions

Based on a modified solvent displacement method and the computational simulations from Danai-Styliari et al, a two-step nanoprecipitation was devised [294, 307]. For the first solvent displacement precipitation, an acetone solution containing 2.75mg of MDTG dissolved in 0.667 ml of acetone was added to 4ml of HPLC grade water under magnetic stirring at a rate of 0.12ml/min. After 3.5 hours, and after confirmation by DLS via a Malvern Nano-ZS of a formation of a colloidal dispersion of a nanosized particle the second solvent displacement, or the coating was performed.

For the coating step, the colloidal dispersion of MDTG was used as an aqueous phase. For the organic phase, 0.667ml of acetone was used to solubilize different masses of dry polymer, either mPEG-PLA (0.22mg, 0.44mg, 1mg), PLGA (0.44mg, 0.88mg, 1mg) or P85 (0.44mg). This organic solution was then added dropwise at a rate of 0.12ml/min to the colloidal dispersion of MDTG, and left overnight for acetone to evaporate. The size, PDI, and zeta potential of coated formulations were also determined by DLS intensity via a Malvern Nano-ZS. Coated nanocrystals samples were not diluted nor filtered prior to DLS readings, rest of conditions were the same as described above. Each sample had three technical repeats. A diagram depicting the potential mechanism can be found in **Figure 15**.



A)



B)

Figure 15. Diagram depicting a two-step solvent deposition method for coating nanoparticles, modified from Fessi et al. and Danai Styliari et al [264, 294, 295]. In similar fashion to the traditional nanoprecipitation, an acetone solution containing MDTG is added drop-wise to deionized water. Deionized water pulls strongly on acetone due to the difference in surface tension, this process forces MDTG to collapse forming an unstable nanocrystal. In this novel method, the aqueous suspension of MDTG nanocrystals will be used as the aqueous phase for a nanoprecipitation. As the organic phase is added dropwise to the nanocrystal suspension, the

nanocrystal is momentarily resolubilized and coated with the polymeric nanocarrier. This results in a coated solid drug nanoparticle, with a hydrophilic core with the aromatic rings of MDTG pointing towards the centre and myristate ends interlocking with the polymeric nanocarriers.

Stability of the nanocoating formulations was assessed by DLS (including zeta potential) in three different media, HEPES, PBS and Dulbecco's modified Eagle's medium (DMEM). DLS assessments were made by diluting the formulation in the aforementioned media, in order to reach concentrations of 10mM HEPES, 150 mM PBS and a 1/10 dilution in DMEM. In the case of 150mM PBS and DMEM no zeta potential was recorded, 150mM PBS was only assessed at time 1 hour. Rest of conditions were assessed at times 0,1,2,3,4 and 24 hours.

3.2.5 Electron microscopy (Scanning electron microscopy/Transmission electron microscopy)

To obtain Field Emission Gun-Scanning Electron Microscope (FEG-SEM) images, freeze dried formulations were mounted on a semi conductive pin, and then coated with a 20nm carbon coating using an Edwards 306A Carbon coating system. Samples were analysed using a FEGSEM Jeol 7100F equipped with an Oxford Instruments AZtec EDX system.

Transmission electron microscopy (TEM) imaging of the MDTG nanocrystals/coated nanospheres was made with a Tecnai G2 80-200keV TEM with a BioTWIN lens configuration. The TEM had a mounted Gatan Digital image capturing system. Samples were mounted on a copper support grid, allowed to dry for 5 min and imaged

immediately. TEM images were obtained under close collaboration and guidance from Denise Mclean.

3.2.6 *In vitro* cytocompatibility and preliminary uptake

Three cell lines were identified as potential targets for assessing biocompatibility of the formulations developed: RAW 264.7 (as a macrophage in vitro model), DC2.4 Mouse Dendritic cell line (as a dendritic cell line) and Caco-2 (as an intestinal cell line model) [164, 308-310]. Due to availability and COVID19 laboratory interruptions only two cell lines could be used. Caco-2 and RAW 264.7. Cells were routinely cultured in growth medium at 37 °C with 5% CO_2 and in 75 cm^2 culture flasks to 70% confluence. Caco-2 and RAW 264.7 cell growth medium consisted of DMEM supplied with 10% (v/v) fetal bovine serum (FBS) and 2 mM L-glutamine.

The PrestoBlue cell metabolism assay, performed to assess biocompatibility of formulations, nanocarriers and compounds, was performed according to manufacturer's instructions. Briefly, Caco-2 cells were seeded at $1x10^5$ cells per well in 96 well plates and RAW 264.7 cells were seeded at a density of $2x10^4$ cells/well. Cells were cultured for 24 hours prior to assaying. Tested compounds/formulations/nano-carriers were applied to cells in HBSS buffer. After 24h exposure cells were washed twice with warm PBS and 100 µl 10% (v/v) PrestoBlue reagent diluted in phenol red free medium applied per well for 60 minutes. The resulting absorbance was measured on a TECAN Spark 10M plate reader (Männedorf, Switzerland) at 560/600 nm (λex/λem). The positive control in the Presto Blue were cells treated with 1% Triton-X. The negative control in the Presto Blue assay are cells treated with the vehicle buffer (HBSS with 2%water for normalization with the nanoparticles). Relative metabolic activity is calculated from

PrestoBlue data by setting values from the negative control as 100% and positive control values as 0% metabolic activity:

$$Relative\ metabolic\ activity = \left(\frac{(x - Positive\ control)}{(Negative\ control - Positive\ control)}\right) \times 100$$

For the LDH assay, Caco-2 and RAW 264.7 cells were seeded at $1x10^6$ cells per well in 96 well plates. Cells were cultured for 24 hours prior to assaying. Tested compounds/formulations/nano-carriers were applied to cells in HBSS buffer. After 24 hours, the plates were centrifuged at 250x g for 10 min. One hundred microliters per well (supernatant) were removed and transferred to the wells of an optically clear 96-well flat bottom plate. To these wells 100 µl of the reaction mixture freshly prepared were added, and incubated for 30 min at 20 °C, protecting the plate from light exposure. The resulting absorbance was measured on a TECAN Spark 10M plate reader (Männedorf, Switzerland) at 490/600 nm (λex/λem).

The negative control in the LDH assay are cells treated with the vehicle buffer (HBSS with 2% water for normalization with the nanoparticles). The positive control in the LDH assay cells treated with 1% Triton-X. Relative LDH release was calculated with the negative control absorbance at 492 nm taken as 0%, and the positive control, assumed to cause total cell lysis, as 100%

$$Relative\ LDH = \left(\frac{(x - Negative\ control)}{(Positive\ control - Negative\ control)}\right) \times 100$$

Formulations tested for uptake were marked with a MPEG-PLA-CY5. In these formulations the total 10% of the total polymeric coating planned was substituted with MPEG-PLA-CY5. Due to COVID19 restrictions, only one formulation was tested for

qualitative and quantitative uptake. This formulation was the MDTG 2.75mg/0.22 mg MPEG-PLA coating. In other words, the polymeric coating mass based on dry polymer, was 0.20mg MPEG-PLA, 0.02mg MPEG-PLA-Cy5.

For both quantitative and qualitative uptake the procedure was similar to what Dr. Cavanagh and Dr. Taresco had done previously [311]. In short, Caco-2 and RAW 264.7 cells were seeded at $1x10^5$ cells per well in 96 well plates. Cells were cultured for 24 hours prior to assaying in DMEM. After this culture period had ended, medium was removed and replaced with 1X HBSS and the MPEG-PLA-Cy5 coated formulation was dosed at 50 μg/ml in HBSS. Uptake was assessed at 24 hours, at which point the MPEG-PLA-Cy5 containing solution was removed and cells were washed three times with cold PBS. For quantitative studies, cells were then permeabilized through the use of a 1% (v/v) Triton X-100 solution in PBS for 10 min at 37°C. Permeabilized cells were centrifugated into a pellet. MPEG-PLA-Cy5 was measured by fluorescent spectrophotometry at 640/680 nm (λex/λem). In order to quantify MPEG-PLA-Cy5 per cell, a calibration curve of known MPEG-PLA-Cy5 concentrations was made diluting the labelled polymer in 1% (v/v) Triton X-100/HBSS solution. Values were normalized regarding viable cell number per well as determined by trypan blue test and hemocytometer cell counting. For the qualitative study the MPEG-PLA-Cy5 formulation was dosed at 50 μg/ml (MDTG concentration) for 24 hours to cells followed by staining with Hoechst 33342 (10 ug/ml) for 30 minutes. Staining solution was washed off with PBS and cells imaged on an inverted Nikon Eclipse TE 300 microscope on DAPI and Cy5 filters. Images were merged using ImageJ software.

Cellular experiments were designed by Dr. Robert Cavanaugh. They were all performed in close collaboration with Dr. Robert Cavanaugh, whose role and expertise in this field is deeply appreciated.

3.2.7 HPLC-UV system

The HPLC-UV system consisted of a Waters Alliance 2695 (Framingham, Massachusetts, USA) separations module coupled with a Waters 996 photodiode array detector. Samples stored in the autosampler were kept at 4 °C. A column oven was used to control column temperature, which was kept between 40°C. Separation column used was a Phenomenex Gemini C18 5um, 250 x 4.6 mm, equipped with a SecurityGuard Gemini 2 × 4 mm, 3 μm (Phenomenex, Macclesfield, UK). Solvent lines were as follows: A: Ammonium Formate 10 mmol buffer, pH3.5 B: Acetonitrile (ACN) C: Methanol D: HPLC grade water. Chromatogram analysis and data processing were performed using Empower TM2 software.

Rat plasma was chosen as a surrogate for lymph due to the relative enzymatic (particularly in esterase concentrations) similarities, for HPLC detection of MDTG and DTG [312]. Two different experiments were set up using this media: DTG drug cleavage from MDTG and drug release from the nanoformulation developed. Full disclosure of the DTG cleavage from MDTG and drug release conditions can be found in section 3.2.9.

Rat plasma samples were analyzed under gradient conditions, with full disclosure of the conditions on **Table 11.** Formulation samples were injected to the HPLC under the following conditions B) 90% D) 10%, Flow Rate 0.8ml/min for 20 min. The total drug

content of the nanoformulations developed was calculated by weight percentage (wt%). Using the following equation:

$$wt\,\% = \frac{Measured\ mass\ of\ drug\ in\ nanoformulations \times 100}{Known\ mass\ of\ formulation}$$

Table 11. Chromatographic gradient conditions for rat plasma data set.

Time in min	**Flow rate**	**A) Ammonium Formate 10mmol/ml Ph 3**	**B) ACN**
0	0.8ml/min	60%	40%
10	0.8ml/min	60%	40%
15	0.8ml/min	10%	90%
45	0.8ml/min	10%	90%
51	0.8 ml/min	60%	40%
57	0.8ml/min	60%	40 %

3.2.8 HPLC formulation sample preparation

Drug content or wt% of formulations was assessed via HPLC. Unbound drug to the polymeric nanocarrier was separated with a PD-10 Sephadex column (as per manufacturer's gravity protocol) and then analysed via Dynamic Light Scattering (DLS), see 3.2.5 and 3.2.6 for more details. Formulations were freeze-dried for 24 hours. Two milligrams of freeze-dried formulations were resuspended in 1ml of methanol. By the addition of methanol, the formulations are then disaggregated, followed by the total dissolution of the drug. Samples were then filtered with a Costar 0.22µm cellulose acetate centrifuge filter at 17500 rcf for 5min. The polymers used have a low solubility in methanol, the filter was chosen as per manufacturer's specifications it can withstand

methanol [266, 313, 314].The combination of both processes, allows for a separation of the polymer and the test drug. Sample was further diluted 1/10 and then 20 ul injected into HPLC.

3.2.9 HPLC rat plasma sample preparation

Rat Plasma was used as a surrogate media for lymph [312]. The HPLC-UV method for rat plasma was based on protein precipitation with acetonitrile and liquid-liquid extraction of the compounds of interest, in this case MDTG and DTG. Liquid-liquid extraction is a method that allows to separate different compounds of interest, by relying on the relative solubility of them in two different immiscible liquids. By separating the phases generated in this procedure, it is possible to selectively separate different compounds by their solubility and affinity to different solvents. The procedure is similar to other methods in our research group [312]. Briefly samples were prepared in the following manner:

100 µl rat plasma was added to a 16x100 mm glass tube at room temperature. Next, 10µl of 20 µg/ml Benzo(a)pyrene (internal standard) was added to the rat plasma. Then, 10µl of different concentrations of MDTG or DTG were added (5,6.2,12.5,25,31.25,62 µg/ml) were added to the mixture. Following the addition of the internal standard and the compound of interest, 300 microliters of cold ACN to each sample and vortex for 1 min, this allowed for protein precipitation in the media.

Following protein precipitation, three ml of ethyl acetate were added to each sample and vortexed for 5 mins. Samples were then centrifuged at 3,000 rpm in $10^{O}C$ for 10 mins. The organic upper layer of the sample was transferred to a new tube and then

evaporated under a stream of nitrogen gas at 0 °C. Sample was then reconstituted with 100 µl of 50%ACN/50% HPLC grade water, vortexed for 5 min and then centrifuged for 1 min at 3000 rpm. Sample was then transferred to an HPLC vial, where 40 µl were injected.

3.2.11 HPLC MDTG cleavage/drug release from formulation studies

For drug cleavage/drug release data sets the sample preparation was similar to the calibration curve described above, but with specific differences.

The 16x100 mm glass tubes were preheated for an hour in a water bath at 37 degrees Celsius, to mimic normal human body temperature. Then, 100 µl of rat plasma were added to the preheated glass tube. Next, 10µl of 20 µg/ml Benzo(a)pyrene (internal standard) was added to the rat plasma in the glass tube. For drug cleavage studies, 10 µl of 5000ng/ml were added to the rat plasma in the glass tube.

For the p85 coated nanoformulation drug release studies, the nanoformulation was first diluted to 5000ng/ml using HPLC grade water (this was adjusted using the wt% that was calculated through a previous measurement by HPLC of the same batch). Ten µl of this diluted coated p85 nanofomulation solution was added to the rat to the rat plasma.

In both cases the tubes were capped and returned to the water bath at 37 degrees Celsius. At the collection time point, 300 µl of ice cold ACN was added for protein precipitation, thus stopping any enzymatic activity. Collection time points were 0,30 min, 1 hour, 2 hours. For the initial time point in both experimental sets, ie. time 0 hours, ACN was added before spiking the solution 10 µl of 20µg/ml Benzo(a)pyrine. This was followed

by the addition of either 10 µl of 5000ng/ml of MDTG or 10 µl of 5000ng/ml of the P85 coated MDTG formulation.

Following protein precipitation, three ml of ethyl acetate were added to each sample, for a liquid-liquid extraction as described for the calibration curve. Sample was then transferred to an HPLC vial, where 40 µl were injected.

3.2.10 Statistical analysis and figure design

Statistical analysis as well as post-hoc analysis were performed with Graphpad Prism (California, USA) 9.1.2 for Windows. Figures were designed with Biorender (Toronto, Canada).

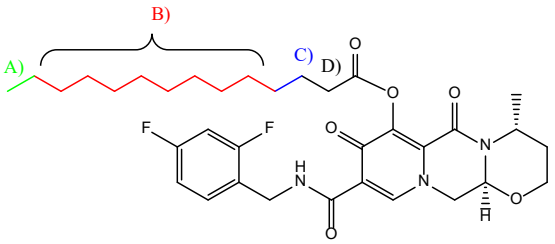
3.3 Results

3.3.1 DTG's lipophilic prodrug

By the addition of a 14-carbon chain to the 7-OH end in Dolutegravir, it was possible to change several physiochemical properties, among them, LogP changed significantly, from -1.37 to 5.51. The molecular weight also changed significantly from 419.23 da in DTG to 630 da in MDTG, full data on **Table 14** and **Figures 14-15**.

Table 14. Summary data for the MDTG prodrug with comparisons, where relevant, to the parent drug.

	Dolutegravir Sodium	MDTG
Molecular weight by LC-MS	419.2	630
LogP (as calculated by ACD labs)	-1.57	5.51
^{1}H NMR (400 MHz, CDCl3)	NA	δ 10.23 (s, 1 H), 8.50 (s, 1 H), 7.37 (dd, J = 15.0, 8.2 Hz, 1 H), 6.81 (app. dd, J = 19.1, 9.3 Hz, 1 H), 5.26 (br. s, 1 H), 4.85-4.89 (m, 1 H), 4.62 (br. s, 1 H), 4.36 (app. d, J = 12 Hz, 2 H), 4.17 (dd, J = 13.3, 5.9 Hz 1 H), 4.0 (app. d, J = 6.3 Hz, 1 H), 2.73 (t, J = 7.6 Hz, 2 H), 2.21 (td, J = 14.5, 7.2 Hz, 1 H), 1.86 (app. t, J = 7.5 Hz, 2 H), 1.51– 1.61 (m, 2 H), 1.40–1.49 (m, 2 H), 1.36 (d, J = 7.0 Hz, 3 H), 1.26 (br. s, 20 H), 0.90 (t, J = 6.6 Hz, 3 H).
C NMR (101MHz, CDCl3)	NA	δ 171.9, 171.1, 163.3, 163.1, 161.6, 161.1, 159.6, 130.6, 130.5, 129.2, 121.3, 121.2, 119.8, 111.1, 111.1, 103.9, 103.7, 103.5, 76.1, 52.4, 36.7, 33.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 24.4, 22.6, 15.8, 14.0
Drug Yield	NA	75%



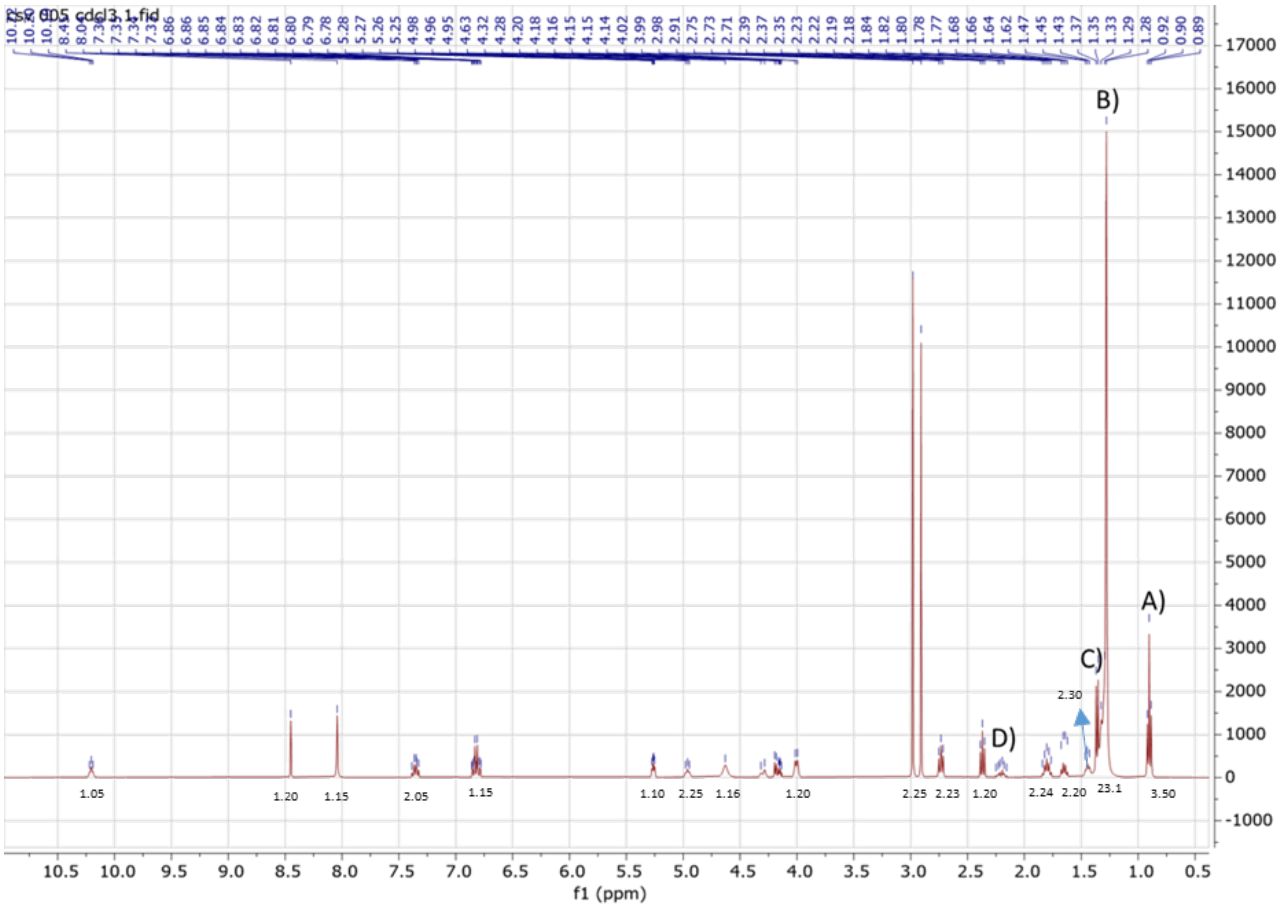


Figure 16. ^{1}H NMR spectra of the DTG Prodrug. Relevant functional groups to the modification are marked in the structure. 1H assignments are displayed on the spectra. Full spectral assignments can be found in **Table 14**.

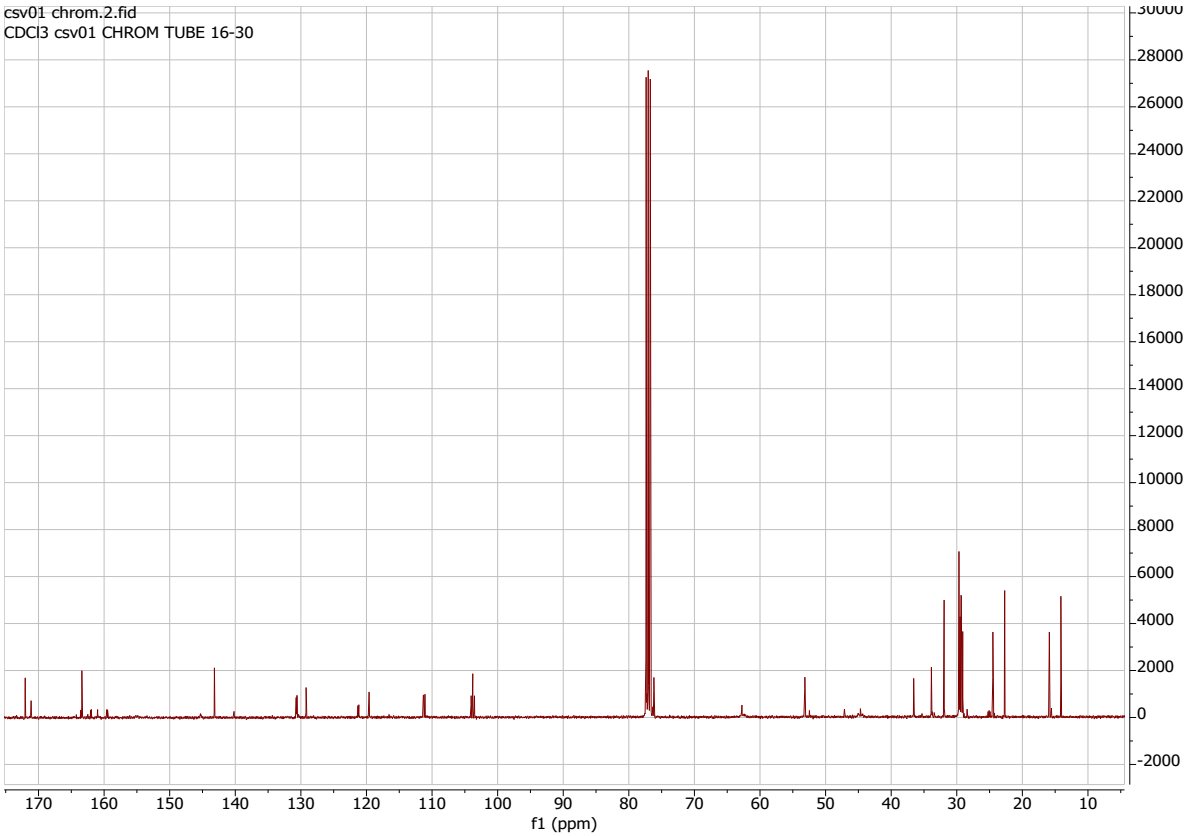
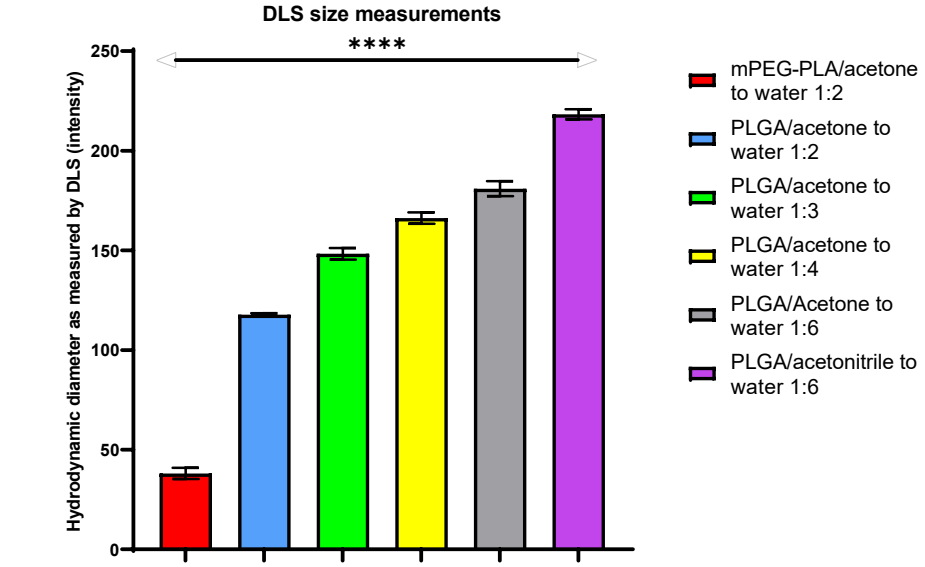


Figure 17. C NMR data of the DTG prodrug (MDTG). Full spectral assignments can be found in **Table 14**.

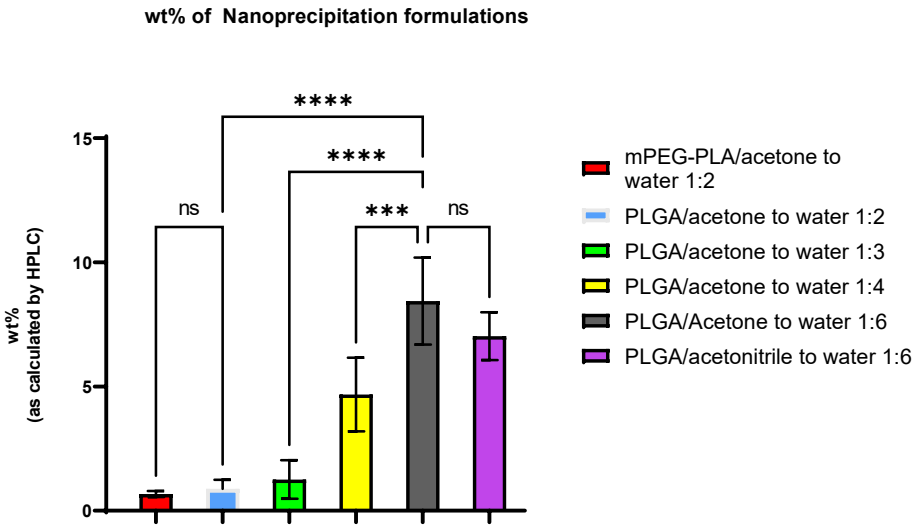
3.3.2 Traditional nanoprecipitation formulations

Two polymeric carriers were tested to produce a nanoaggregate formulation via nanoprecipitation, MPEG-PLA and a commercially available PLGA. MPEG-PLA produced the smallest nanoaggregates (38nm +/- 5nm) as measured by DLS (**Figure18. A**). Drug content was measured by HPLC (**Figure18 B**), where MPEG-PLA nanoaggregates had a minimal drug content (<1% wt%), thus these formulations were not tested further. PLGA formulations were fine-tuned by lowering the ratio of organic phase to aqueous phase. A ratio of 1:6 acetone to aqueous phase produced a significant weight percentage, 8.4% wt% (+/- 1.7%), and increased the size to 181 nm (+/- 4) (**Figure 18 A**). This formulation was imaged through FEG-SEM, where a correlation with the DLS data was found, as individual nanoaggregates also are 180nm in size. A

distinct morphology was found with this formulation as the imaged nanoaggregates displayed an olive shape (**Figure 19**). The change from Acetone to ACN as an organic phase produced the biggest nanoaggregates by DLS, at 218nm (+/- 4), however this formulation did not improve the wt% in a statistically significant way. All samples had a PDI <0.3. Full results can be seen on **Figure 18 and Figure 19**.



A)



B)

Figure 18. DLS size by intensity and wt% by HPLC UV of nanoprecipitation conditions tested. All samples have an N=3. In the case of DLS measurements N=3 and n=3 for each sample. Data presented as mean ± S.D. A) Represents the size by DLS of the different formulations. The difference between groups is statistically significant by ANOVA p= <0.00001. A post hoc Bonferroni correction revealed that the difference in between groups is also significant, as all comparisons had a **** significance. B) Represents the drug content, expressed in wt% of the formulations developed. The difference between groups is statistically significant by ANOVA p= <0.00001. Bonferroni correction is displayed on the graph.

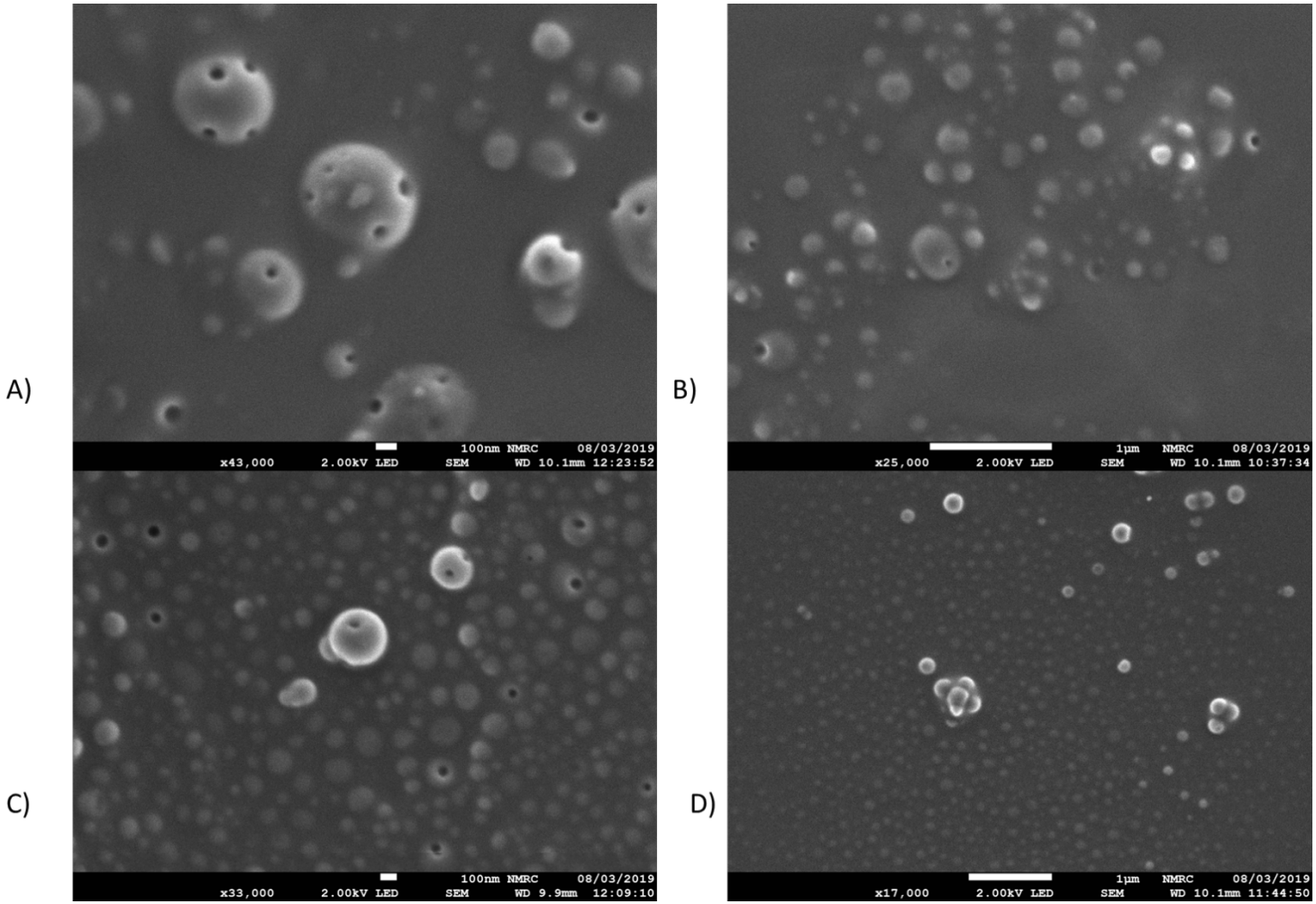


Figure 19. SEM micrographies for nanoaggregate formulations. Panels A) and B) show representative images of a PLGA nanoprecipitation formulation generated through a 1:6 acetonitrile to water ratio. Panels C) and D) show a representative PLGA nanoprecipitation formulation generated through a 1:6 acetone to water ratio. Nanoaggregates formulated this way, display a spherical shape with a kissed punctuated morphology. Scale bars (white bars) are displayed on image. Magnification displayed on image.

3.3.3 MDTG nanocrystal precipitation

MDTG was precipitated into an unstable nanoemulsion through a solvent displacement method. TEM images show geometrical morphology of the MDTG particles (**Figure 20**). These images are suggestive of a three dimensional regular definite pattern of a rod-like structure, suggesting the formation of a nanocrystal at this stage [289, 315]. The nanocrystal size is measured at approximately 190nm both by DLS and TEM. The unstable emulsion breaks after 4 hours without any mechanical stirring, and 72 hours with mechanical stirring, as visible insoluble aggregates are seen to the naked eye. The nanocrystal suspension had a zeta potential of -37 mv (+/-6) by DLS (**Table 15**).

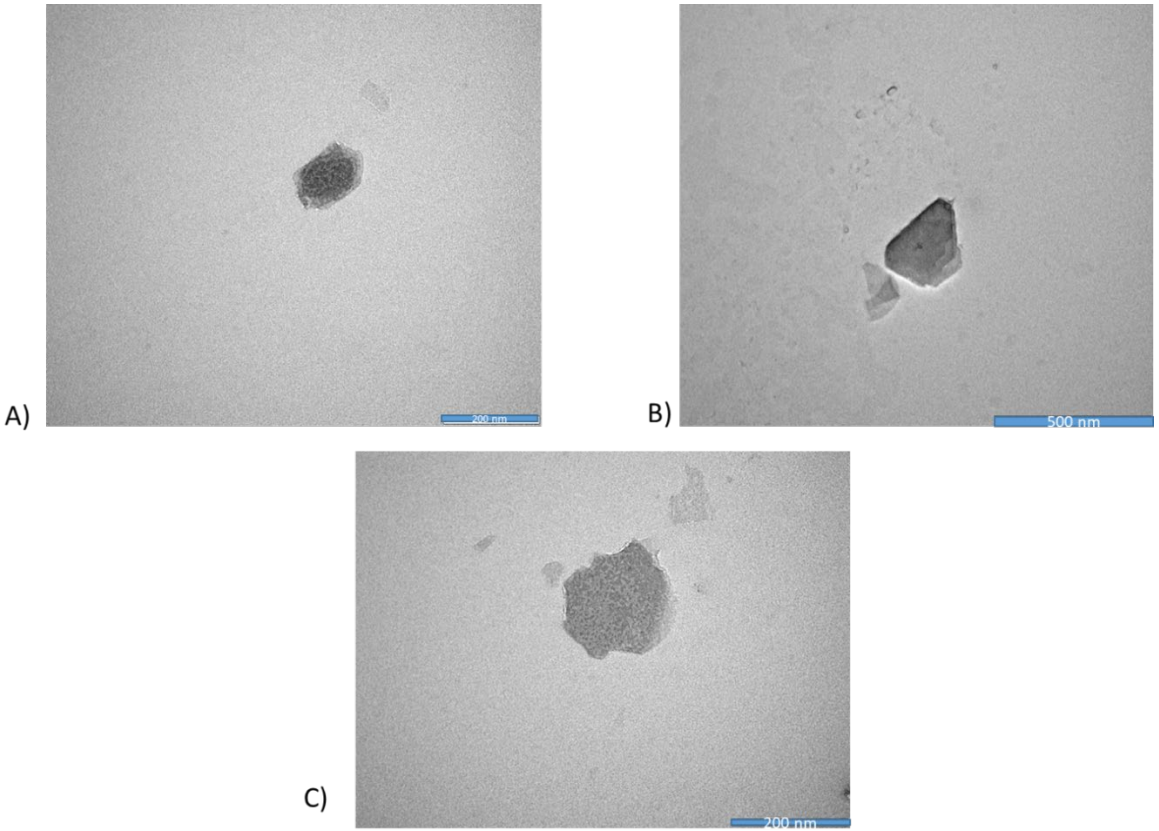


Figure 20. TEM images taken at 8 hours after precipitation of MDTG. A): Image taken at 105K magnification. B): Image taken at 60K magnification. C) Image taken at 87K magnification. A defined geometrical rod-like structure, consistent with "top-down" produced nanocrystal descriptions is found in images obtained. TEM images of sharp edges after a "top-down" formulation of a nanocrystal have been published elsewhere [294]. Further confirmation of a nanocrystal structure can be done with polarized optical microscopy [316]. The structure obtained has a hydrodynamic diameter of approximately 200nm. The suspension is broken easily, as without magnetic stirring it forms visible aggregates within 4 hours.

3.3.4 Polymeric coating of MDTG nanocrystals

Once the nanocrystal emulsion has been formed, and the organic phase has evaporated successfully, the second solvent displacement precipitation was prepared. On average, the coating was performed at 3.5 hours after MDTG precipitation. These "coated nanocrystals" formulations measure on average 215 nm by DLS, thus being bigger than the original nanocrystal (**Figure 21. A**). All samples had a PDI <0.3. Of note is that despite coating with different materials and different polymeric masses, the size of formulations by DLS has no statistically significant difference between coated formulations. By DLS all coated formulations have a statistically significant difference when compared to the MDTG nanocrystal (**Figure 21. A**). Further evidence of successful coating can be found in the TEM micrographies as a "double halo" appearance is seen, without any negative staining. Such an appearance suggests the presence of two electron densities, or two different materials. Of note is that the morphology of the formulations changes from a rod shape found in MDTG, to a well-rounded spherical structure **(Figure 22)**. Further evidence of successful coating was provided by the change of Z potential measured by DLS, from -37 mv (+/-6) as an uncoated MDTG nanocrystal, to -50 mV (+/-2) with PLGA, -62 mV (+/- 2) with P85 and -45 (+/- 2) mv in MPEG-PLA groups (**Table 15**).

All coated nanocrystals formulations developed contained a significant drug content, as they all surpassed 20% wt%, as measured by HPLC-UV (**Figure 21.B**). Within the coated formulations MPEG-PLApresented the highest wt% of all the formulations, with 78% (+/-14%) and P85 the lowest wt% with 29% (+/-6%). The mPEG-PLA formulation

was initially selected for further testing in *in vitro* systems, such as Caco-2 and RAW 264.7 cell lines.

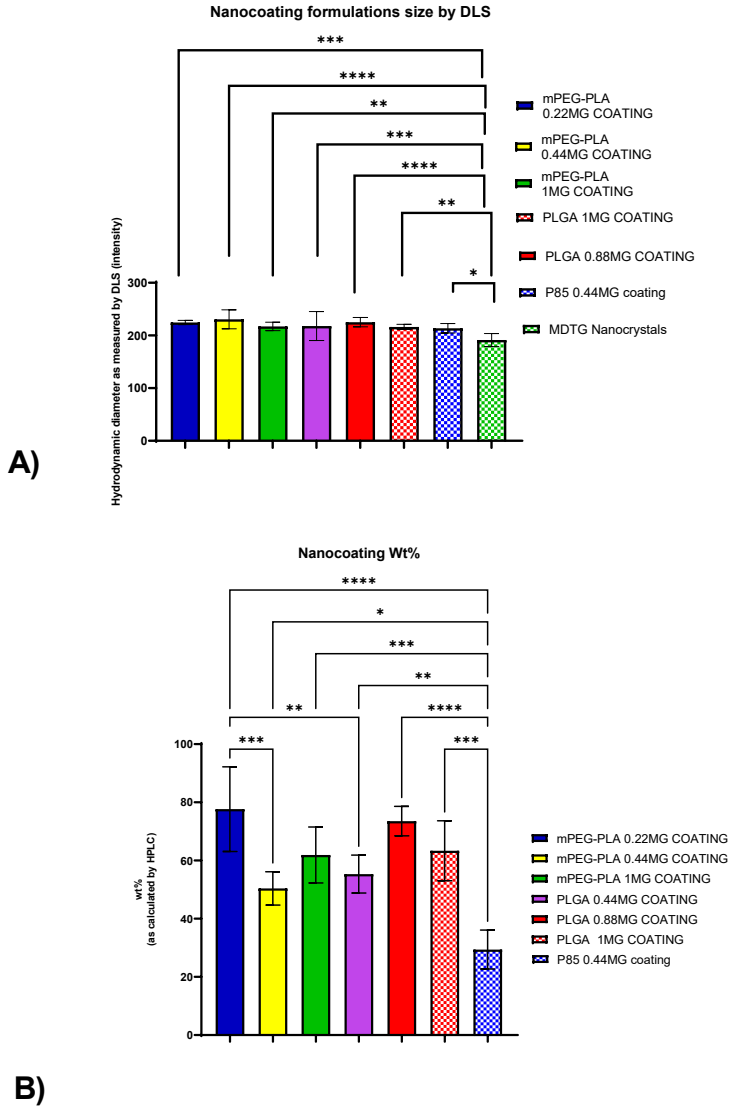


Figure 21. Size by intensity DLS and wt% as measured by HPLC-UV of of coated nanocrystal formulations prepared under different conditions. The coating mass represents the dry polymer added per sample. All samples have an N=6. In the case of DLS measurements N=3 and n=3 for each sample. Data presented as mean ± S.D. A) Size by DLS comparison of the MDTG nanocrystals with the coated nanocrystal formulations. The difference between groups is statistically significant by ANOVA p= <0.0001. Post Hoc Bonferroni corrections are displayed on the graph. B) Represents the drug content, expressed in wt% of the coated formulations developed. The difference between groups is statistically significant by ANOVA p= <0.00001. Relevant Bonferroni corrections are displayed on the graph.

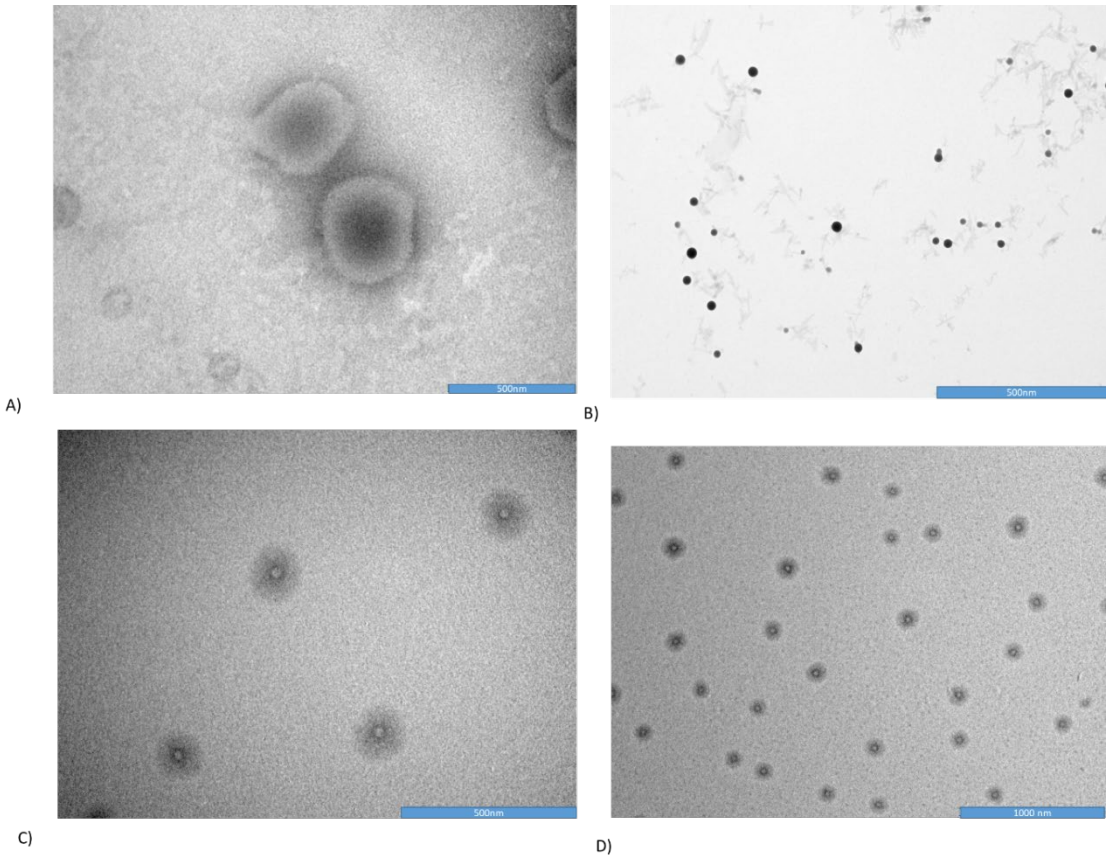


Figure 22 TEM micrographies of representative coated nanocrystals formulations. Coating represents the dry weight of the polymer added per sample. A*)* and *B)* were taken 12 hours post-coating. No negative staining was used. In TEM micrographies a "double halo" morphology is observed. Samples have a hydrodynamic diameter of approximately 210nm *C)* and *D)* were taken 1 week after coating (but under mechanical stirring). *A)*: Sample coated with 0.22 mg of MPEG-PLA imaged at 43K magnification. *B):* Sample coated with 0.44 mg of PLGA (75:25) imaged at 26.5K magnification. *C)* Sample coated with 0.44mg of MPEG-PLA Imaged at 20.5K magnification. *F)*: Sample coated with 0.44 mg of PLGA (75:25) Imaged at 60K magnification

3.3.5 In vitro cytocompatibility/preliminary cellular uptake

Before testing the coated MPEG-PLA formulation, the nanocarrier was subject to testing in relevant concentrations with the Presto Blue assay and the LDH assay in two cell lines, RAW 264.7 and Caco-2. Cellular metabolism rates remained high in both cell lines, over 90%, while there was no significant LDH release associated with the exposure to the polymeric carrier (**Figure 23 A)- B**). Once this potential confounding factor in the formulation was assessed, RAW 264.7 and Caco-2 cells were exposed to "free" unformulated MDTG, MDTG nanocrystals, DTG and the MPEG-PLA coated formulation in different concentrations **(Figure 23 C)- D).** In both cell lines cellular metabolism started to be adversely affected from 10 μM exposure to both the "free" MDTG and DTG. At 100 μM this effect was more pronounced in both cell lines as DTG had lowered the cellular metabolism to 65% and MDTG to 70%. The coated formulation and the MDTG nanocrystal did not present such adverse effects even at 100 μM. The LDH assay did not reveal any significant signs of cellular necrosis with either the free MDTG, the coated formulation or the MDTG nanocrystal (**Figure 23 E) -F**). Of note is that measured LDH release levels were very similar between groups, and in no case were suggestive of significant cellular toxicity .

To assess uptake of the formulation, 10% of the total formulation coating was substituted with a MPEG-PLA-Cy5 (**Figure 14**). This labelled formulation was assessed at 50μM with Presto Blue and the LDH assay for adverse effects on cellular metabolism, using HBSS buffer and Triton X as controls (**Figure 24**). At 50μM the coated formulation did not present any relevant adverse effects that would be observed through a lower metabolism or cytosolic LDH release.

Qualitative uptake of the formulation was assessed with fluorescence microscopy 24 hours after exposing the cells to 50μM (based on MDTG concentration). Qualitative internalization of particles showed evidence that the RAW 264.7 cells had a higher uptake rate than the Caco-2 cells at 24 hours (**Figure 25**). Quantitative uptake was assessed via spectrophotometry at 24 hours. Quantitative uptake showed similar results as the RAW 264.7 cell line had internalized 71 pg/cell of formulation, whereas the Caco-2 cell line had internalized 43 pg/cell, with the difference being statistically significant ($p < 0.05$, **Figure 26**). However, these results need to be taken with caution as they may only reflect the fate of the polymeric carrier. These experiments were conducted under controlled environments as the cells were dosed with HBSS buffer in all cases.

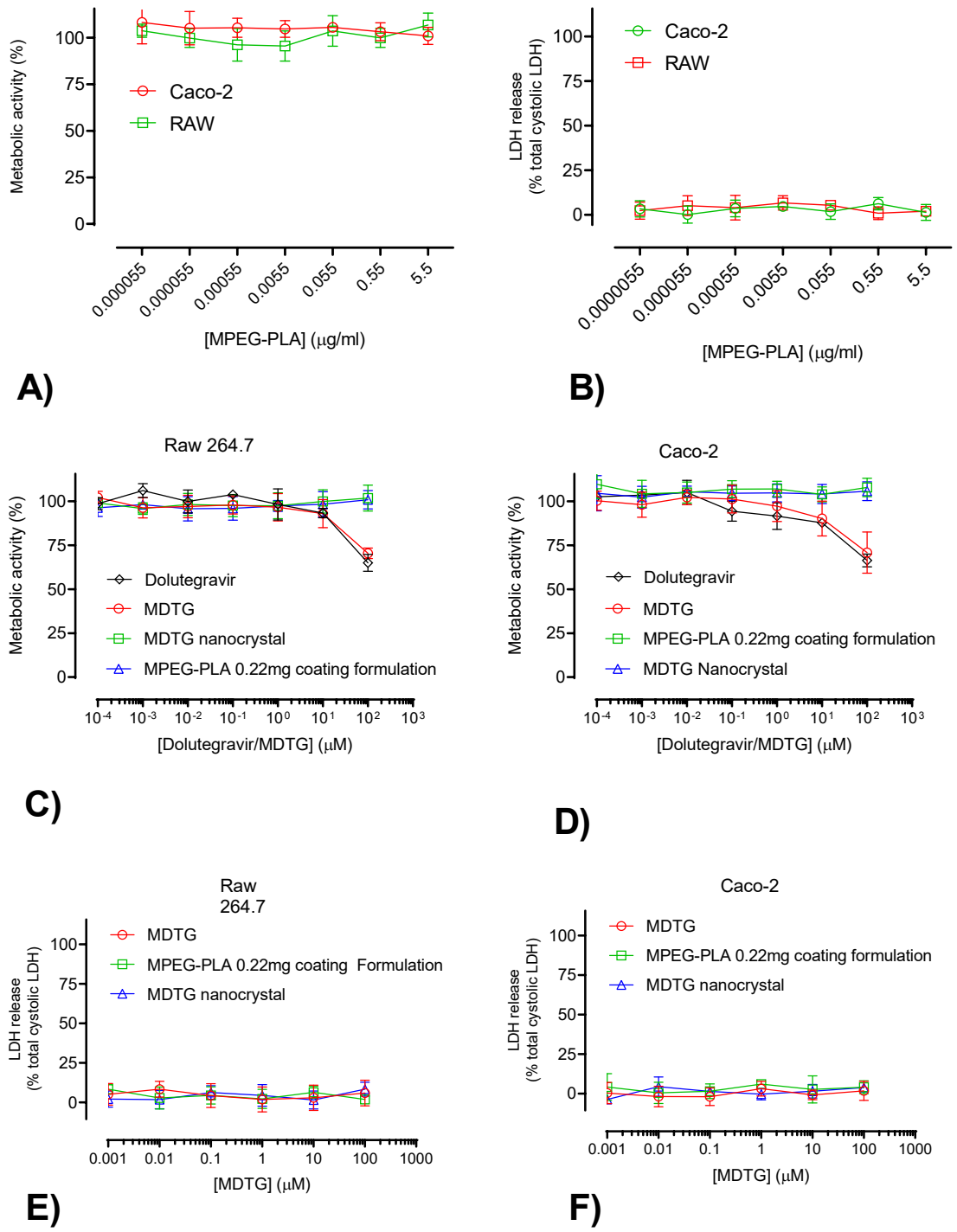


Figure 23. Cytocompatibility data on Raw 264.7 and Caco-2 cell lines at 24 hours. Coating represents the dry weight of the polymer added per sample. Panel A) Shows the metabolic activity measured through the Presto Blue assay of two cell lines to relevant concentrations of MPEG-PLA .B)LDH release of two cell lines to relevant concentrations of mPEG5000-La100. C) Metabolic activity of the RAW 264.7 cell line measured through the Presto Blue assay of two cell lines to relevant concentrations of parent drug, prodrug and MPEG-

PLAconcentrations. D) Metabolic activity of the Caco-2 cell line measured through relevant concentrations of parent drug, prodrug, MPEG-PLAconcentrations. E) LDH release of RAW 264.7 cell line when exposed to relevant concentrations of formulation, prodrug, and MDTG nanocrystal. F) LDH release of Caco-2 cell line when exposed to relevant concentrations of formulation, prodrug, and MDTG nanocrystal. Data presented as mean ± S.D (N=3, n=3) for each data point.

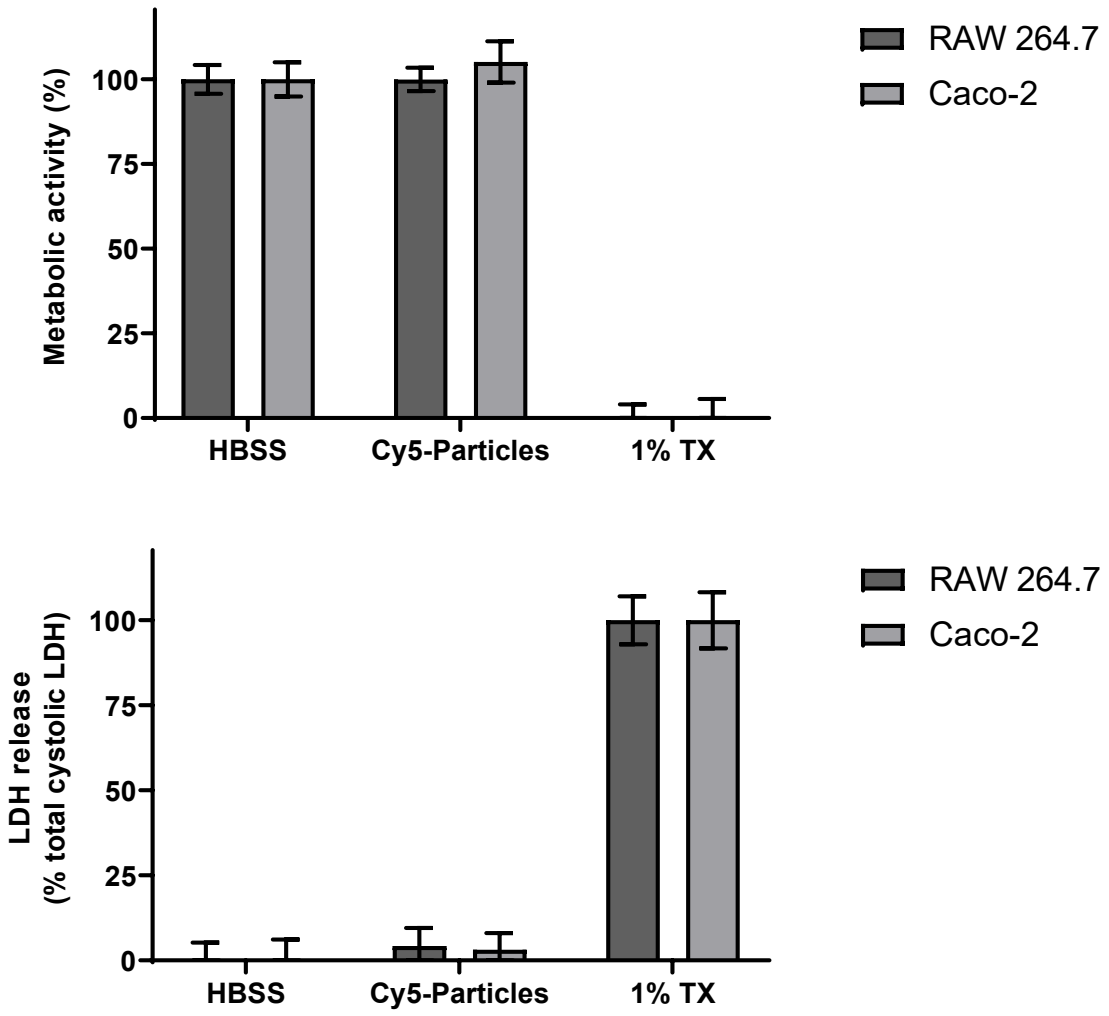


Figure 24. Biocompatibility assessment of 50μM at 24 hours of the MPEG-PLA-Cy5 coated formulation. Ten percent of the total polymer used (0.22mg) for coating was substituted with a MPEG-PLA-Cy5 for tracking uptake. Data presented as mean ± S.D (N=3, n=3) for each data point. *Upper panel*: Metabolic activity as assessed by Presto Blue assay. More details on the methods in figure 14. *Lower Panel*: Relative cytosolic LDH release.

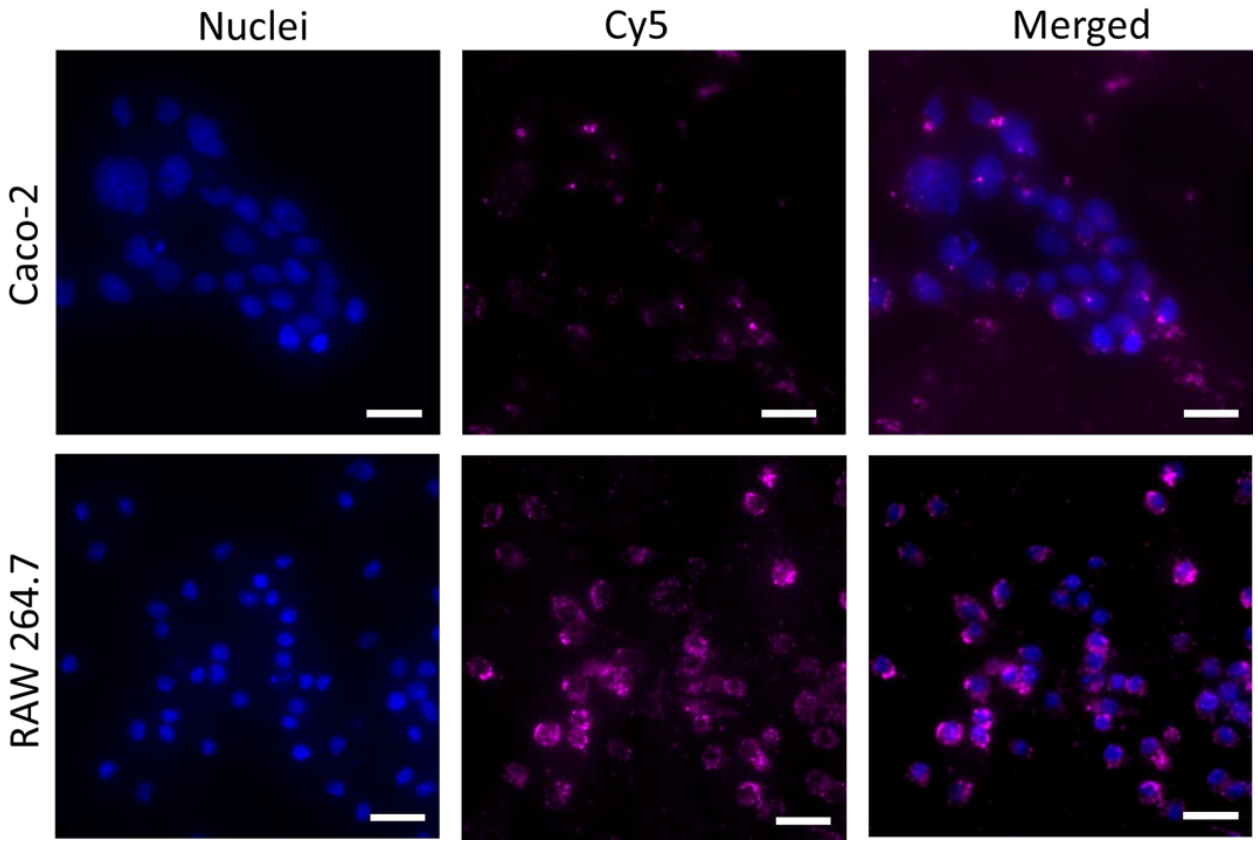


Figure 25. Micrographs of polymer internalization in Caco-2 intestinal cells and RAW 264.7 macrophage cells. Formulation was dosed at 50 µg/ml (MDTG concentration) for 24 hours to cells followed by staining with Hoechst 33342 (10 ug/ml) for 30 minutes. Staining solution was washed off with PBS and cells imaged on an inverted Nikon Eclipse TE 300 microscope on DAPI and Cy5 filters. Images were merged using ImageJ software. Scale bar = 50 um

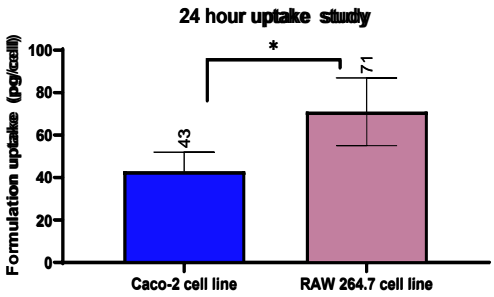


Figure 26. Quantitative uptake of MPEG-PLA-Cy5 at 24 hours. Raw 264.7 and Caco-2 cell lines were incubated with NP suspensions(50 ug/ml) for 24 hours, followed by removal of solutions, three washes with ice cold PBS and lysis with 1% Triton X. Fluorescence of wells was then measured on Tecan Spark 10M plate reader. Data presented as mean ± S.D (N=3, n=3) for each data point. Nanoformulation concentration per well calculated via calibration curve. Un-lysed wells in the experimental plate were trypsinised and counted to determine cell number per well and enable normalisation of uptake to cell number. T student test was statistically significant (p=<0.05).

3.3.6 Stability of the polymeric coated formulations in strong ionic environments

Two buffers, HEPES and PBS, along with DMEM were selected as high-pressure ionic environments for the formulation to be tested. After the formulation was prepared, it was diluted into these three environments. The MPEG-PLA formulation remained stable judging by size and zeta potential for up to 72 hours in 10mM HEPES buffer. There was no statistically significant difference between the size by DLS, and the zeta potential remained negative, despite the positive ionic presence. In 150mM PBS the formulation had a change in size, from 205 nm to 271 nm ($p < 0.05$). Such results were not found when analysing the stability of the formulation in cell culture media (DMEM), as the formulation quickly formed visible aggregates within the test vial (**Figure 27**).

Within the library of formulations, a second formulation was chosen to test under these conditions, as both vaginal fluids and/or anal mucus are high pressure environments for nanoformulations. The 2.75mg MDTG/P85 0.44mg coating was chosen to test further as it presented a favourable zeta potential in control conditions. The P85 formulation when exposed to 10mM HEPES buffer did not experience any statistically significant size changes after four hours. As with the MPEG-PLA formulation, the zeta potential remained negative in 10mM HEPES buffer through four hours. The formulation did not experience any statistically significant changes in 150mM PBS (**Figure 28**).

Repeated size by DLS readings were made in DMEM for the tested formulation, with no statistically significant difference found despite prolonged exposure (24 hours) to DMEM. The formulation was then studied for changes due to day-to-day variability, in three separate dates. With a two-way ANOVA, there was no statistically significant difference between groups, or between dates studied (**Figure 29**). A summary of the library of formulations with their results, can be found in **Table 15**.

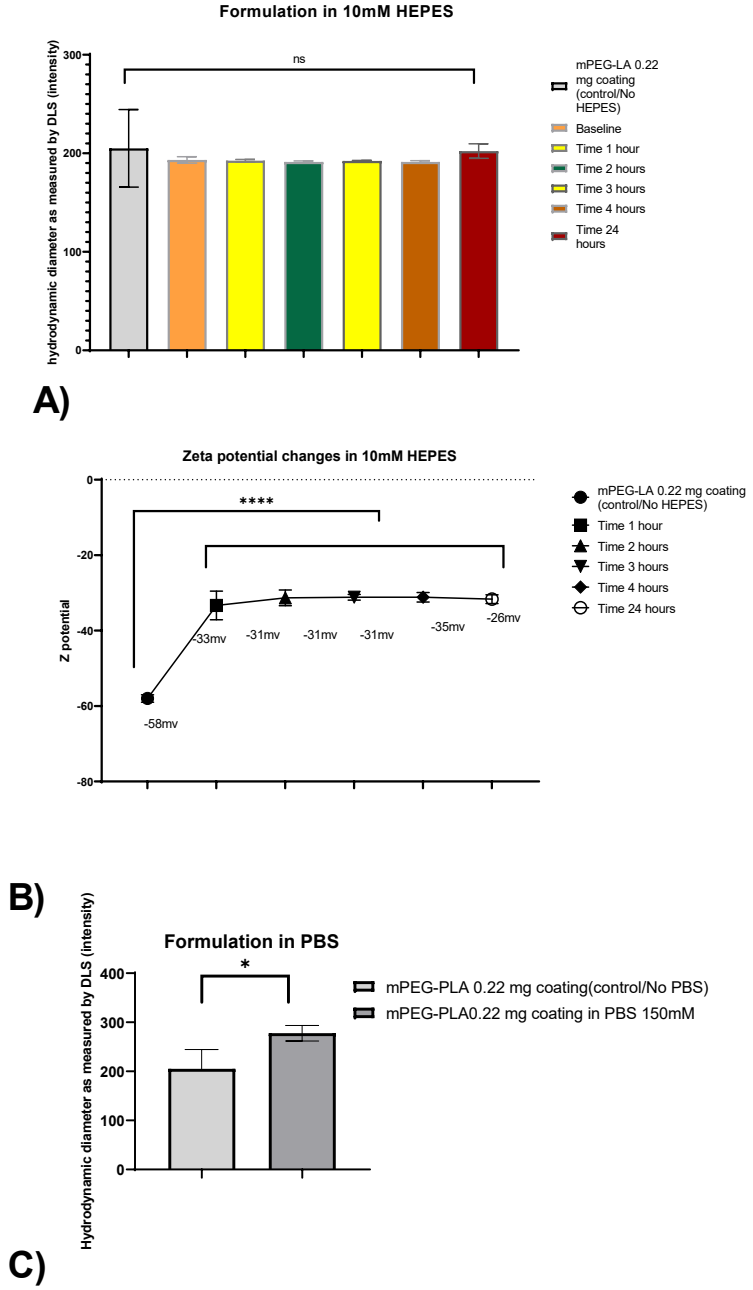
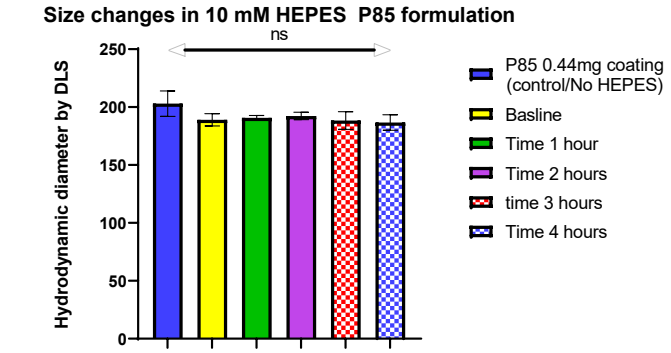
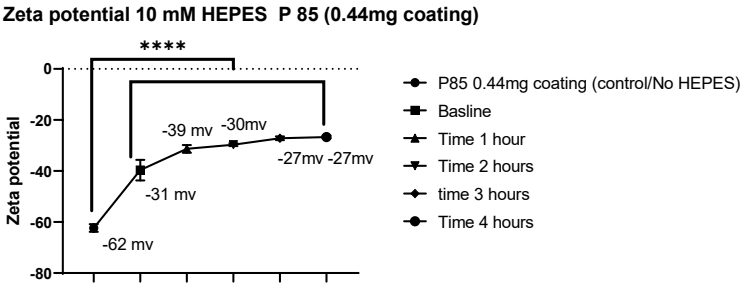


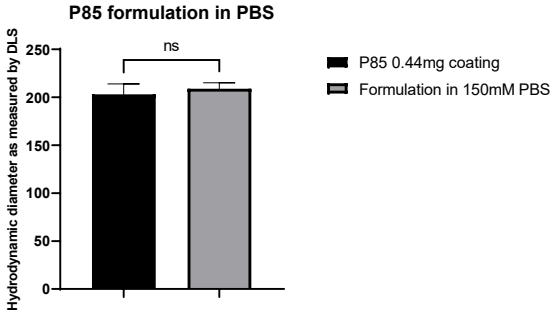
Figure 27. Stability studies of the MPEG-PLA 0.22mg coated formulation in 150mM PBS, and 10mM HEPES buffer. A) Size by DLS of formulations in 10mM HEPES buffer. B) Zeta potential changes of the MPEG-PLAformulation in 10mM HEPES buffer. The difference between groups is statistically significant by ANOVA p= <0.0001. Post Hoc Bonferroni corrections are displayed on the graph C) Size by DLS comparison of the MPEG-PLAformulation in control environment and in 150mM PBS after 5 min. An unpaired T-Student test showed statistically significant differences between the two groups ($p<0.05$). Data presented as mean ± S.D (N=3, n=3) for each data point



A)



B)



C)

Figure 28. Stability studies of the P85 formulation in 150mM PBS, and 10mM HEPES buffer. A) Size by DLS of formulations in 10mM HEPES buffer. B) Zeta potential changes of the P85 formulation in 10mM HEPES buffer. The difference between groups is statistically significant by ANOVA p= <0.0001. Post Hoc Bonferroni corrections are displayed on the graph. C) Size by DLS comparison of the P85 formulation in control environment and in 150mM PBS. An unpaired T-Student test showed statistically significant differences between the two groups ($p<0.05$). Data presented as mean ± S.D (N=3, n=3) for each data point

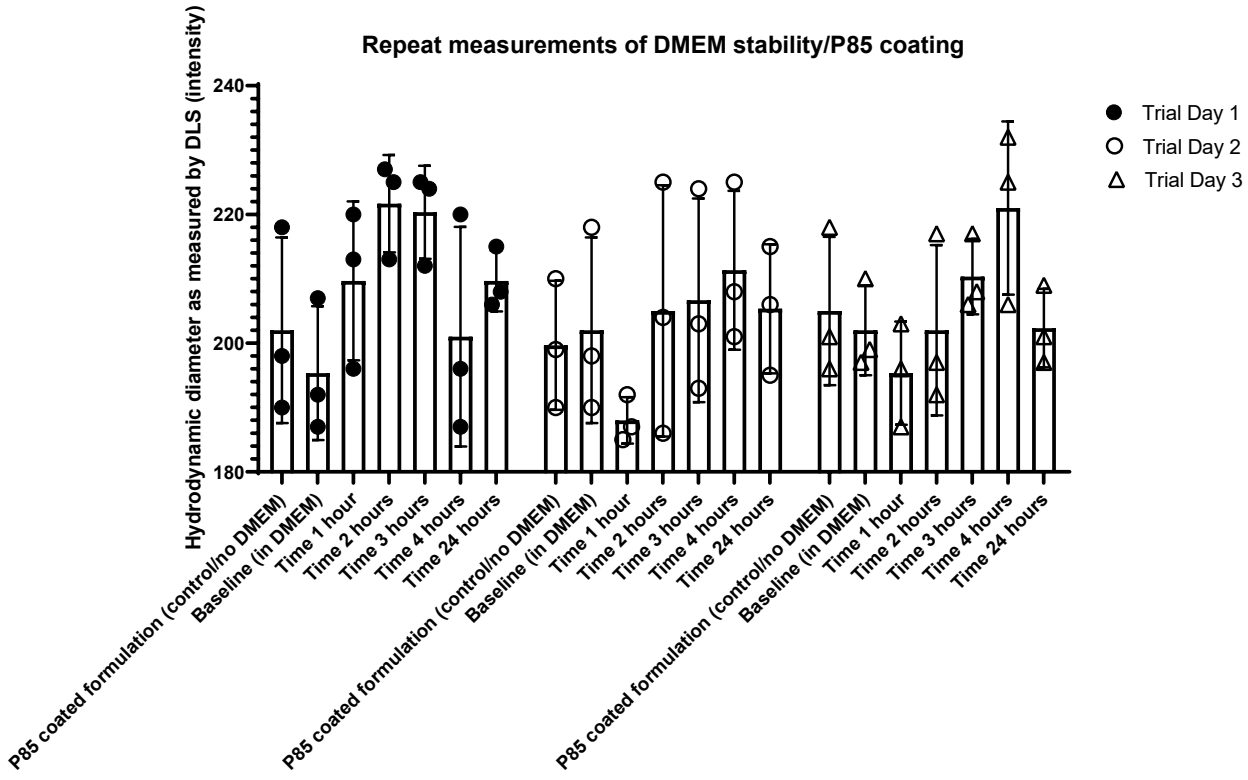


Figure 29. Size comparison by DLS of repeated DMEM stability studies of the P85 0.44mg coated formulation. The experimental set up was repeated on three different trial days with an N=3 and n=3 for each day, this approach allowed for a robust data set. Data presented as mean ± SEM for each data point**.** Symbols depict individual samples. Sample sets tend to normality according to Shapiro-Wilk test, thus a two-way ANOVA is performed, with a p=>0.05. Therefore, the means of observations grouped by time points are not statistically significant between each other. The means of observations grouped by date are not statistically significant between each other. There is no statistically significant interaction between the two factors analysed.

Table 15. Summary table of DLS, Zeta potential measurements and biocompatibility tests of the formulations developed. Data presented as mean ± S.D (N=6, n=3)

Formulation	**Size by DLS (nm)**	**Zeta potential (mV) N=3 n=3 (S.D+/-)**	**TEM**	**Biocompatibility/Preliminary uptake**	**Preliminary drug release studies**
MDTG nanocrystals	191 nm (+/-12 nm)	-37 mV (+/- 6)	Images show a hexagonal structure with size matching the DLS measurements. (**Figure 20**)	Biocompatibility in Caco-2, Raw 264.7 macrophages at 24 hours. **Figure 23**.	NA
MPEG-PLA0.22 mg coating per vial	224 nm (+/- 4 nm)	-47 mV (+/- 2.5)	Images show a spherical structure, displaying a "double halo", suggestive of two different materials. (**Figure 22**)	Biocompatibility in Caco-2, Raw 264.7 macrophages at 24 hours. Preliminary uptake at 24 hours, quantified by spectrophotometry in Caco-2 and Raw 264.7 macrophages. **Figures 25-26**.	NA
MPEG-PLA0.44 mg coating per vial	230 nm (+/- 18 nm)		NA	NA	NA
MPEG-PLA0.88 mg coating per vial	217 nm (+/- 8 nm)		NA	NA	NA
PLGA (75;25) 0.44 mg coating per vial	218 nm (+/- 27 nm)	-50 mV (+/- 2)	Images show a spherical structure, displaying a "double halo", suggestive of two different materials. (**Figure 22**)	NA	NA

PLGA (75;25) 0.88 mg coating per vial	225 nm (+/- 9 nm)		NA	NA	NA
PLGA (75;25) 1mg coating per vial	216 nm (+/- 5 nm)		NA	NA	NA
P85 0.44mg coating per vial	214 (+/-9 nm)	-62 mV (+/- 2)	NA	NA	**Figure 30-31**

3.3.7 MDTG Cleavage/Drug release from the formulation

Drug cleavage and subsequent release from the prodrug was assessed in inhibitor free rat plasma, a model media for lymph. Similarly, the P85 formulation was spiked in rat plasma, both MDTG concentrations were normalized to the same concentration. Under these conditions, a significant percentage of DTG is released by 1 hour, as 90% of MDTG has been converted to the parent drug. By cross-referencing the expected nanomols/ml with the measured value, no alternative pathway for MDTG was found **(Figure 30)**. The P85 coated formulation displayed a similar fast conversion rate as 60% of the total available DTG has been converted from the MDTG released in the media. Using a method of residuals a virtual data set was generated, to represent the unreleased MTDG from the formulation into the media. The virtual data set was generated, by subtracting MDTG concentrations in the coated formulation at each time point minus the MDTG concentration measured in free prodrug. This virtual data set performs in a very similar way to the P85 coated formulation. Calibration curve used and HPLC chromatograms can be found in **Figure 28**. Full results can be found in **Table 16 and Figure 30**.

Table 16. Generation of the "virtual" data set of MDTG residuals and the calculated half-lives of the three data sets. Half-lives of free MDTG and P85 coated MDTG in plasma are calculated by $0.693/K = T1/2$.

Method of Residuals				
Time in minutes	**MDTG "released" from P85 formulation Nanomol/ml (mean, N=3)**	**"Free" MDTG Nanomol/ml (mean, N=3)**	**MDTG "residual" Nanomol/ml (mean, N=3)**	**K value**
0	893.3	790.6	1.66	0.08141
30	115.3	332.6	217.3	0.05547
60	10	63	54.6	0.04641

Projected Half Lives of data sets	
Data set	**Half life**
Free MDTG	8.5 minutes
P85 coated formulation	12.4 minutes
MDTG residuals in P85 formulation	14.93 min

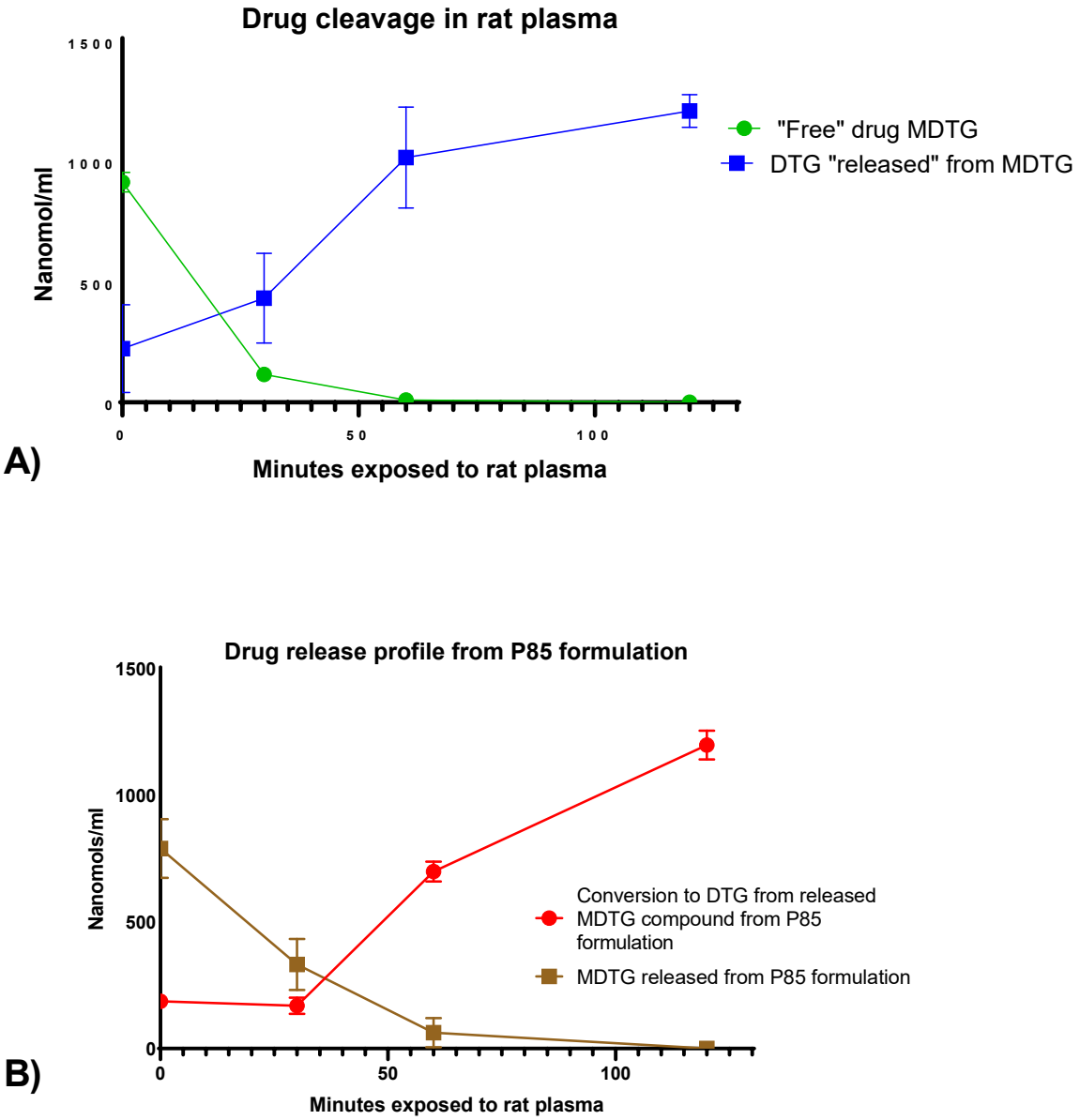


Figure 30. Drug cleavage from MDTG and drug release from P85 formulation. Data presented as mean ± S.D (N=3) A) Drug cleavage and subsequent release of DTG from the prodrug MDTG. The covalent link of the MDTG prodrug gets cleaved by esterase enzymes found in rat plasma very quickly, as nearly 86% of the total possible DTG is released by 1 hour of exposure to rat plasma. B) Drug release profile from P85 formulation. Despite the P85 coating, MDTG gets released very quickly into the media, and thus this prompts the cleavage of the 14-carbon chain from the prodrug. The method of residuals (**Table 16**) shows a very similar DTG release between the P85 coated MDTG and the "free" MDTG.

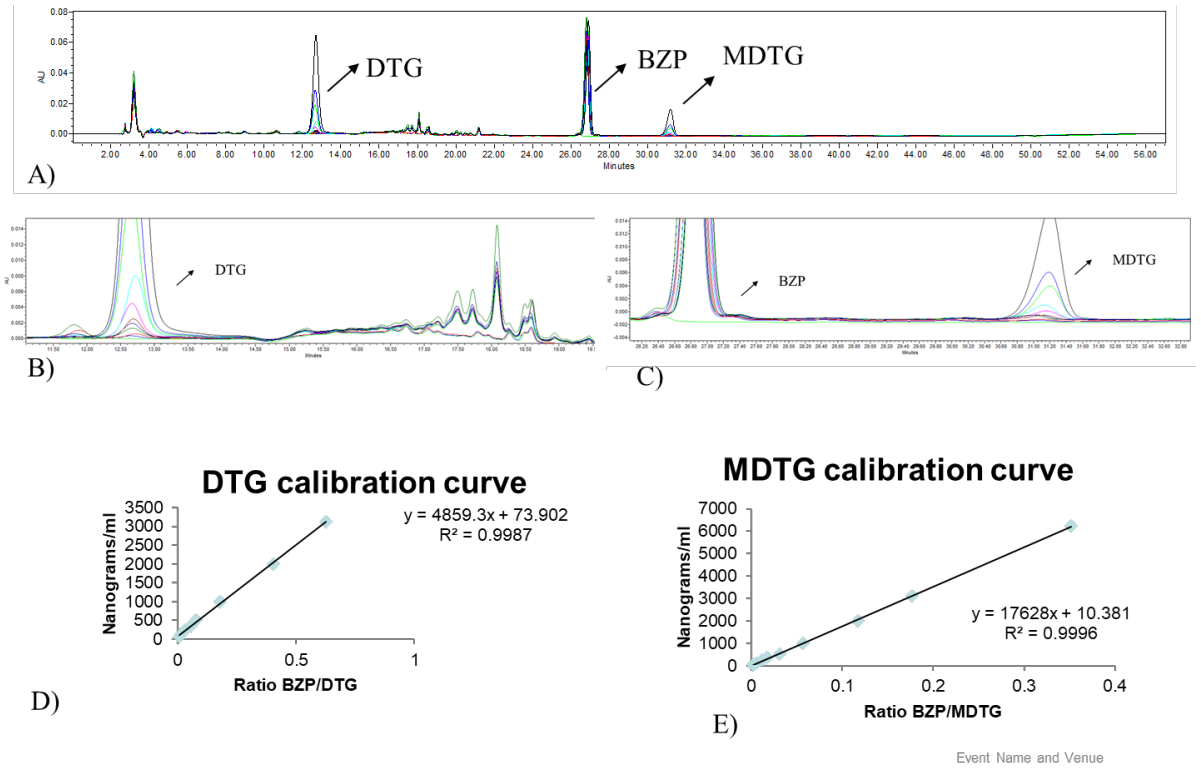


Figure 31. Chromatograms and calibration curves for both MDTG and DTG. A) Full chromatogram of rat plasma under conditions described in 3.2.3. B) Zoomed section of interest for DTG elution. DTG is expected to elude between 12.1 min and 13.5 min with a peak at 12.6 min. C) Zoomed section of interest for MDTG elution and Benzo(a)pyrine (BZP) which acts as the internal standard. MDTG is expected to elude between 30.8min and 31.60 min with a peak at 31.20min. BZP is expected to elute between 26.6 min and 27.2 min with a peak at 26.8 min. D) Calibration curve values for DTG quantification. E) Calibration curve values for MDTG quantification.

3.4 Discussion

3.4.1 A modified DTG as a means of solving physiochemical shortcomings.

DTG is a prime antiretroviral agent, currently used as a first line therapy treatment for most cases. Among the relevant characteristics are its high genetic barrier to HIV resistance, a significant antiviral potency, highlighted by a low IC-90, at 64 ng/ml, and favourable adverse effects profile. Such characteristics have made DTG a model compound for a "potential functional" cure for HIV [64-66]. However, DTG is a highly hydrophilic compound (logP -1.57), which presents a challenge for formulating the drug through traditional methods for nanoformulations such as a solvent displacement method. A solution to this problem is to modify the drug by attaching a 14-carbon chain through an ester bond at the 7'OH of the parent drug. To have a higher chance to effectively saturate the local lymph nodes, any PEPSE formulation should carry a significant payload. The reasoning behind this desired characteristic is that the hypothesized uptake of the formulation is dependent on Peyer's patches found in anal tissue. Given the minimal proportion of Peyer's Patches in the intestinal mucosa, a significant payload would be more likely to saturate the local lymph nodes and thus abort impending infection [40, 317, 318]. Lymphatic transport is benefitted directly from the lipophilicity of a compound; thus a hydrophobic prodrug approach is a potential solution to both of these issues [40, 51, 86].

As seen in **Table 14**, the physiochemical characteristics of MDTG were changed significantly when compared to the parent drug. Due to resource limitations, our laboratory was unable to confirm the antiviral properties of MDTG that Sillman et al. had reported. Sillman et al.'s experimental set up was heavily based on Gendelman's

monocyte-derived macrophages (MDMs) reverse transcriptase assay [319]. In short, the set up involved MDMs being exposed to 100 μM of DTG or MDTG followed by a HIV challenge. Following the viral challenge, the cells were washed and incubated with relevant concentrations of parent or prodrug. Antiretroviral efficacy was then calculated by measurements of the reverse transcriptase activity, through culture fluids collected daily for a month. Interestingly MDTG produced significant reverse transcriptase inhibition (83% activity inhibition at day 30). These results suggest that MDTG has intrinsic activity against HIV, providing evidence that a hydrophobic approach could solve delivery issues without compromising the antiviral activity. Further research into MDTG's properties would allow for it to become a significant tool in both prophylaxis and therapy of HIV.

3.4.2 Nanoprecipitation as a means of formulation for a Dolutegravir Prodrug

Published literature suggests that a hydrophilic compound, such as DTG, will present major difficulties when formulating it into a nano capsule [253, 263, 266, 320]. Some approaches have tried to combat this issue through a double emulsion system, water in oil in water, or through a modified nanoprecipitation system. In the case of the water in oil in water double emulsion, there appears to be a low cap for drug content (around 5% wt%), additionally scaling the formulation for industry production would prove difficult [168]. While a modified nanoprecipitation can be a solution to produce nanoaggregates with a hydrophilic compound, this technique also presents important limitations in drug content [320]. MDTG on the other hand, having a calculated logP of 5.51, allows for traditional approach for nanoprecipitation to be performed[252, 263, 264].

Nanoprecipitation relies heavily on a polymeric nanocarrier that is biologically inactive, biocompatible (non-toxic) and ideally biodegradable. Adequate biocompatibility is not correlated with a good degradation rate [321]. Among FDA approved materials, PLGA, MPEG-PLA and some surfactants such as the pluronic family have been deemed adequate for human use. Thus, the selection of potential nanocarriers was performed based on these factors, narrowing down on a commercial PLGA, a Pluronic 85 and MPEG-PLA that has been published elsewhere [306].

Within the nanoprecipitation conditions, the MPEG-PLA did not achieve high drug contents in early screenings, thus the focus was moved to the comercial PLGA. In the experimental data set using acetone as an organic phase, decreasing the ratio of organic phase to aqueous phase produced encouraging results. The ratio of 1:6, proved to be the most successful in producing a relatively high drug content for nanoaggregates by nanoprecipitation, at 8.43% wt%. However, such results are still far from the results hypothesized for success in a PEPSE scenario, as those formulations do not reach 10% wt%.

A different strategy was using a different organic solvent, substituting acetone with acetonitrile as published works have suggested that its use can produce nanoaggregates with a higher wt% [253]. In the experimental data set, such measure did not improve the formulation when compared to acetone at the same organic to aqueous ratio. Therefore, in order to achieve a better drug content as hypothesized (>25% wt%), a different approach should be taken. One such approach can be a two-step nanoprecipitation for coating a solid drug nanoparticle of MDTG. However, in order to do such a formulation, MDTG must be able to be precipitated in an aqueous solution.

3.4.3 Solid drug nanocrystals of MDTG

Our results provide evidence that through a solvent evaporation method a colloidal dispersion of a coated solid drug nanocrystal of MDTG can be generated. Such results are in line with newly published literature that suggest that some compounds such as etoposide and olaparib are potential candidates for such approaches [295]. The exact physiochemical characteristics that the compounds need to have to be successfully precipitated into a nanocrystal are still unknown. A potential explanation may lie within the modification that MDTG has undergone, by having a 14-carbon chain attached, and therefore has amphiphllic proprerties. This allowed MDTG to be precipitated into a nanostructure, as evidenced by the TEM images, via a solvent displacement method thus forcing the compound to collapse around itself and precipitate (**Figure 20**). The resulting nanoemulsion as shown by our TEM images, provided evidence towards one of our secondary hypotheses. Further research is required to define the characteristics that allow for any compound to be precipitated into a nanoemulsion through a low energy solvent displacement method.

MDTG has poor solubility in water, thus the nanocrystal generated in the first nanoprecipitation will tend to form insoluble aggregates that are visible to the naked eye. As with other nanocrystal production methods, a solution to this problem is adding a polymeric stabilizing agent. To solve this issue, inspiration was drawn from the coating of inorganic nanoparticles performed by Abrushida et al [322]. Similarly, Danai-Stlyliari et al. performed computational models that allowed for the comprehension of a potential coating process in order to stabilize solid drug nanoparticles through computational modelling [294]. Within our research group, we

discussed the possibility of simplifying the process further, by generating a nanocrystal emulsion and coating it through a second solvent displacement precipitation. Such initial results have recently been published [295].

3.4.4 A polymeric nanocarrier as a means of stabilizing a nanocrystal emulsion

To stabilize the nanocrystal form of MDTG, a second solvent displacement experiment was designed, using the nanocrystal emulsion as the aqueous phase, with an acetone solution containing a polymer. While the mechanism is poorly understood, a potential hypothesis towards this would be that the organic phase droplets momentarily solubilize the nanocrystal, which follows with a supersaturation of the droplet and subsequent precipitation of the polymer around the MDTG nanocrystal. This two-step solvent displacement coats the nanocrystal with a polymeric carrier. TEM micrographies suggest coating of the MDTG nanocrystal, as a "double halo" appearance is seen (**Figure 22**). The "double halo" appearance is indicative of two different electron densities, i.e., two different regions of scattering ability. Those micrographies in conjunction with the size change and zeta potential change measured by DLS, provide evidence that the MDTG nanocrystal was coated by the polymeric nanocarrier. This provides proof of concept of one of our hypotheses.

The coated nanocrystals formulations obtained, have a significant drug content (>25% wt%) , as the polymeric mass is only intended as a stabilizer. HPLC-UV provides evidence of this significant drug content (**Figure 21**). Such encouraging results allow for a coating method that can effectively be performed in most laboratory settings. With growing evidence, the coating process via a two step nanoprecipitation allows for a low energy, scalable, and resource friendly way to prepare high drug loading formulations.

The formulation set up presents drawbacks, as being a newer method, the perfect ratio of drug to polymeric coating, as well as polymer used, remain to be studied further.

3.4.5 In vitro cytocompatibility and uptake in Caco-2 RAW 264.7 cell lines

The Caco-2 and RAW 264.7 cell lines, displayed a similar behaviour to what was previously published by Sillman et al. regarding the biocompatibility of MDTG [169]. The formulation coated with 0.22 mg of MPEG-PLA was chosen to test further in vitro studies. Previous reports using the similar polymer builds show encouraging results in biocompatibility, a higher polymer degradation rate, and a fast release of the compound when compared to formulations with homopolymers such as PLA [269, 299, 323, 324].

Evidence produced within our facilities, as well as published data suggests that DTG, and its prodrug, MDTG, when not in a nanoformulation, have an impact on cellular metabolism at 100mM [169, 283]. Both MDTG's nanocrystal and the formulation developed maintained high cellular metabolism at 100mM. Preliminary biocompatibility assays on two cell lines have demonstrated promising results, even when analysing the nanocrystal form of MDTG, however more in-depth experiments are needed to confirm this data. In our data set, both the nanocrystal and coated formulations of MDTG were significantly less adverse to cellular metabolism than the non-formulated parent drug or prodrug. LDH data revealed that the interference in cellular metabolism, associated with the prodrug treatment, was not associated with cellular necrosis and subsequent release of LDH. The presence of LDH release was also low with the nanocrystal and coated MDTG treatments. Taking both data sets into account, the MDTG formulation tested did not produce cellular necrosis, nor did it alter

significantly cellular metabolism, and thus had evidence of potential biocompatibility with in vivo models, however further research is needed to confirm the trend.

Qualitative uptake produced encouraging but incomplete results as only show the fate of the polymeric nanocarrier. As the MPEG-PLA-Cy5 formulation appeared to have a better penetration into the RAW 264.7 macrophages than the Caco-2 intestinal cells. These results were reproduced quantitatively, as the Raw 264.7 cells contained more labelled polymer when compared to the Caco-2 intestinal cell lines ($p<0.05$). Thus, providing evidence that MPEG-PLA may indeed be internalized by the target cells via phagocytosis. However, the uptake studies on a dendritic cell line are still pending. Given that HIV has the possibility of infecting both a macrophage and a dendritic cell, the dendritic cell results are imperative for evaluating the formulation's uptake [23]. The fate of the antiretroviral payload is still to be investigated and thus these results do not reflect what behaviour would MDTG have.

3.4.6 Coated nanocrystals formulations in strong ionic environments

The MPEG-PLA formulations when being subject to strong ionic environments, such as HEPES and PBS buffers, formed visible aggregates after 4 hours. The effect was found almost immediately in cell culture media; thus, this developed formulation is likely to encounter issues where the media is associated with salts. Such mayor issue suggest that the formulation would produce visible aggregates in anal or vaginal environments.

The P85 formulation did not produce such aggregates in the HEPES or PBS buffers. While visible aggregates were found after 24 hours of exposure to cell culture media,

this effect is not likely to be relevant in animal models, as 24 hours would allow for absorption of the compound/formulation delivered. It is likely that that the triblock copolymer structure of P85, with two distinct hydrophilic arms, was vital for providing further stability to the coated formulation of MDTG in relatively aqueous environments, particularly in PBS and HEPES buffers [298]. This is in stark comparison to the diblock PLGA and mPEG-PLA used. P85 is also a smaller molecule than the PLGA or mPEG-PLA used, this fact could potentially lead to a better "coating" process as they could self-assemble and coat MDTG with less difficulty than bigger molecules [294, 298]. A Pluronic agent albeit more lipophilic, F127, was also used in Sillman et al.'s MDTG formulation[169].

Evidence suggests that the HIV virus would cross and invade the target lymphatic nodes within the first 72 hours, with systemic replication during the first week [23, 170]. Experiments on healthy human subjects using an ejaculate of HIV surrogate particles, show that the virus can potentially remain in the vaginal tissue for 4 hours and 24 hours in the anal tissue [325, 326]. Therefore, a minimum of 24 hours of stability in situ would be required for any PEPSE formulation.

A formulation with sustained release could also prove ideal in a PEPSE scenario, as the threat of HIV infection remains after a sexual exposure [23]. The saturation of local lymph nodes with sustained release at the paracortex areas, where TCD4+ lymphocytes are prevalent, is a priority for any localised PEPSE formulation [37].

3.4.7 Parent drug cleavage from MDTG/Release from the formulation.

Our results present a similar behaviour as what was reported by Sillman et al [169]. In our data set, more than 90% of MDTG was cleaved by 1 hour; by time 2 hours, no MDTG is present in our set up. Sillman et al.'s results have small variations, as in their set up, in whole mouse blood, most (80%) of MDTG was cleaved in one hour, thus releasing DTG into the media. When measuring the DTG output in nanomols/ml all of the initial MDTG input was accounted for and thus no other potential pathways of MDTG conversion were found. This is another finding that is heavily correlated with what was reported by Sillman et al. [169]. Due to COVID19 related constraints, and the stability observed with the P85 formulations, the P85 coated formulations were tested for drug release from the formulation.

A speedy release of the MDTG payload is also found with the P85 coated formulation, as all the MDTG content is released within 2 hours. Such effect is also reflected in the half-lives of MDTG and the calculated half-life of the P85 coated formulation, as the difference between them both is approximately 5 minutes. By using the method of residuals, a virtual data set was generated, representing the MDTG that is yet to be released by the P85 coating. The difference between this virtual data set and the free MDTG is minimal, approximately 7 minutes. Thus, this quick release may not allow for the coated particles to cross all the epithelial barriers and reach the local lymph nodes. The whole timeline of HIV infection is difficult to model effectively. The full process between initial epithelial uptake and the impending systemic infection is hypothesized to be 72 hours, with 54 hours being used for the initial daughter virions to be produced, and 1 week to be detected in blood with modern assays[23, 170]. In fact,

it is considered that during the first 24 hours, PEPSE could be most effective in aborting impending infections (17). Controlled release of the compound inside of a nanoformulation is key to success [253]. In this scenario, the formulations produced did not provide evidence that they could be uptaken by the lymphatic cells within the suggested timeframe, as they would have likely released the drug content prior to encountering target cells.

The formulations developed proved to be unlike Sillman et al.'s formulation in terms of stability and controlled release. However growing evidence suggests that a modified two step nanoprecipitation can be used in some instances to generate nanocrystal formulations that are coated with a polymeric stabilizer [294]. The formulations developed require significant research into potential coating agents, as well as the coating mass, to produce a formulation that would be a candidate for PEPSE.

3.5.1 Conclusions

Nanoprecipitation as a means of generating high drug content formulations has important limitations. While some of those limitations can be successfully overcome by nanocrystal formulations or other solid drug nanoparticle techniques, these are often cost prohibitive or would present issues for production scaling-up. In our work such limitations are significant and rule out nanoprecipitation as a technique for a novel localised PEPSE.

This work follows the line of recent published literature, that suggests that by precipitating a compound of interest and coating it through a modified solvent

displacement method, high drug content is possible [294, 295]. The physicochemical properties that allow for this method to work as intended are still under further study, however it could be theorized from this work that a lipophilic compound is needed for effective coating of a nanocrystal produced via solvent displacement. The formulated nanocrystal had no significant adverse effects within the metabolism of two cell lines, a contrast from the “free” drug and “free” prodrug (**Figure 23**).

The formulations developed had a significant drug content (>20 wt%), however their stability on strong ionic environments is a concern, as visible aggregates are seen when in contact with DMEM. Within the formulations tested, the P85 coated formulation was stable for 24 hours under high anionic environments, such as PBS or DMEM. However, when analysing drug release, these results were not as encouraging, as there was a limited difference between the non-formulated MDTG and the P85 coated formulation. This evidence suggests that the formulations generated are not ideal for a sustained release effect for PEPSE.

This work presents a proof of concept of a formulation that can be produced with a low energy setting achieving high drug content, however further study as for the coating agent used (as well as the mass used) is needed for the advancement of the technique. The perfecting of the technique would allow for a new weapon against the sexual transmission of HIV.

Chapter 4

"Role of a novel PEPSE formulation in the future of the HIV pandemic"

4.1.1 An unprotected target population for PEPSE

One of this thesis's objectives is to estimate the size and views of a sexually active population that is currently not covered by pharmacological prevention schemes for HIV such as TasP, PrEP or PEPSE. TasP is mainly a preventive measure for serodiscordant couples, as treatment of the HIV positive individual, will lower the transmission risk significantly [3]. PrEP is an intervention mainly geared towards key populations that due to specific characteristics, that have a marked higher risk of HIV acquisition, and thus not available for the general population, nor available in every country. [91, 92, 273]. PEPSE is a prevention scheme designed for prevention of HIV acquisition in high-risk sexual exposures (risks that are higher than 1/1000). PEPSE requires the immediate administration of antiretroviral agents in the first 72 hours after a high-risk sexual exposure, to abort HIV acquisition [95, 106].

The population in a low to moderate risk of sexual HIV acquisition has been acknowledged previously in UNAIDS reports, however its size and views have not been studied further [2].

Two separate studies were performed, using an online survey system, in Thailand and in the UK (**Chapter 2**). To assess the size of a population that was in unplanned low to moderate sexual risk for HIV acquisition, populational filters were designed. The

characteristics that the population must have to be classed as unprotected with current pharmacological interventions are: sexual activity within the last three years with at least one person, they self-report as a condom user, they are not PrEP users (in the Thai study) and did not self-report an HIV positive status. The population in question must also have reported events of unplanned sexual exposure to HIV, namely condom failure, "forgetting to use a condom" and "wanting to use a condom but none available". One limitation of these studies was that the possibility a non-UK or non-Thai national participating in the study can't be ruled out. Both studies did not provide information about the number of sexual partners in the last year, as this data can reveal crucial risk scenarios.

A particular limitation of the UK study is the lack of age data, as while the assumption is that many of the participants are representative of a younger population, it is not possible to assess how many participants are mature students (> 25 years old). Additionally, as it is a self-reported study, there is a possibility that the data collected on certain questions, such as HIV status, is dependent on the participant's accurate depiction of events, which can be unreliable.

Both Thai and UK questionnaires had an important focus on nonpharmacological interventions for HIV prevention, which are mainly male condoms, as they remain the most effective way to protect individuals from HIV acquisition in a sexual exposure [184, 192]. However, condom failure events, and low or inconsistent condom use rates have an important impact on the potential sexual transmission of HIV. As reviewed in **Chapter 1**, since 2012, no major progress has been made to lower the worldwide burden of newly diagnosed cases [1]. Scenarios such as inconsistent condom usage, or condom

breakages can lead to low to moderate risk scenarios of sexual HIV acquisition. Such scenarios are not covered by current PEPSE (or other pharmacological intervention), as they often have lower to moderate risks of HIV acquisition, which are between 1/1000 and 1/10000 [95]. In such scenarios often, only medical follow-up, is suggested as no further alternative intervention currently exists. The populations that have a higher risk of HIV acquisition are covered with PrEP, traditional PEPSE or TasP. This situation effectively leaves a population that has an unplanned unprotected sexual risky scenario with no options for prevention, but medical follow up. Under current antiretroviral formulations, the risk to benefit ratios do not favour systemic exposure to the antiviral agents as often they have significant side effects [95].

These unplanned and unprotected scenarios, condom break/failure, “wanting to use a condom but none available” and “forgetting to use a condom” were scenarios that were commonly reported in both studies. Condom failure events were of particular concern within both the UK and the Thai study, as the reported rates of condom breakage are significantly over what has been reported previously [216]. The high rates found in both studies (15% in the Thai study and 14% in the UK study) , in populations with different backgrounds and risk behaviours, are alarming for HIV prevention scenarios. Such a high rate of condom failure events should be explored further, as it could lead to the design of better condom models and better HIV prevention schemes.

Other unplanned unprotected scenarios reported in our study, such as “wanting to use a condom but none available” and “forgetting to use a condom” were also reported in significant numbers. Both scenarios, representative of inconsistent condom use present several limitations for further analysis. Future research into these unplanned risky

events should follow-up these scenarios with questions regarding relationship type (stable, casual, commercial) and whether alcohol and/or drugs were consumed prior to intercourse [218, 222]. Additionally, condom resistance tactics such as “reduced sensitivity” or risk assessment reassurance by a partner, can lead any individual to report inconsistent condom use [220, 221]. Future research into this unplanned inconsistent condom use events will allow to further improve HIV and STDs prevention programmes. An additional hypothesis to be tested in future research relates to the use or abuse of drugs prior to a sexual act. Such hypothesis has been explored in specific populations, such as Black African American heterosexual men, with a correlation with alcohol abuse and a higher risk of HIV acquisition [327]. Expanding this hypothesis to different populations can uncover further gaps in prevention schemes both for substance abuse and sexually transmitted diseases prevention.

In this work unplanned low to moderate risk scenarios for sexual HIV acquisition occurred in a significant population, as in both studies, more than 10% of the total population reported them (29% of the UK study and 15% of the Thai study). While these two studies do not represent every variable found in the world’s population, they do reveal a sizable population that can be potentially exposed to HIV, where no pharmacological intervention is prescribed.

Given the size of these unprotected population, they constitute a priority research interest for the better design of HIV preventions schemes. Currently the ongoing SARS-CoV-2 pandemic has negatively impacted public health prevention programmes, with the full extent of the disruption likely to be felt for years to come [101, 103]. Tackling

this unprotected population in future prevention campaigns is imperative for achieving the 2030 UNAIDS goals and eventually end the HIV/AIDS pandemic.

As the COVID19 pandemic eases the pressure on health services worldwide, emphasizing the use correct use of condoms, and the use of PrEP in high-risk populations is a priority intervention, particularly for key populations [328, 329]. While experimental tools such as PrEP “on demand” and PIP will allow for a more tailored approach, these interventions further rule out a population in unplanned lower to moderate risk scenario for sexual HIV acquisition.

Current prevention campaigns often overlook the potential importance of PEPSE in ending the HIV pandemic [330]. Literature suggests that PEPSE awareness, while improving within the LGBT community, remains a major issue to resolve [331]. PEPSE has many barriers associated to the prescription, including side effects, virological resistance concerns, cost effectiveness of the intervention and the limited timeframe for effectiveness [328, 330]. Current PEPSE agents are significantly more tolerated than the antiviral compounds that were used in the early stages of the HIV pandemic, while such advances are encouraging, patient adherence to PEPSE remains an issue to resolve [328]. Virological resistance concerns in the context of PEPSE are not studied often, as PEPSE continues to be an under prescribed option, however with the current treatment regimens based on tenofovir, emtricitabine and one INI (usually DTG or Bictegravir) such issues should not be encountered often [64-67]. These PEPSE regimes make resistance unlikely, and should it occur viral fitness is likely impacted, additionally the medical evaluations during PEPSE allow for prompt discovery of a breakthrough infection [67, 95, 114, 117]. Cost effectiveness of PEPSE has been positively evaluated

previously for high-risk scenarios, however in resource limited scenarios the cost of current therapies can be a factor in prescription of preventive antiretrovirals [332]. The limited timeframe for PEPSE effectiveness is an immoveable roadblock, as the countdown for HIV integrating its proviral DNA to a target TCD4+ lymphocyte in the lymph nodes, sometimes does not allow for PEPSE to be prescribed at all. Not accessing PEPSE within 72 hours of an exposure to HIV, has been liked to HIV acquisition [328]. Prior to the administration of any pharmacological prevention measure, it is imperative to assess the baseline HIV status of an individual [94]. This would allow for correct follow-up of cases, as cases that are HIV-positive at baseline would not benefit from a PEP or a PREP scheme, but rather a therapy scheme [94].

Two important barriers to HIV prevention in a PEPSE scenario are patient adherence to the treatment, and a misjudged risk perception of an unprotected sexual act by the public [198, 199, 225, 330, 333]. The latter can be tackled with appropriate prevention campaigns focusing on the importance of safe sex and early diagnosis. However patient adherence is a more complex issue. Several factors are associated with less adherence to the PEPSE course, as young people (<25 years of age) are less likely to complete the treatment, probably related to inaccurate risk perception [334]. An opposite trend is observed in older individuals who had intercourse with a sex worker or MSM, which were more likely to complete the treatment scheme [335]. Currently no PEPSE regime is significantly associated with low patient adherence, and low adherence is linked to breakthrough infections [328].

With this context, a targeted localised PEPSE approach for a population whose behaviours place them in an unplanned low to moderate risk scenario for HIV

acquisition could present a solution for many of the aforementioned issues. A drug delivery system that is administered to vaginal or anal tissue can potentially bypass systemic side effects, thus improving the risk to benefit ratio of the administration of PEPSE. Additionally, such a delivery system is favoured as an option by a majority (over 85%) of participants in both studies, should they find themselves in an unplanned lower to moderate risk for HIV acquisition. For such a formulation to succeed, uptake to the paracortex area of lymph nodes is key to success [40]. This formulation, if available would allow for a pharmacological intervention for a population whose size is significant, and whose protection would allow further decrease new cases of HIV worldwide.

4.2.1 Coating an MDTG nanocrystal as a means of generating a PEPSE formulation.

The second aim of this thesis is to develop a nanoformulation that could protect the population that was uncovered with the two survey studies in the UK and in Thailand but is likely found worldwide. Nanoformulations are gaining evidence in different fields, they can possess very favourable side effect profiles, a property that would be key in HIV PEPSE formulations [69]. Additionally, in some cases, nanoformulations can allow for a controlled release of the compound, such as the novel cabotegravir/rilpivirine formulation for treatment and PrEP [68]. This intramuscular nanosuspension was FDA approved for treatment in January 2021. Preliminary data suggests that the PreP intramuscular nanoformulation containing cabotegravir and rilpivirine is superior in preventing HIV acquisition than the traditional oral formulation of tenofovir and emtricitabine. Due to the sustained release of both antiretroviral agents,

superiority versus the traditional scheme and patient acceptability, it can be expected that the role of this formulation in the treatment and PrEP guidelines will become more prominent. However, this formulation has not been tested for PEPSE purposes. Formulating a nanoparticle based PEPSE, should focus on specific characteristics to have a higher chance of success (**Table 17**).

For this project DTG was chosen as a model antiviral compound, due to its safety profile, antiviral efficacy, and high genetic barrier [64-67]. However, DTG had to be chemically modified based on Sillman's illustrative work, to generate a lipophilic prodrug, as lipophilic compounds are more likely to be entrapped nanoaggregates such as the ones generated by nanoprecipitation [169, 252, 253]. Dolutegravir sodium is a salt form of the parent compound DTG. It is one of the generic compounds that has been recently pushed in low to middle income countries in order to cover the treatment needs of people living with HIV [336]. In countries such as Thailand, it has been approved for the treatment of people living with HIV given its similar pharmacokinetic profile when compared to DTG [288]. The modified reaction to generate MDTG, allows for dolutegravir sodium to be used instead of pure DTG, which is expensive and challenging to obtain. To exemplify this, 250mg of pure DTG is priced at £1,552.72 by MERCK, while dolutegravir sodium is valued at $400 from Chemshuttle. Additionally, unlike Sillmnan's reaction, no deprotonating agent was needed, further simplifying the reaction [169]. Therefore, this route of synthesis allows for a resource friendly reaction for MDTG.

MDTG had, in our work, a very similar cytocompatibility profile as to what was described by Sillman et al, as it starts to impact cellular metabolism, in Caco-2 and

RAW 264.7 macrophages, at approximately 100μM [169]. No significant LDH release was recorded in either RAW 264.7 macrophages or Caco-2 cell lines, suggesting that the effect is not related to necrotic events (**Figure 21**). In regard to DTG release from MDTG, our results are similar to what Sillman et al. reported. Sillman et al. reported that in whole mouse blood 79.9% of MDTG gets hydrolysed by carboxylesterases by one hour, thus releasing the parent compound DTG. In our results in rat plasma, a similar profile was observed, as 86% (+/- 12%) of the total MDTG had released DTG into the media. A lipophilic prodrug approach has several advantages as seen in Chapter 3. Overall, for any prodrug approach to work as intended, the chemical linker, whether it is an ester, carbamate or amide, must be hydrolysed in relevant biological tissues, so that the active compound can have the desired effect [275-277]. In MDTG's case, the carboxylesterases found in rat plasma (a lymph surrogate), break the 7 OH' ester bond releasing the parent compound DTG. Due to resource limitations, MDTG's antiviral properties were not tested in our laboratory facilities. Interestingly, in Sillman et al.'s work, MDTG's activity was measured indirectly through an in vitro test focusing on the reverse transcriptase activity [169]. In Sillman's work, there was a sustained inhibition of the reverse transcriptase as by day 30, there was an 83% activity inhibition. Due to the potential usefulness in treatment and prophylaxis of MDTG, further research is needed to confirm these results. With MDTG's lipophilic physiochemical characteristics traditional approaches such as nanoprecipitation could be attempted.\ However many parameters had to be covered for a PEPSE formulation to be successful (**Table 17**).

Table 17. Ideal characteristics for a novel nanoformulated PEPSE.

Characteristics	Reasoning
Non-toxic, biodegradable and biocompatible nanocarrier	Any ideal excipient should not present any significant adverse effects on the individual [337]. This would justify the use of a novel PEPSE under a risk to benefit ratio.
Significant drug content (>25% wt%)	A high drug content allows for a high chance for the developed formulation to saturate the paracortex area of local lymph nodes with the antiretroviral compound [40].
Significant uptake by cellular macrophages or antigen presenting cells.	Dendritic cells, macrophages and other antigen presenting cells are vital for HIV transportation to the lymph nodes[23]. In some cases, HIV can replicate in such cell lines. Therefore, the uptake of the formulation should be significant in these cell lines.
Adequate penetration into the paracortex of the lymph nodes	The paracortex area harbours a vast majority of the TCD4+ lymphocytes. As the TCD4+ lymphocytes are the viral end point of disease, these areas are prime targets for distribution of a PEPSE nanoformulation [40].
The ideal PEPSE formulation should have 4 to 24 hour stability in local tissue	Evidence suggests that HIV viral particles can remain in vaginal tissue for four hours, while in anal tissue they can remain for up to 24 hours [325, 326]. The presence of aggregation before these crucial time points will impact the uptake into relevant cell lines and thus lymphatic tissue.
Sustained release for at least a week	The exact timeline of HIV infection is difficult to determine [23]. In some very sensitive viral detection assays, HIV status can be confirmed within the first week after contact. PEPSE guidelines suggest that the first 72 hours are key to abort an impending HIV infection, as late initiations of PEPSE can lead to HIV acquisition [95, 328]. Therefore, a novel PEPSE formulation should have a sustained release for at least the first week after exposure.
Formulation to be easily scalable and cost effective	Current PEPSE schemes also have a high economic cost associated with them. For any novel PEPSE scheme to have an impact in the HIV pandemic, economic cost should be accessible for resource limited locations.

In order to generate a formulation that had such characteristics, the first step was the selection of non-toxic polymeric nanocarriers. For this project, three polymeric nanocarriers were used, one triblock copolymer in P85 and two diblock copolymers in

MPEG-PLA and PLGA. All three of them present a distinct hydrophobic branch in PLA component for MPEG-PLA and PLGA, and the polypropylene oxide (PPO) in the case of P85. All three of them have a good biocompatibility profile and have been approved by advisory organisms such as the FDA [298, 301]. The first polymer to be chosen for this project was PLGA, as it has a wide range of versatility regarding drug delivery systems, being used as a nanocarrier for both microparticles and nanoparticles formulated via nanoprecipitation [252]. PLGA has been recently used as a nanocarrier for a PrEP formulation generated via a water in oil in water double emulsion. This formulation used emtricitabine a hydrophilic NRTI compound, achieving a relatively high (for the technique) wt% at 5%[168]. However, this formulation was intended as intramuscular, and not to be delivered via vagina or anus.

The second polymer that was selected was the MPEG-PLA. Its two components, PEG and PLA are both polymers that have been considered biocompatible by several regulatory agencies such as the FDA [338, 339]. PLA was the first polymeric nanocarrier described for nanoprecipitation formulations [264]. MPEG-PLA nanoformulations have been described as having favourable sustained release of the compound. One such example of this is Dong and Feng's MPEG-PLA nanoformulation for paclitaxel. In their study there was a relatively slow and sustained release of paclitaxel from the MPEG-PLA formulation, as 95.9% of the total drug content was released in 14 days [269]. Such a characteristic drug release profile would present an opportunity for any nanoformulated PEPSE, as it would cover the necessary period for HIV prevention.

Pluronics are a family of triblock copolymers that have two arms of a PEO flanking a PPO block. In this polymeric family the two branches of PEO act as hydrophilic arms, while the PPO acts as a hydrophobic block [298]. This allows for them to be classified based on their hydrophilic to lipophilic ratios, which has an impact on their physical characteristics as well. In the pluronic family, these triblock copolymers are classified also by their physical state, with L standing for liquid, P for paste and F for flake. P85 was chosen as a potential candidate as it has been linked to TCD4+ cell activation, when used as a delivery for gene therapy [303]. Recent data regarding P85's use as an excipient in vaccine formulations has found that it has been linked to TCD4+ lymphocyte responses and activation by unknown mechanisms, potentially by acting as a toll like receptor agonist, at least in rodent models[303-305]. P85 was not tested as a carrier for nanoprecipitation purposes.

Within all the nanoprecipitation formulations, a maximum drug content was capped at 8.43% wt%, which is in line with published literature [253, 263].Due to the characteristics that MDTG has, it was proposed that a nanocrystal emulsion could be formed via nanoprecipitation and then coated with a minimal polymeric mass. In doing so, a solid drug nanoparticle formulation would be generated, maximizing drug content, as similar strategies have found encouraging results [294, 295]. In our work coated nanocrystal formulations had a significant drug content, all of them above what traditional nanoprecipitation has been reported to achieve. Such a high drug content in a nanoformulation, is rarely reported on traditional methods such as nanoprecipitation [252, 253].

Due to the COVID19 pandemic related restrictions, only one formulation was tested further for cellular biocompatibility and uptake. This formulation, a MDTG nanocrystal coated with 0.22 mg (per vial) of MPEG-PLA, did not significantly alter the cellular metabolism, as per the Presto Blue assay, and was not associated with significant cytosolic LDH release. Encouraging results were also found in the uptake studies, as in both quantitative and qualitative studies, RAW 264.7 cells had internalized particles. A major limitation of these studies is that particle tracking was made through a Cy5 blue labelled polymer, and thus the fate of the antiviral payload may not be the same as the polymeric nanocarrier. A potential way to further analyse the location of the antiviral payload would be through mass spectrometry imaging [40]. While this method is based on *ex-vivo* drug measurements, it can provide evidence regarding the localization of the prodrug and parent drug within the lymph node. This is crucial information as TCD4+ cells are the endpoint of both HIV and a novel PEPSE formulation, thus saturating the paracortex area of a lymph node is critical for success.

This MPEG-PLA formulation did not present stability under high pressure environments such as cell culture media (DMEM), as visible aggregates were seen shortly after spiking the formulation. This result is not encouraging for the development of a localised PEPSE, as both vaginal and anal tissues have a mucous layer that could destabilize the formulation in a similar fashion to cell culture media [121, 124, 147].

The P85 formulation on the other hand did have stability over 24 hours in cell culture media, as measured by DLS. Stability in situ of the formulation is a desirable result, as evidence suggests that HIV-like particles can be localised 4 hours in vaginal tissue and for up to 24 hours in anal tissue [325, 326]. Pluronics are not inert polymers, as some,

namely those with a low hydrophilic to lipophilic balance (like L61), can have toxic consequences to cells [340]. In terms of P85, Kabanov *et.al* reported that it can present a selective cytotoxic effect in multidrug resistant breast cancer cells via a mitochondrial disruption that leads to the generation of reactive species of oxygen [341]. In terms of enzymatic degradation, there is little evidence for the generation of toxic by-products of Pluronics [298, 341]. To further assess biodistribution of Pluronic co-polymers, Arranja et. al radiolabelled two Pluronics (namely F127 and P94) and followed their behaviour via SPECT-CT in Wistar rats. The results of such experiment suggest that all Pluronics experience high uptake by the liver via the reticuloendothelial system during the first 24 hours after IV administration through the tail central vein. This work concluded that the elimination rate of Pluronics via renal filtration, is heavily dependent on the molecular weight of the hydrophilic block, PEO. Pluronics with a higher molecular weight of PEO will experience a delayed renal clearance after a single IV administration, when compared to Pluronics with lower PEO chains[342].

All carriers show fast uptake in the organs from the reticuloendothelial system followed by a steady elimination through the hepatobiliary tract and renal filtration. The PEO block length affects the initial renal clearance of the compounds and the overall liver uptake.

The P85 formulation created did not have a controlled and sustained release, as all of the prodrug payload was converted before 2 hours in a lymph surrogate (rat plasma). This suggests that the coating is vulnerable to enzymes found in plasma, and thus the formulation does not achieve all the required characteristics for delivery to lymph nodes in a PEPSE scenario.

PEPSE prescriptions must be initiated during the first 72 hours after a high-risk intercourse[95, 328]. These 72 hours are key for PEPSE success, thus securing adequate distribution into the paracortex areas of the local lymph nodes is paramount for success in that timeframe [40]. Current estimations suggest within the first 54 hours, daughter virions of HIV are generated within an infected cell [170]. By the end of first week after exposure, the most sensitive diagnostic tests can detect viral RNA of HIV in blood. This suggests that there is a difference between HIV's timeline of infection between the local site and the systemic circulation [23]. Therefore, any localised nanoformulation of PEPSE should maintain sustained release for at least 72 hours locally, as the intention is to provide a one dose formulation for HIV prevention. This formulation did not achieve this goal.

While localised therapy can potentially solve the issue of patient adherence to PEPSE, this can also be tackled through long-acting antiretroviral formulations. In Sillman et. al.'s illustrative work such a formulation was generated using MDTG. This formulation, with a significant drug content, 82.2 ± 4.4% encapsulation efficiency, was administered intramuscularly to non-human primates and male Balb/cJ mice. In both species, this formulation achieved significant concentrations, over the IC-90 of DTG (64ng/ml), for 8 weeks in Balb/cJ mice and for 35 days in non-human primates [169]. While intended as a formulation for treatment or PrEP, this formulation could prove useful in a PEPSE situation such as the drug release profile, however certain questions remain for it to be successful in this scenario. Sillman et al. did not measure the localization of their formulation within the lymph nodes, this information is paramount for aborting an impending HIV infection. For a novel PEPSE to succeed in generating an option for the

population in unplanned lower to moderate sexual risk scenarios, besides the nanoformulation effectiveness, cost effective measures are needed.

Several potential hypotheses could explain the coated formulation's failure. An initial hypothesis would lie in an incomplete/inconsistent coating of the MDTG nanocrystal. Sillman *et. al*'s formulation was generated with a high-pressure/high-energy system that generated a uniform coating, in contrast with the low energy coating via solvent displacement method that was used in this thesis [169]. Sillman *et al.*'s formulation had approximately a 1:10 polymer to drug ratio, while the formulation that is described in this thesis has a 1:16 polymer to drug ratio. It is likely that the combination of a higher polymer mass and a high energy setting allowed for the generation of a more stable coating. A second hypothesis is that Pluronics have a characteristic that can be exploited for prolonged release, as when in sufficient concentration they can form a thermoreversible gel (meaning it will revert back when temperature is decreased) at body 37°C [343]. It is possible that a higher concentration of P85 would allow for both a more stable coating, and a long and prolonged release via the formation of a thermoreversible gel at body temperature.

A third hypothesis would lie in the molecular composition of the Pluronic used. Sillman et al.'s formulation used F127 as the polymer, while the formulation described in this thesis used was P85. F127 is a polymer that has a tendency to self-assemble into micelles in aqueous environments and thus is often used as a surfactant, this characteristic could also have helped in the coating process (**Figure 32**) [298].

4.3.1 Future work

There are several aspects to improve the formulation developed. The first characteristic would be to improve the drug release profile of the formulation. In order to do so, a potential solution would be to experiment with varying masses of P85 coating, as this was the coating that could stabilize the formulation further in plasma or lymph [294].

A different approach to solving this issue can be found in using a different Pluronic. Pluronic polymers are classed depending on their PEG and PPO branches, as they give them different properties. A potential polymeric nanocarrier to be used is Pluronic F127 (F127) sometimes known as poloxamer 407. This was the main excipient for Sillman et al's formulation and thus presents a favourable option as it is considered to be an inactive ingredient for different types of formulations, including rectal [169]. Pluronics have different critical micellar concentrations (CMC), based on the PEO content they have. Due to the relatively high PEO content that F127 (**Figure 32**) has, its CMC value is relatively low at 0.26-0.8 weight per volume percentage. Finding out a correct coating mass with this Pluronic can present a research opportunity, that would allow for a cost-efficient formulation.

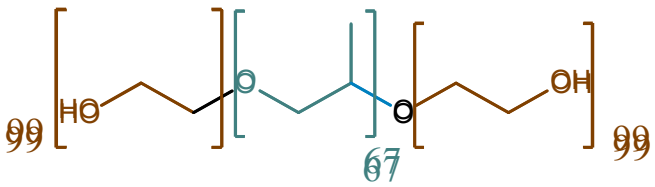


Figure 32. Chemical structure of F127. F127 is a triblock copolymer that has been used to solubilize hydrophobic agents in micelles, and it is the main excipient used in Sillman et al's formulation[169, 298]. Further exploration of this triblock copolymer would present a research opportunity for coated nanocrystals MDTG formulations.

In vitro models of anal tissue for delivery usually do not account for many issues found in the local tissue. The intestinal mucus, for example is a significant barrier to any nanoformulation delivered to the rectal mucosa. The gastrointestinal mucus is comprised of glycoproteins that forms a first barrier for the absorption of compounds. This mucus has a known permeability for negatively charged particulates, which have a tendency to diffuse up to 20 times quicker than positively charged particulates. Unlike the cervicovaginal mucus, the gastrointestinal mucus experiences no cyclical changes and thus has a more heterogenous permeability [163].The gastrointestinal mucus, has also been reported to be more permeable to specific drug delivery systems, as highly hydrophobic polystyrene based nano-particles have a tendency to aggregate in the mucus layer, and thus not be uptaken by cells [300]. Other more hydrophilic drug delivery systems like ones based on PEG-PLA have significantly better penetration into mucus when compared to polystyrene nanoparticles [300]. In terms of size particles sized up to 500 nm in size have been known to cross it [162].

A feature that is difficult to replicate in vitro are Peyer's patches, which are a form of specialized gut associated lymphatic tissue [154]. The epithelium of Peyer's patches is significantly different from the epithelium found in the rest of the intestinal tissue, as it is mainly comprised of specialized M Cells. M Cells serve the purpose of uptaking antigens in transit and bring them into contact with a relevant antigen presenting cell, such as a dendritic cell [156, 318]. M-cells have been shown to be a vital component to Peyer's Patches, as even if they comprise only 5% of the total gut epithelium, they significantly contribute to uptake of nanoparticles and antigens[344, 345]. A model *in vitro* culture was developed by Schimpel et al, which includes mucus secreting goblet

cells, M cells and Caco-2 cells. Schimpel et al's co culture mimics two vital characteristics for nanoparticle uptake: M Cells and the mucus barrier[344]. While there is no antigen presenting cell that would mimic the lymphatic pathway, the model developed could effectively represent the initial route that the formulation would traverse after a rectal administration. Uptake experiments on this triple co-culture model would provide a lot of insight into the formulation that has been developed, as it is assumed that the formulation should follow HIV's route to the lymph nodes in order to be effective. It is very likely that for the full understanding of the uptake mechanisms, two different experiments should be performed, one focusing on the fate of the polymeric carrier, and the other focusing on the fate of the drug payload. Current data on the coated nanocrystals formulation, only has insights on the fate of the polymeric nanocarrier.

Another in vitro model that would allow for important information to be gathered would be the dendritic cell line DCC 2.3. While this cell line is a rodent cell line, the information gathered would be very revealing in regards to biocompatibility and uptake of the formulation. The main caveat that this cell line has, is the short passage stability that it displays, as per manufacturer's instructions it can only be used reliably between the 4-10 passages.

The distribution of the formulation within the lymph node is also of significance, as a successful formulation will target the paracortex area of a lymph node [40]. To confirm this location, mass spectrometry imaging presents a unique opportunity, as it has been reported to quantify drug and metabolites in a system. Ntshangase et al performed an illustrative example of this technique localizing elivitegravir in different areas of a rat

brain. Elvitegravir belongs to the same antiretroviral family as DTG, and thus the same proof of concept can apply in a lymph node. To further this data, a combination of mass spectrometry imaging with immunohistochemistry can allow for confirming the internalization of the formulation within the paracortex area. A similar, proof of concept work was performed by Thomson et al, as they colocalised the distribution of different antiretroviral agents with HIV viral RNA and TCD3+ cell linages in non-human primates, HIV+ humanized mice and HIV positive patients 98. The aforementioned experimental set-ups can improve the properties of the formulation and give significant insight into the biocompatibility and distribution of the formulation inside a lymph node.

4.4.1 Conclusions

The size of a population that finds themselves in unplanned lower to moderate risk for sexual HIV acquisition is staggering, being between 15-29% in two survey studies with different populations. Targeting this population in future pharmacological prevention schemes is needed to reach the strict UNAIDS 2030 goals.
DTG is a model compound for HIV prevention, however its physiochemical properties are obstacles for generating a targeted formulation towards the lymph nodes. To combat this issue, a prodrug approach was taken to attach a 14-carbon chain producing MDTG, thus changing physiochemical properties. Due to the lipophilic characteristics of MDTG a nanocrystal was developed via a solvent displacement method. This process generated formulations with significant drug content. The formulations developed were resource friendly, however they were either not stable in strong ionic environments, or had a quick uncontrolled release, as the entire payload was released in less than 2 hours.

For any localised PEPSE formulation to have a high chance of success, it needs a controlled release of the antiviral payload, with sustained release for approximately a week. This would have a higher chance to saturate local lymph nodes and abort impending infection. The formulations developed did not achieve this goal. Further research is needed to develop a localised PEPSE that targets the local lymph nodes. Such formulation would cover the needs of a sizeable population.

4.5.1 COVID19 impact statement

As the UK SARS-CoV2 cases were rising in the UK in mid-March 2020, the University of Nottingham was advised to close for research purposes that were not deemed essential. This situation did not allow for any laboratory-based data from the period of March to August 2020. As the UK lockdown was announced, cellular metabolism and uptake studies were being performed. Due to the restrictions they had to be cancelled until further notice.

In August 2020 when the research facilities opened again, they opened with important limitations such as a maximum number of researchers per laboratory room. A booking system was mandatory for all experimental set ups, which did not account for failed experiments or other users needing the laboratory space/equipment. Additionally,not all of the research facilities had the same restrictions nor opened at the same time, and due to this facts most experimental data plans had to experience heavy corrections and adaptations. In the case of this project, experimental plans for cellular uptake and biocompatibility data on a dendritic cell line, DC2.4. had to be cancelled completely. The main reasons for the cancellation of the dendritic cell line experiments were related

to the low passage number where the cell line is representative for experimental data and low cell laboratory availability.

During the early stages of reopening of facilities, different facilities had different containment protocols, thus making the work in between buildings difficult. An example of such conditions were the Working hours were limited to 8 am to 4:15 pm thus limiting certain experimental set ups where certain time points were needed such as cellular uptake experiments or drug release studies.

In the case of the Thai online study, the recruitment was severely impacted by the COVID19 pandemic. This situation paralyzed our Thai collaborators, and with none of the UK research team being fluent in Thai it was for both situations to resolve before we could continue recruiting participants.

Some of the COVID19 pandemic's negative impact was mitigated by prioritizing experimental data, such as the stability of the formulations in strong ionic environments or the drug release of the formulation, however the impact of the pandemic was profound on this project.

Supplemental information

Questionnaires and consent statements for the UK and Thailand.

To note is that if a participant does not accept all the 6 ethics statements, their answers will not be recorded, and the survey progression will be closed in either of the survey studies. The UK questionnaire was written in English, while the questionnaire in Thailand was written in Thai language.

A) UK research statement, questionnaire, and ethics statements:

Survey on Sexual Health and HIV prevention

Conducted by: School of Pharmacy, University of Nottingham, UK

Thank you for your interest in this exciting research project which aims to collect information about the sexual practices of individuals in order to develop better prevention protocols for HIV. The information we collect will contribute to the development of a more detailed research project on sexual health and practices.

The questionnaire should take no more than 5 minutes to complete. Please feel free not to answer any questions you are uncomfortable with. All the information collected will be kept confidential and anonymous, and you will not be identified in any way.

We are very grateful for your important contribution to this research. Thank you for your time.

Research team:

Dr. Pavel Gershkovich, School of Pharmacy, pazpg@exmail.nottingham.ac.uk

Dr. Carlos Sanders-Velez, School of Pharmacy, paxcs6@exmail.nottingham.ac.uk

Prof. Andrew Kam-Tuck Yip, School of Sociology & Social Policy, andrew.yip@nottingham.ac.uk

Please indicate your consent to take part in this survey by ticking all the following statements:

- ☐ 1) I am over 18 and I voluntarily agree to take part in the study.
- ☐ 2) I give the investigators permission to use the results of my participation in the study anonymously and confidentially.
- ☐ 3) I understand that I can ask for further instructions or explanations at any time by contacting the research team.
- ☐ 4) I understand that I do not have to answer every question.
- ☐ 5) I understand that I am free to withdraw at any time by simply clicking X on the browser screen and my data shall not be uploaded.
- ☐ 6) Data will only be uploaded after successful completion of the questionnaire when I have clicked the 'Finish' button.

7) You are:
   - o Male
   - o Female
   - o Trans Male
   - o Trans Female
   - o Other. Please state: _____

8) How do you define your sexuality?
   - o Heterosexual
   - o Gay /Homosexual/Queer
   - o Lesbian
   - o Bisexual
   - o Asexual
   - o I prefer not to define my sexuality
   - o Other. Please state: _____

9) Have you had any sexual contact with at least one person in the last three years
   - o Yes Please answer **Question 10**
   - o No Please do to **Question 11**

10) When you have engaged in sexual activities with others, which of the following are

    relevant to you? Please tick **all** that apply (if any).

   - o Perform penetrative anal intercourse
   - o Receive anal intercourse
   - o Perform penetrative vaginal intercourse
   - o Receive vaginal intercourse

- Other activities. Please state: _____

11) Do you use condoms during sexual activities?
    - Always
    - Never
    - Most of the time
    - Sometimes/seldom

12) If you have had unprotected penetrative sex (no condom/damaged condom) at least once in the last three years, which of these have applied? Please tick **all** that apply.
    - You forgot to wear a condom
    - The condom broke during a sexual intercourse
    - Your partner and you mutually consented to not wearing a condom
    - You wanted to use a condom but it was not available
    - You do not regularly use condoms
    - Not applicable

    If any of the previously described scenarios in **question 12** apply to you, please answer the following questions:
    12 (a) how many times have these happened to you in the past three years?
    _____

    12 (b) What did you do in any of these scenarios? Please tick all that apply.
    - You went to the GP and the GP suggested that you get tested for HIV and other STDs.
    - You went to the GP and the GP diagnosed an STD and suggested treatment.
    - You did not get any professional advice but did go for testing for HIV and other STDs.
    - You did not get any professional advice.

13) We are interested in developing a medication that could be inserted into the vagina or the anus, after a potential exposure to HIV, in order to prevent infection. This new medication could be used up to after 72 hours after intercourse.
    Would you be interested in using such a medication if you found yourself in such a situation?
    - Yes
    - No

14) Have you been tested for HIV in the past year? If so, what was the result?

- Yes I have been tested. It was negative.
- Yes I have been tested. It was positive.
- Yes I have been tested. I do not remember the result.
- No I have not been tested.

Please now ensure that you click the 'Finish' button to submit your answers.

Thank you for participating in this survey!

B) Thai research statement, questionnaire, and ethics statements, translated to English language:

Survey on Sexual Health and HIV prevention

Conducted by: School of Pharmacy, University of Nottingham, UK

Thank you for your interest in this exciting research project which aims to collect information about the sexual practices of individuals in order to develop better prevention protocols for HIV. The information we collect will contribute to the development of a more detailed research project on sexual health and practices.

The questionnaire should take no more than 5 minutes to complete. Please feel free not to answer any questions you are uncomfortable with. All the information collected will be kept confidential and anonymous, and you will not be identified in any way.

We are very grateful for your important contribution to this research. Thank you for your time.

Research team:

Prof. Emeritus Praphan Phanuphak, Thai Red Cross AIDS Research Centre, ppraphan@chula.ac.th

Dr. Nittaya Phanuphak Pungapapong, Thai Red Cross AIDS Research Centre, nittaya.p@prevention-trcarc.org

Dr. Reshmie Ramautarsing, Thai Red Cross AIDS Research Centre, reshmie@prevention-trcarc.org

Dr. Tanat Chinbunchorn, Thai Red Cross AIDS Research Centre, tanat.c@prevention-trcarc.org

Dr. Pavel Gershkovich, School of Pharmacy, pazpg@exmail.nottingham.ac.uk

Dr. Carlos Sanders-Velez, School of Pharmacy, paxcs6@exmail.nottingham.ac.uk

Prof. Andrew Kam-Tuck Yip, School of Sociology & Social Policy, andrew.yip@nottingham.ac.uk

Please indicate your consent to take part in this survey by ticking all the following statements

Please indicate your consent to take part in this survey by selecting your answer to statements 1 - 6.

- ☐ *1) I am over 15 and I voluntarily agree to take part in the study.*
- ☐ 2) I give the investigators permission to use the results of my participation in the study anonymously and confidentially.
- ☐ 3) I understand that I can ask for further instructions or explanations at any time by contacting the research team.
- ☐ 4) I understand that I do not have to answer every question.
- ☐ 5) I understand that I am free to withdraw at any time by simply clicking X on the browser screen and my data shall not be uploaded.
- ☐ 6) Data will only be uploaded after successful completion of the questionnaire when I have clicked the 'Finish' button.

7) You are:
   - o Male
   - o Female
   - o Trans Male
   - o Trans Female
   - o Other. Please state: _____

8) How old are you?

____

9) How do you define your sexuality?
   - o Heterosexual
   - o Gay /Homosexual/Queer
   - o Lesbian
   - o Bisexual
   - o Asexual
   - o I prefer not to define my sexuality
   - o Other. Please state: _____

10) Have you had any sexual contact with at least one person in the last three years?
   - o Yes Please go to **Question 11**
   - o No Please go to **Question 12**
   - o I don't know Please go to **Question 12**

11) When you have engaged in sexual activities with others, which of the following are

relevant to you? Please select/tick **all** that apply (if any).

- Perform penetrative anal intercourse
- Receive anal intercourse
- Perform penetrative vaginal intercourse
- Receive vaginal intercourse
- Other activities. Please state: _____

12) Have you heard about PrEP (A pill that prevents HIV)?

- Yes I have

- No I have not

13) Are you currently on PrEP ?

- Yes I am

- No I am not

14) Have you taken PEP before (Pills to prevent getting HIV AFTER having sex)?

- Yes I have

- No I have not

- I have never heard about PEP before

15) Do you use condoms during sexual activities?

- Always
- Never
- Most of the time
- Sometimes/seldom

16) If you have had unprotected penetrative sex (no condom/damaged condom) at least once in the last three years, which of these have applied? Please select/tick **all** that apply.

- You forgot to wear a condom
- The condom broke during a sexual intercourse
- Your partner and you mutually consented to not wearing a condom
- You wanted to use a condom but it was not available
- You do not regularly use condoms
- Not applicable

If any of the previously described scenarios in **question 16** apply to you, please answer the following questions:

16 (a) how many times have these happened to you in the past three years?

_____

16 (b) What did you do in any of these scenarios? Please select/tick all that apply.

- You went to a healthcare professional and they suggested that you get tested for HIV and other STDs.
- You went to a healthcare professional and they diagnosed an STD and suggested treatment.

- You did not get any professional advice but did go for testing for HIV and other STDs.
- You did not get any professional advice and did not get tested for HIV and other STDs.

17) We are interested in developing a medication that could be inserted into the vagina or the anus, after a potential exposure to HIV, in order to prevent infection. This new medication could be used up to after 72 hours after intercourse.
Would you be interested in using such a medication if you found yourself in such a situation?
    - Yes
    - No

18) Have you been tested for HIV in the past year? If so, what was the result?
    - Yes I have been tested. It was negative.
    - Yes I have been tested. It was positive.
    - Yes I have been tested. I do not remember the result.
    - No I have not been tested.

Please now ensure that you click the 'Finish' button to submit your answers.

Thank you for participating in this survey!

Logistic regression vs Linear regression

Different types of mathematical regressions are usually performed for the correct interpretation of data. We used a multiple logistic regression model with a logit link function to analyse and understand the subpopulation that exhibits certain behaviours concerning condoms when carrying out sexual activities.

The most mathematically used regression, is a linear regression, such as a calibration curve. In a linear regression, a line is fitted through the known "X" and "Y" and the equation that produces this line is used to predict the unknown variable (**Figure A**) [346].

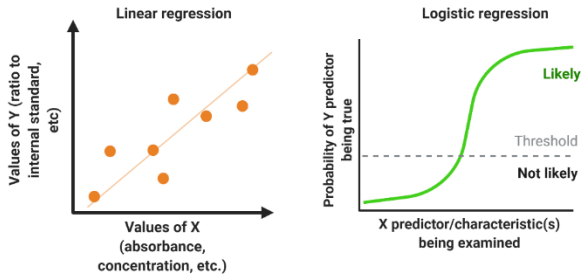


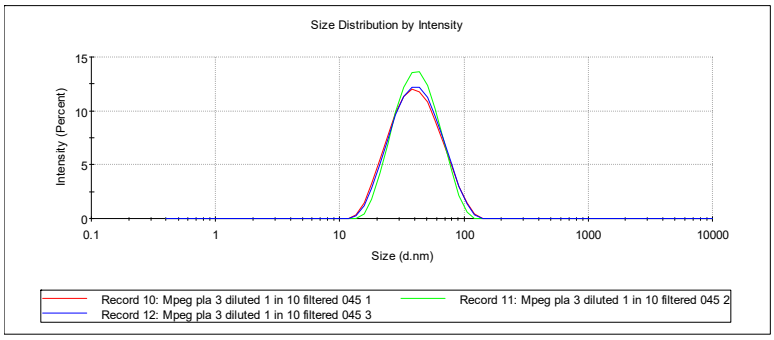
Figure A): Comparison of a logistic regression and a linear regression. A linear regression is most used when trying to predict the values of X or Y from a given data set. A common example of a linear regression is an HPLC calibration curve. A logistic regression on the other hand attempts to predict the probability of a given outcome or characteristic being present, represented on the Y axis as 0 or 1. Characteristics or predictors are plotted on the X axis, and a logistic curve is fitted on the data set. This allows for a mathematical threshold to be set and thus predict the possibility of a predictor/characteristic being associated with the outcome of interest.

A logistic regression is a mathematical model that most commonly tries to predict an outcome (in this case a behaviour or characteristic) based on fitting a sigmoidal line based on a logistic equation using the problem data set. In this scenario, the "X" axis stands for the characteristics/behaviours (also known as predictors) analysed in the problem data set and the "Y" axis for the behaviour/characteristic of interest. The line is fitted to assign the characteristic of interest after a threshold is met. In summary, rather than predicting a specific value of "X" or "Y", a logistic regression is based on the relationship of association that predictors have on a problem characteristic or behaviour [347].

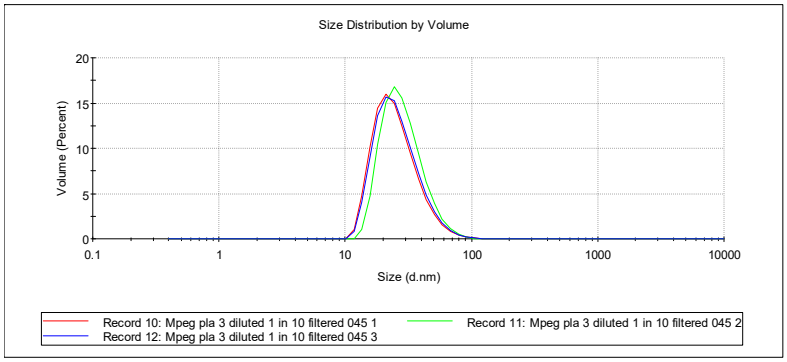
Dynamic Light Scattering data (Intensity, Number, Volume and Correlogram) of relevant formulations produced.

A) mPEG-PLA /Acetone/ Acetone to water ratio 1:2

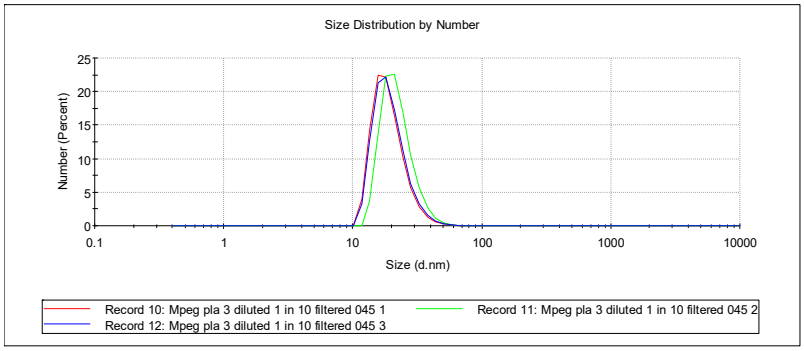
Size by intensity



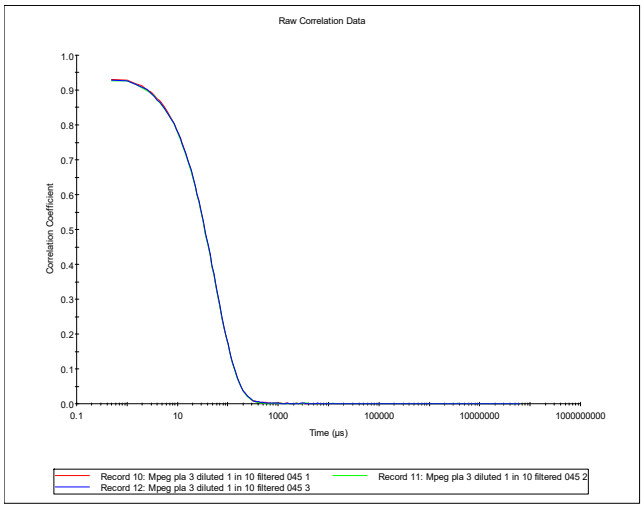
Size by volume



Size by Number

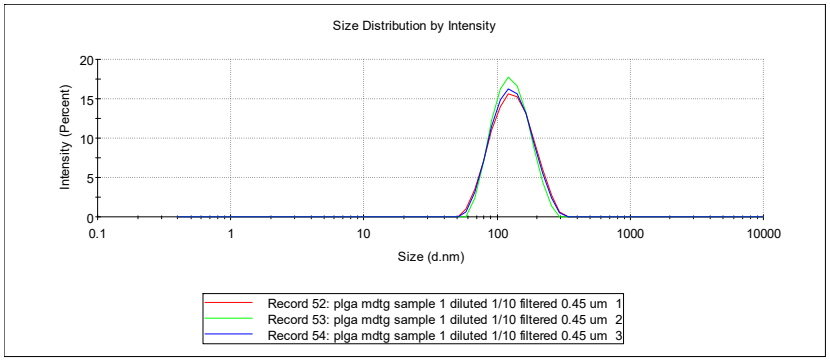


Correlogram

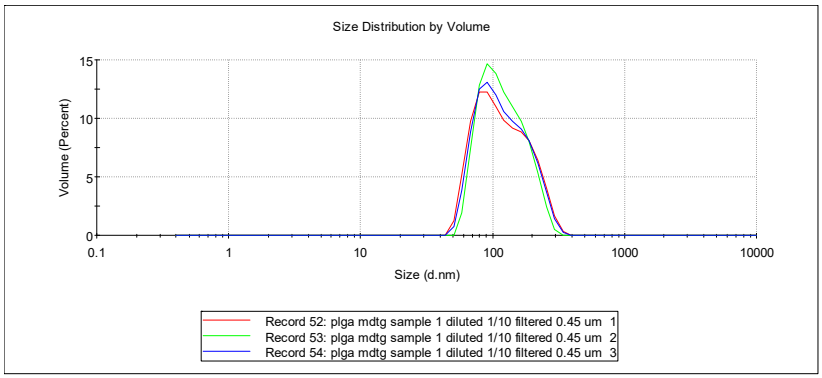


B) PLGA Acetone/Acetone to water ratio 1:2

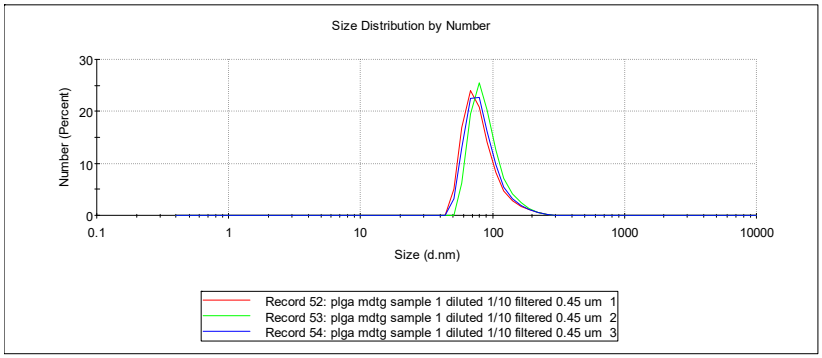
Size by intensity



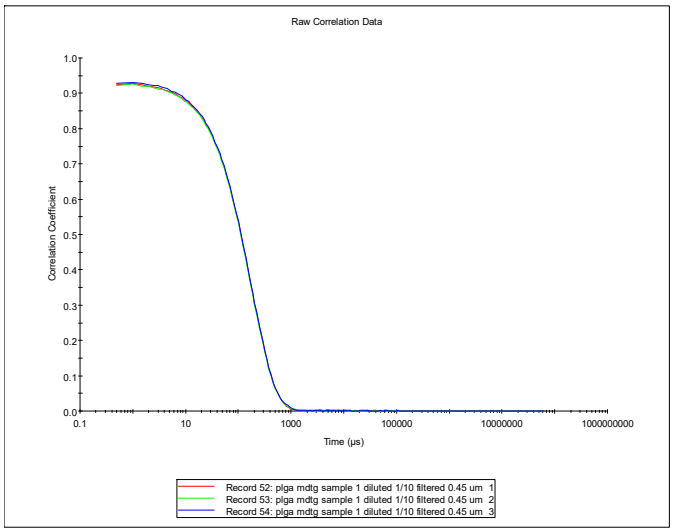
Size by volume



Size by number

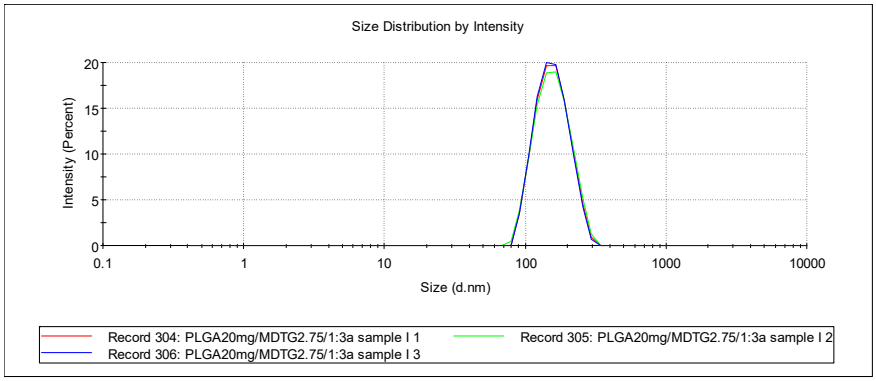


Correlogram

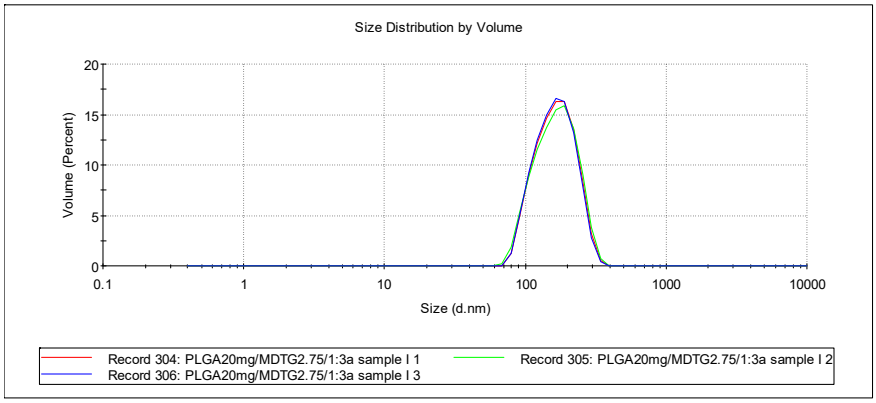


C) PLGA /Acetone/Acetone to water ratio 1:3

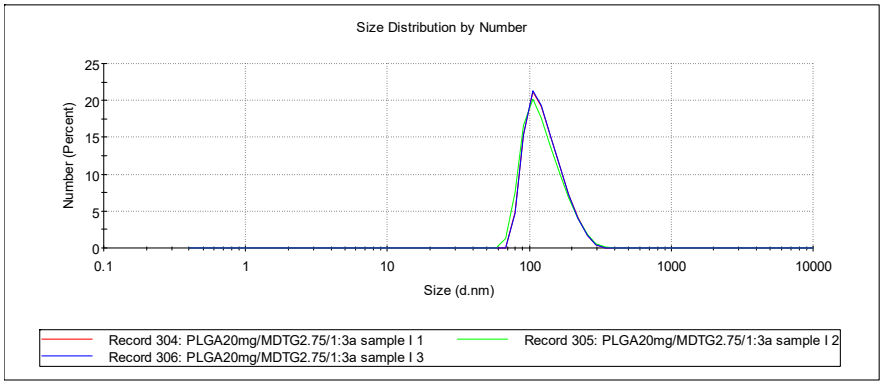
Size by intensity



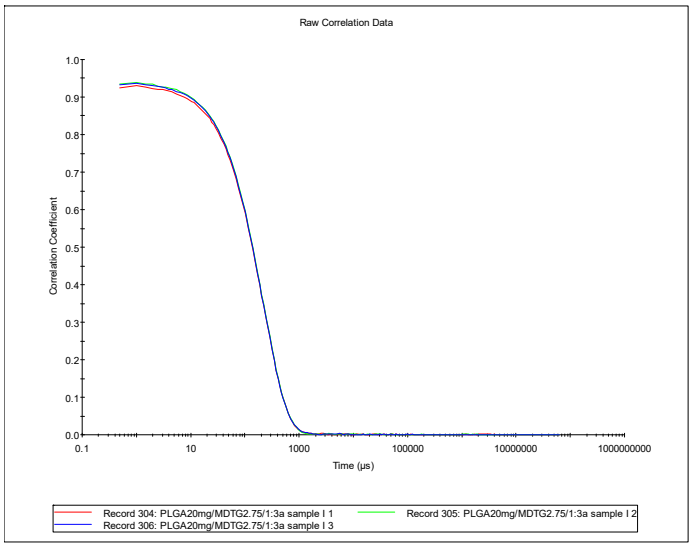
Size by volume



Size by number

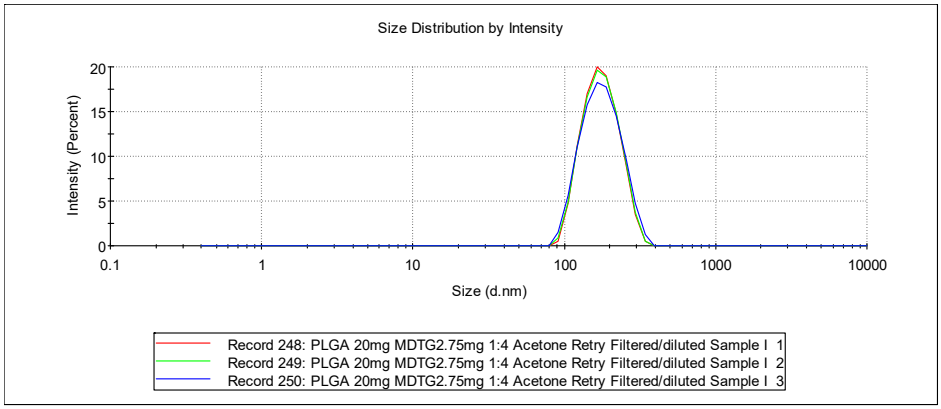


Correlogram

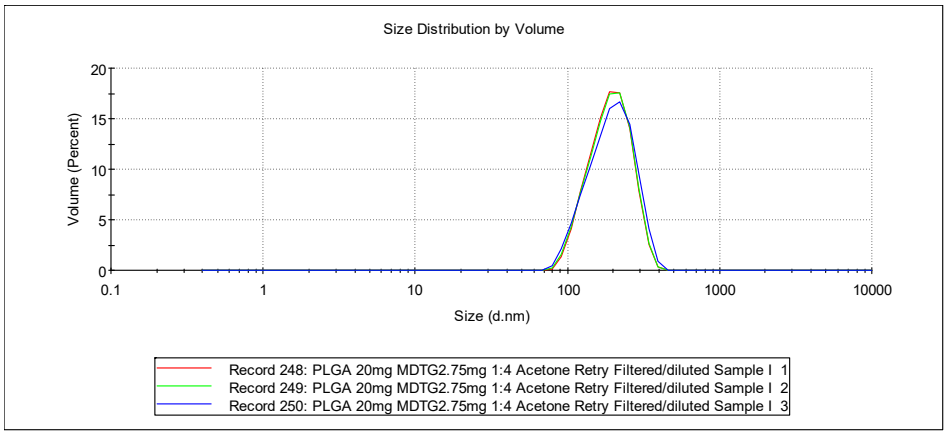


D) PLGA/Acetone/Acetone to water ratio 1:4

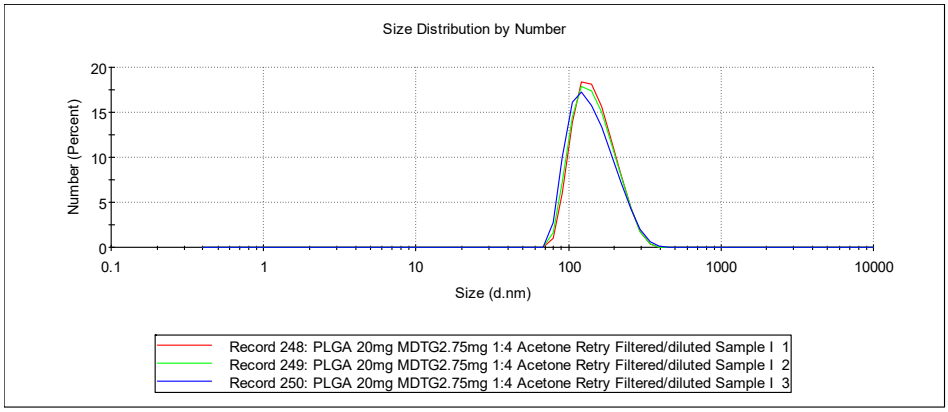
Size by intensity



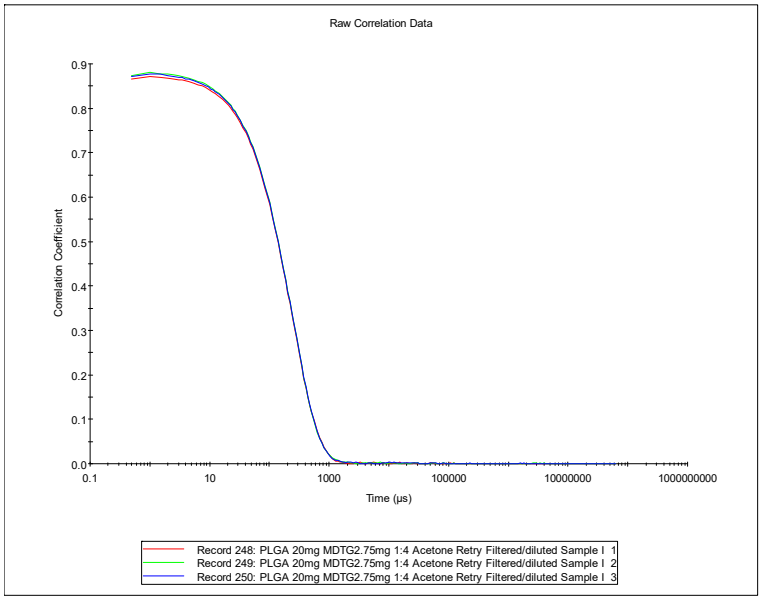
Size by volume



Size by number

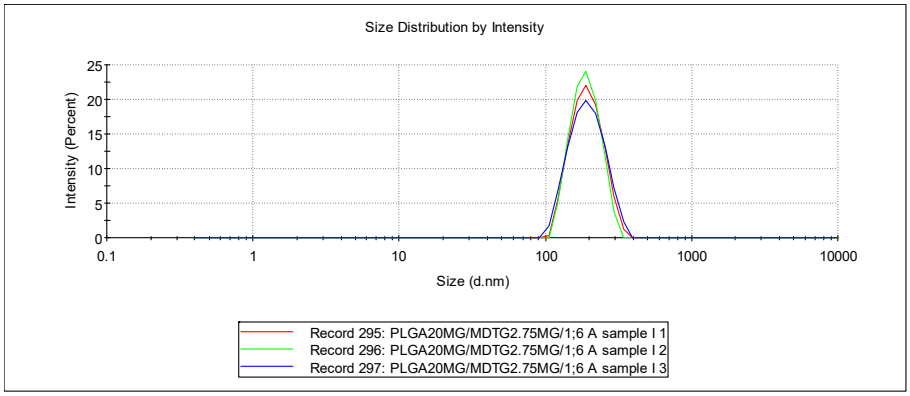


Correlogram

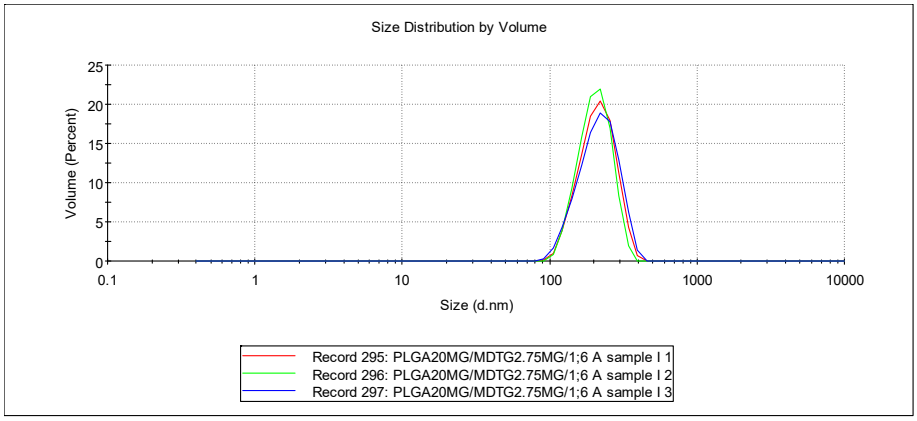


E) PLGA/Acetone/Acetone to water ratio 1:6

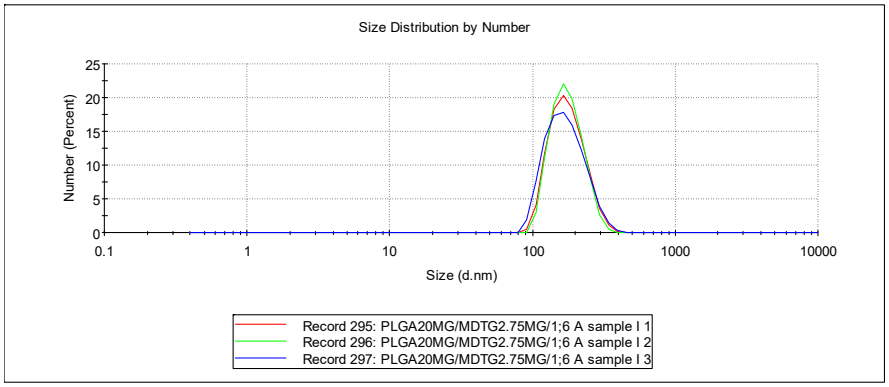
Size by intensity



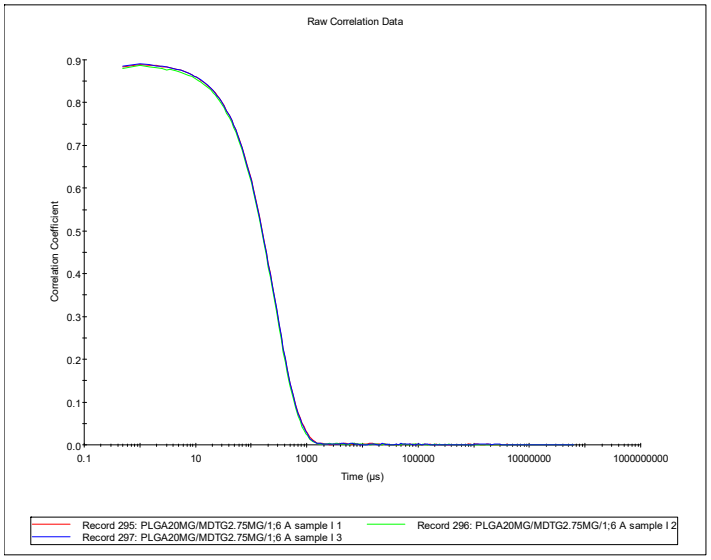
Size by volume



Size by number

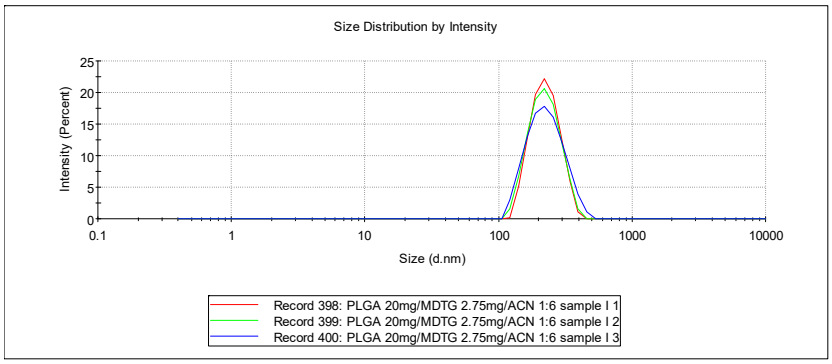


Correlogram

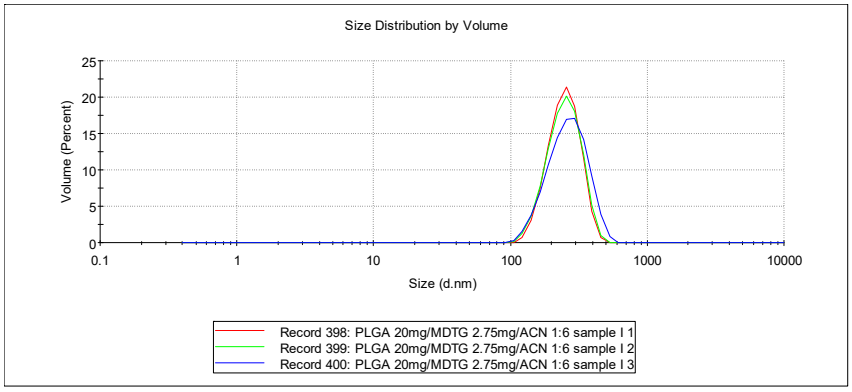


F) PLGA/ACN/ACN to water ratio 1:6

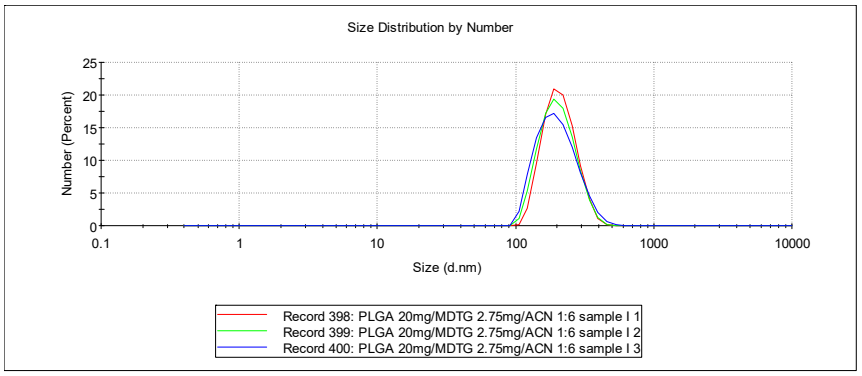
Size by intensity



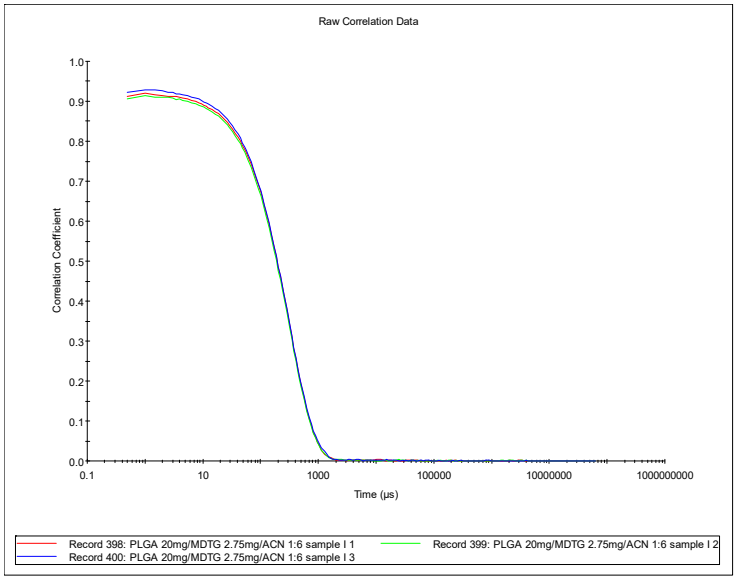
Size by volume



Size by number

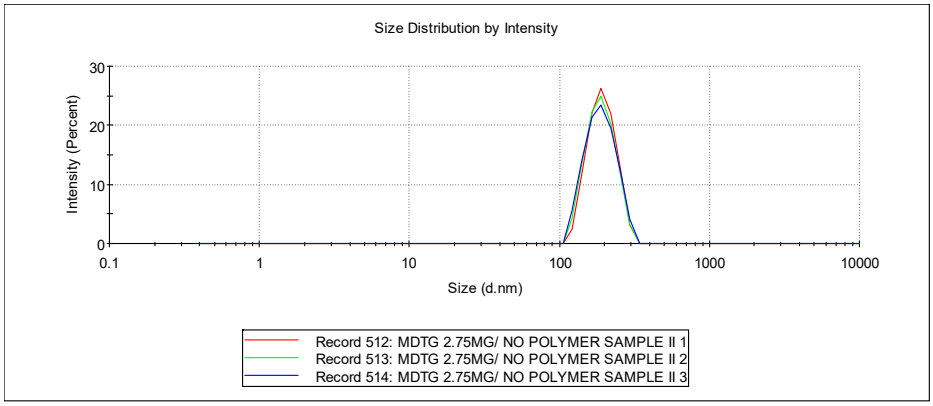


Correlogram

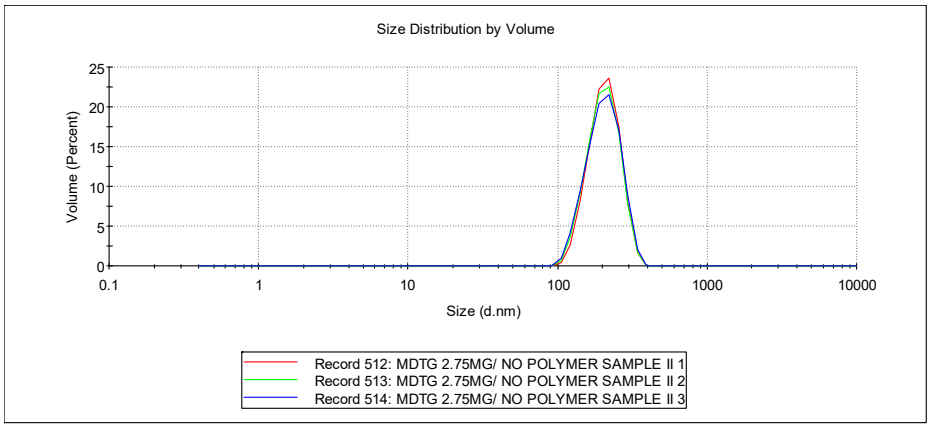


G) MDTG nanocrystal

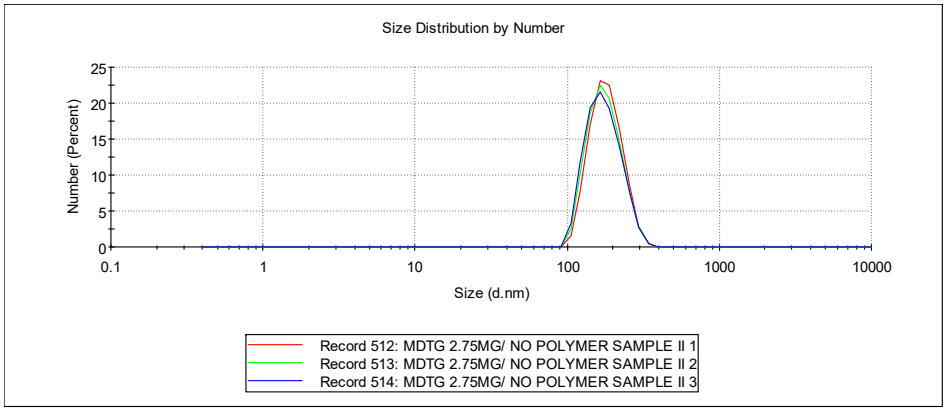
Size by intensity



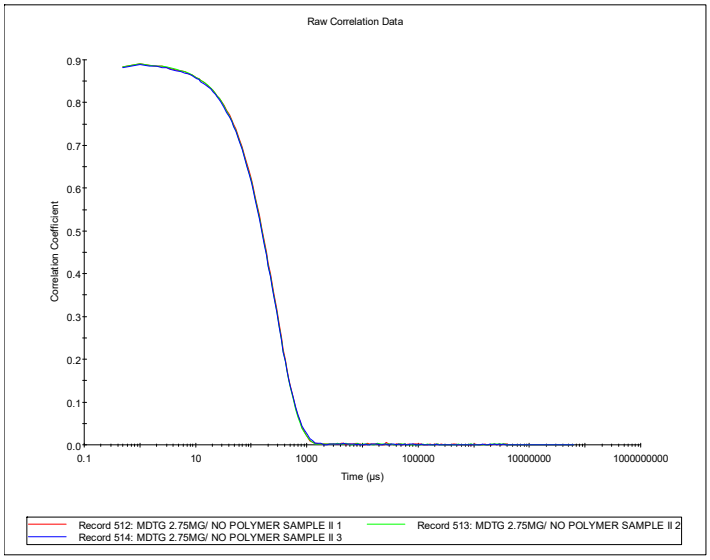
Size by volume



Size by number

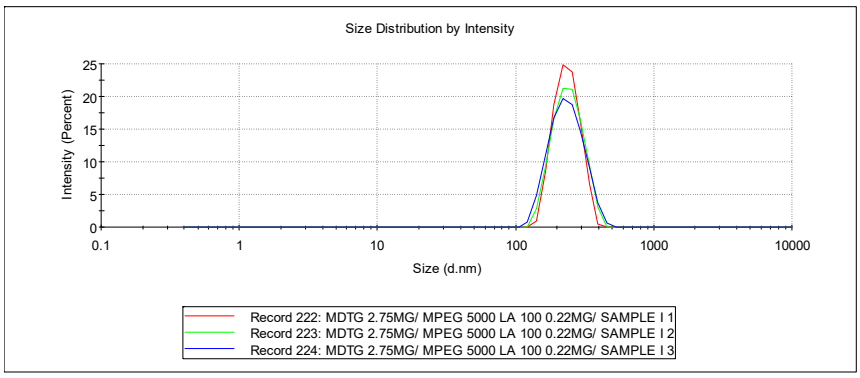


Correlogram

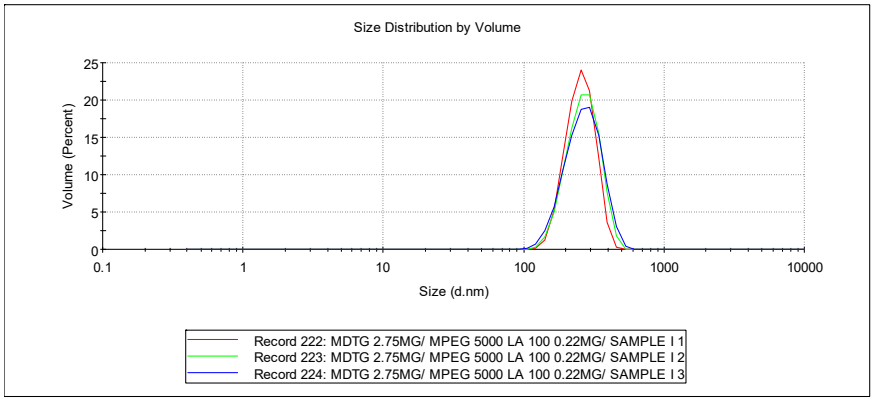


H) mPEG-PLA 0.22mg coating

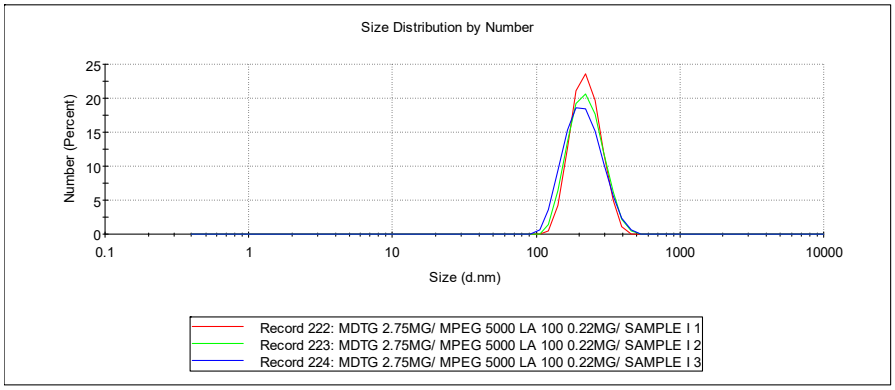
Size by intensity



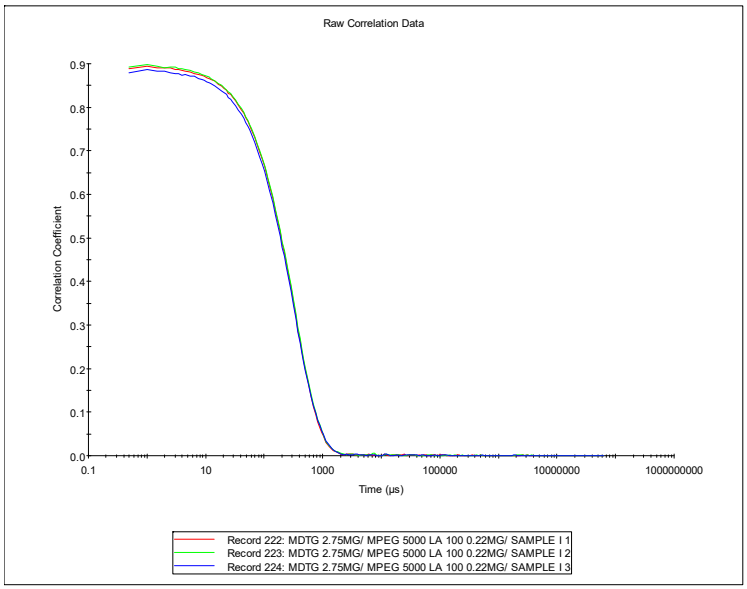
Size by volume



Size by numbers

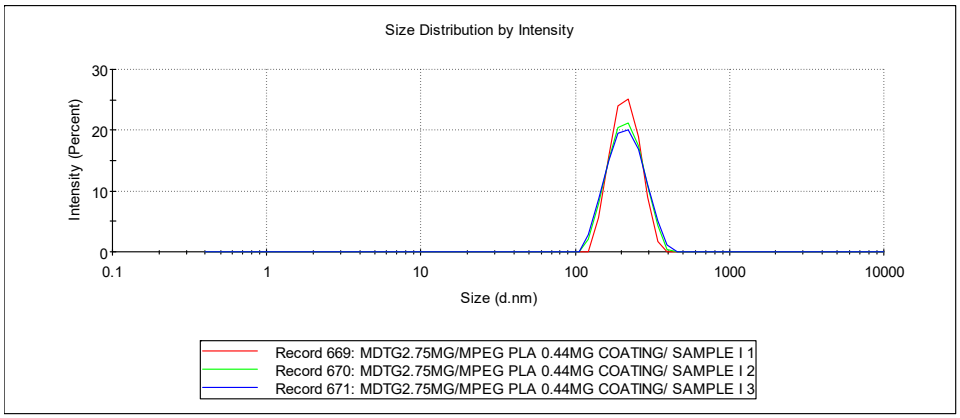


Correlogram

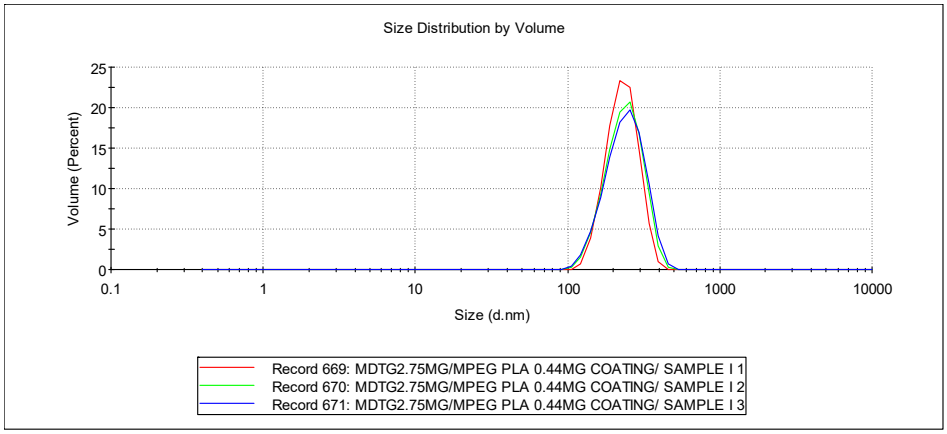


I) mPEG-PLA 0.44mg coating

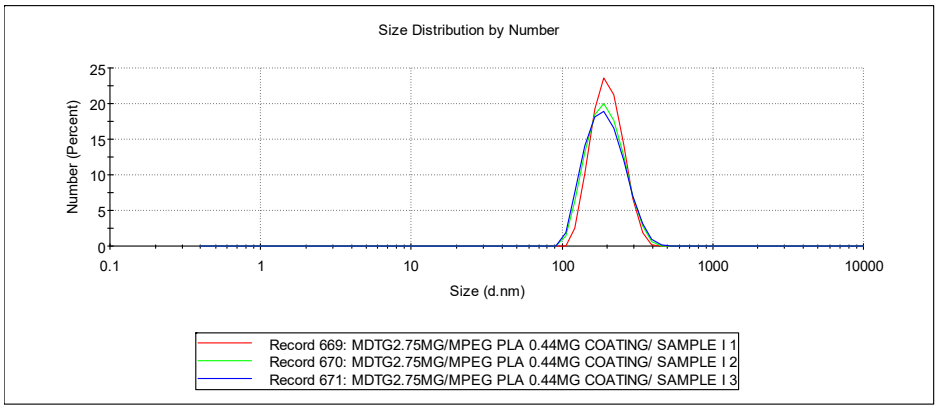
Size by intensity



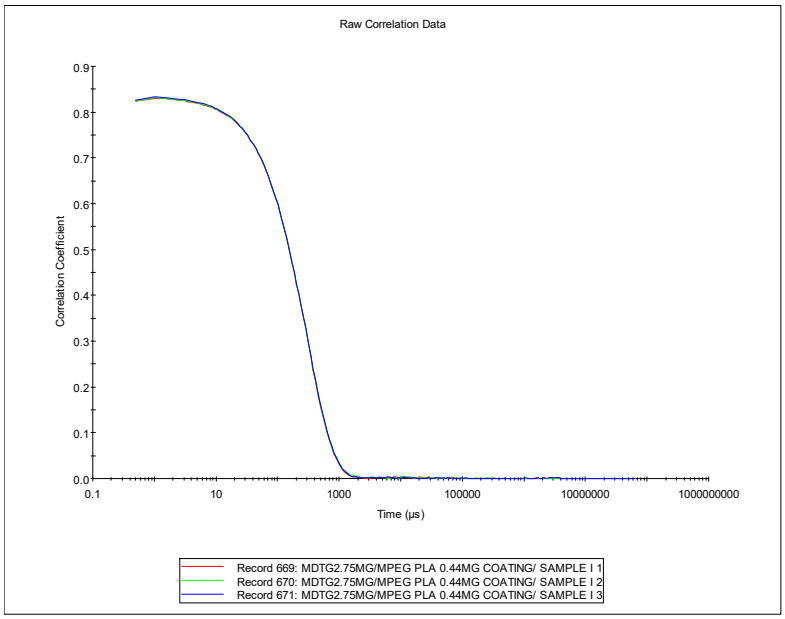
Size by volume



Size by numbers

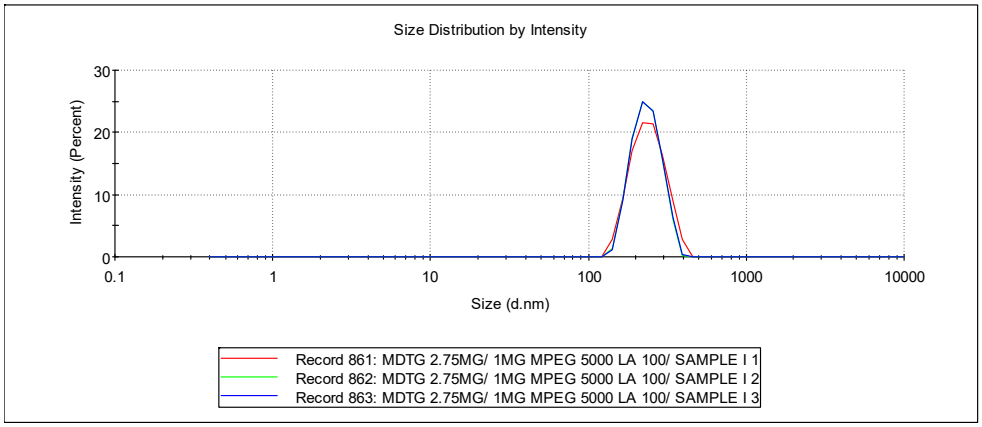


Correlogram

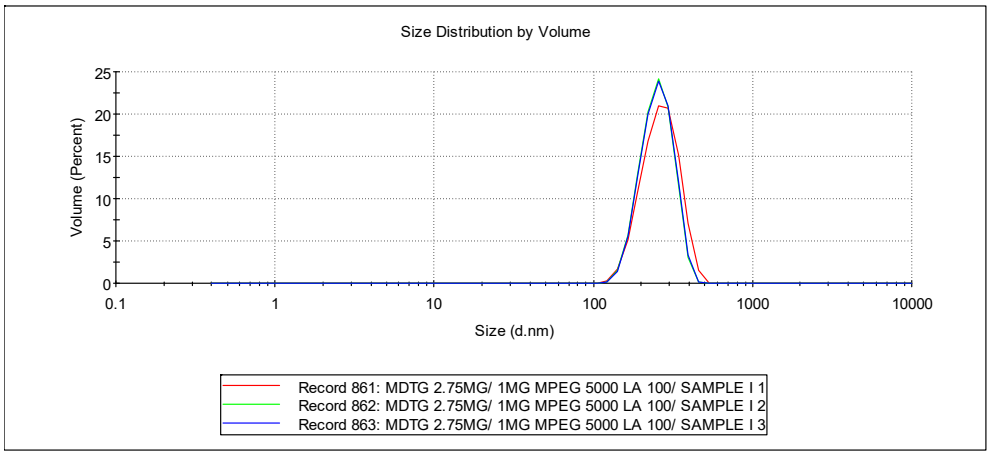


J) mPEG-PLA 1mg coating

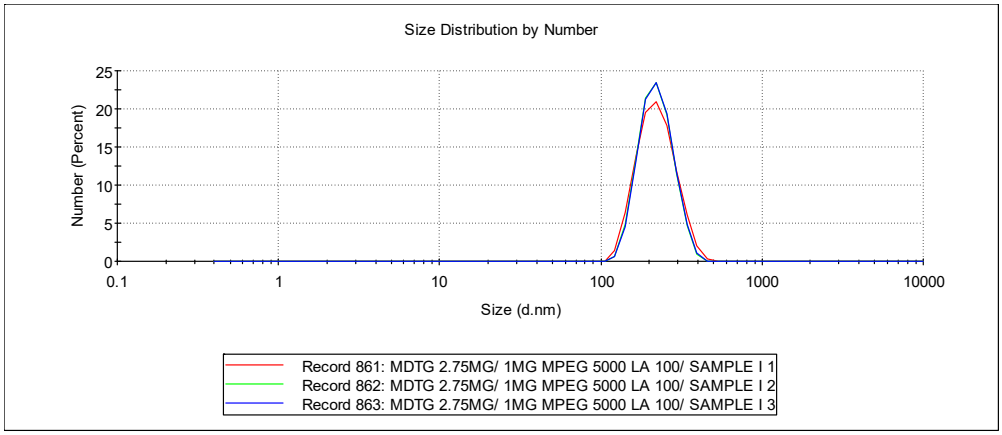
Size by intensity



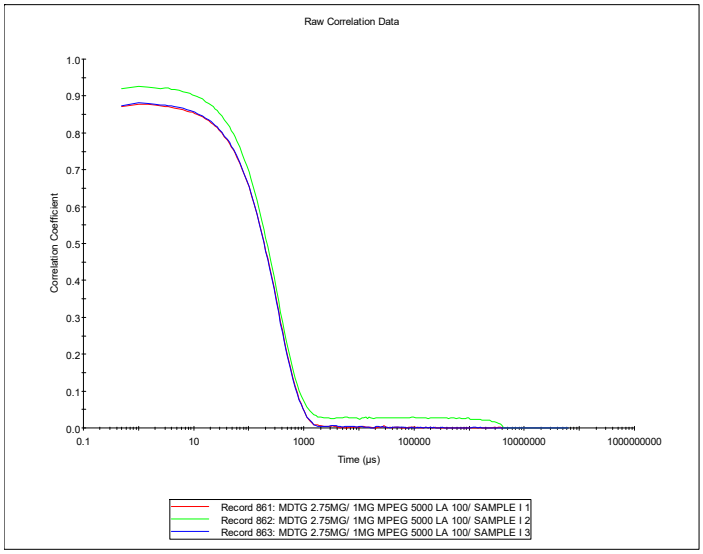
Size by volume



Size by numbers

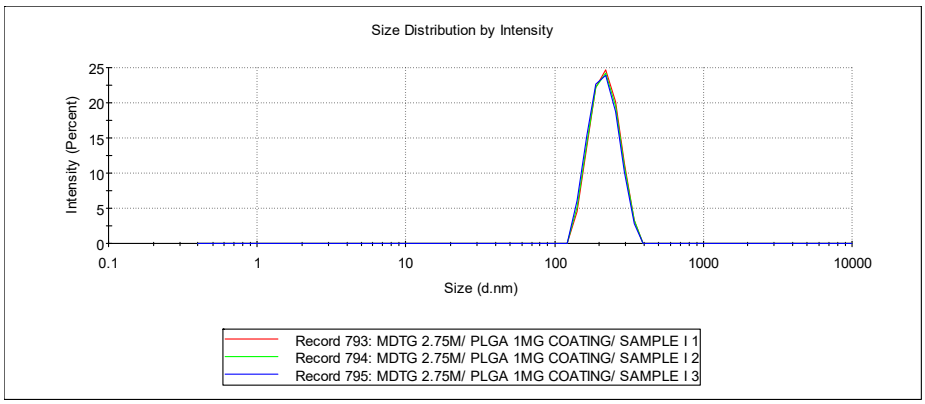


Correlogram

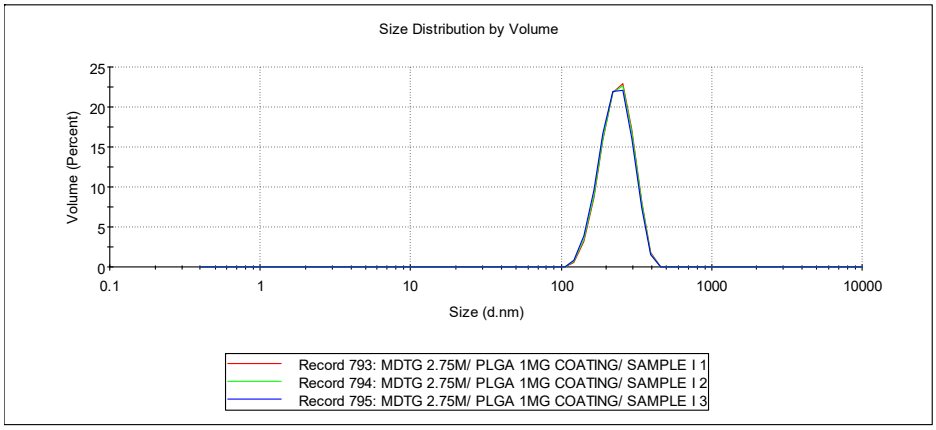


K) PLGA 1mg coating

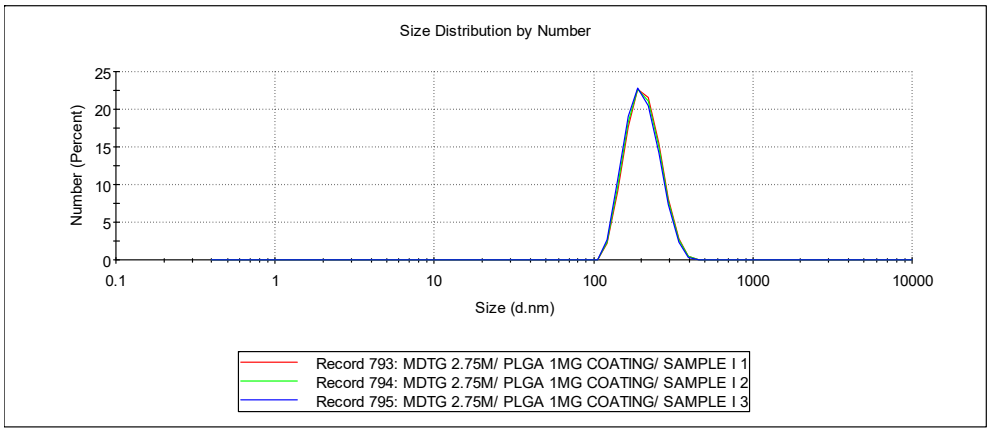
Size by intensity



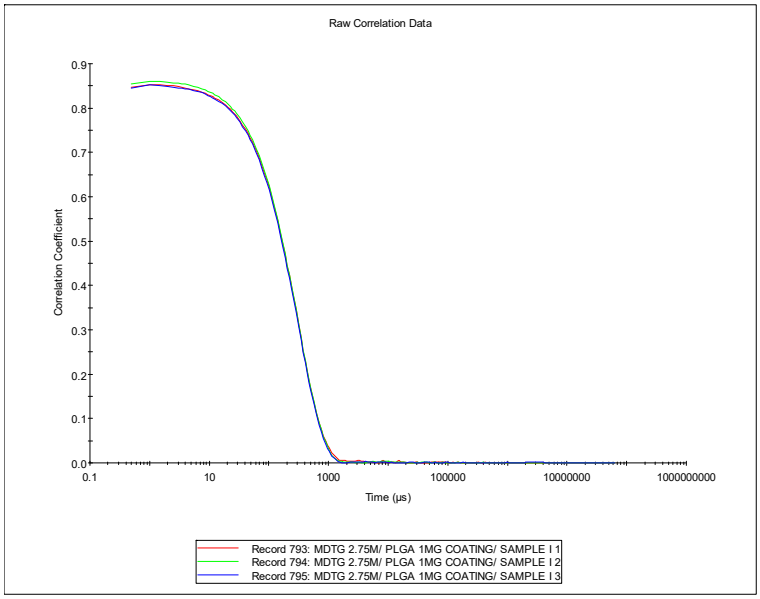
Size by volume



Size by numbers

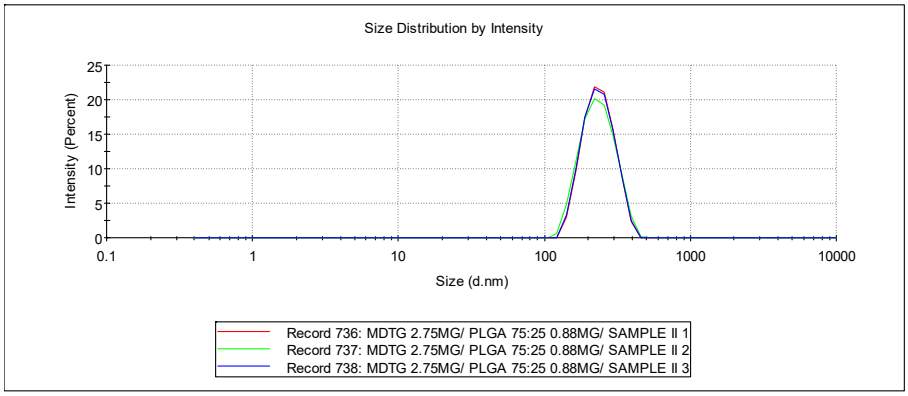


Correlogram

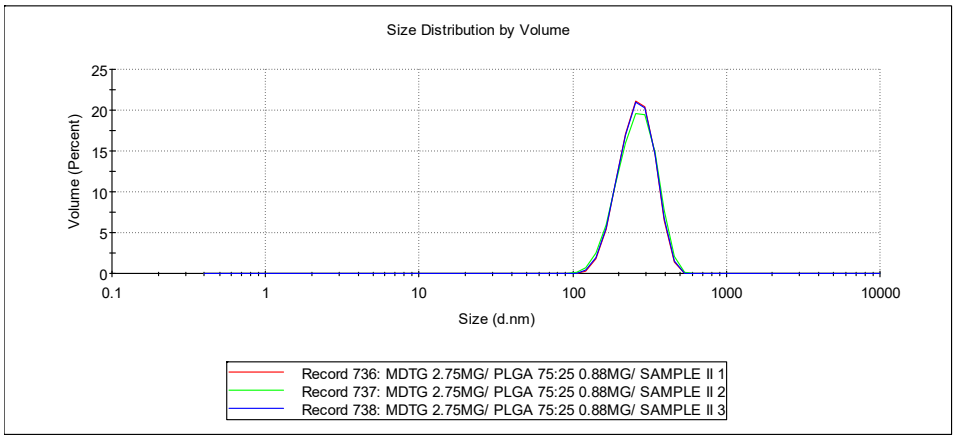


K) PLGA 0.88mg coating

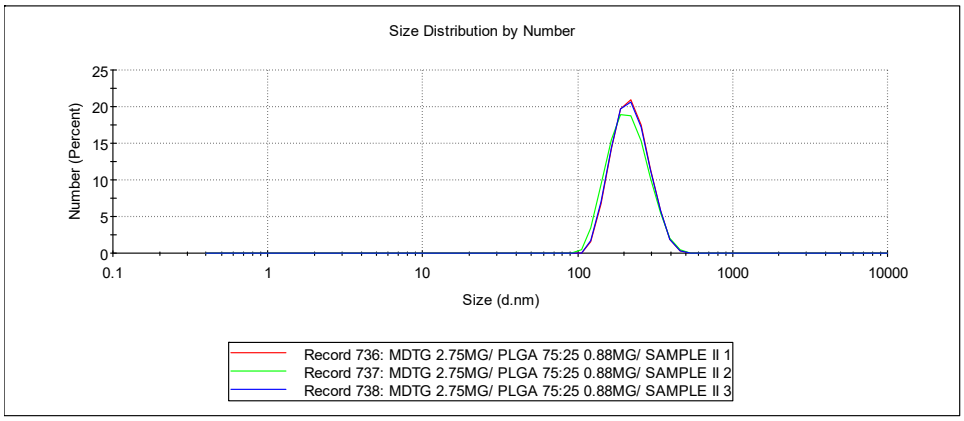
Size by intensity



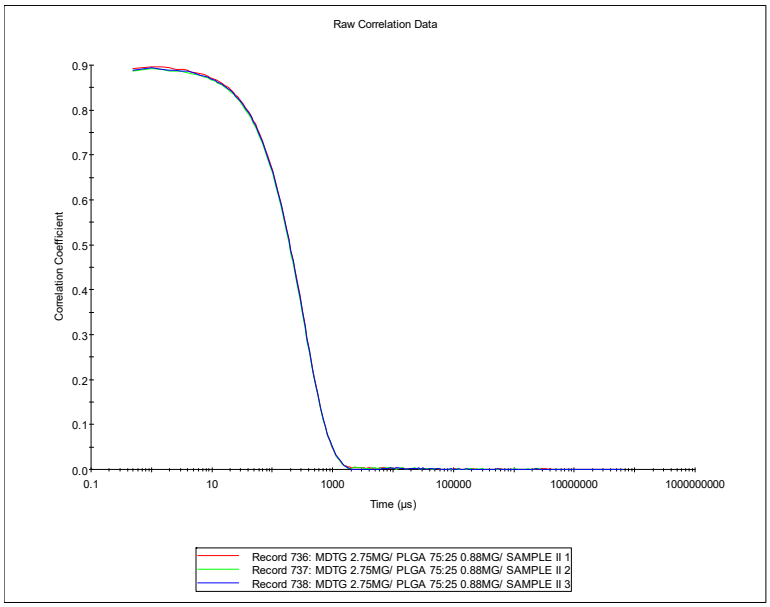
Size by volume



Size by number

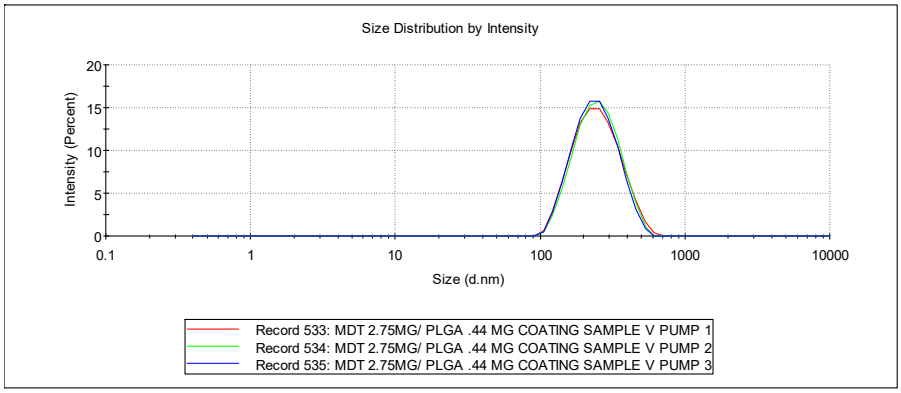


Correlogram

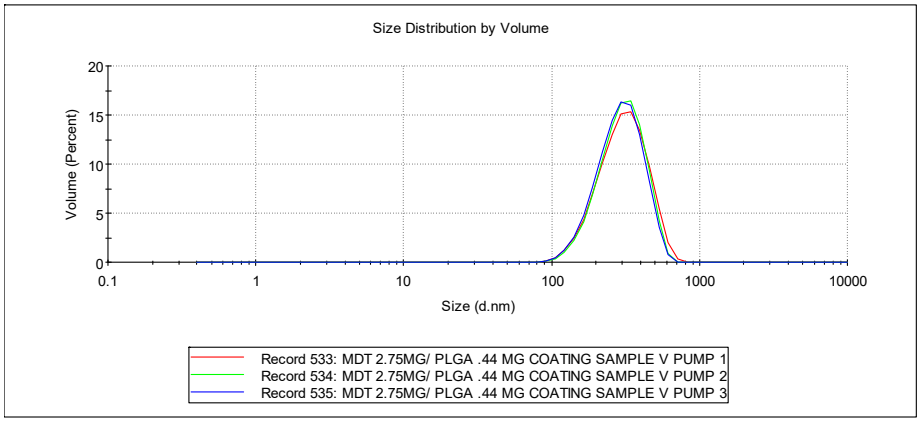


L) PLGA 0.44mg coating

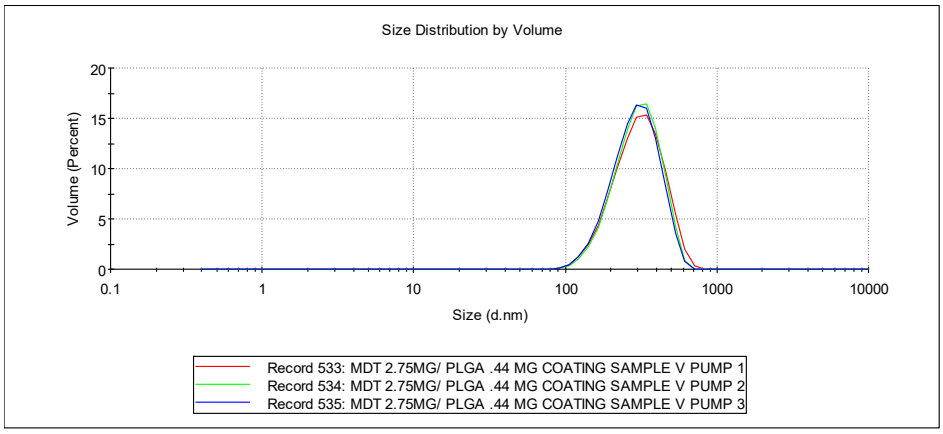
Size by intensity



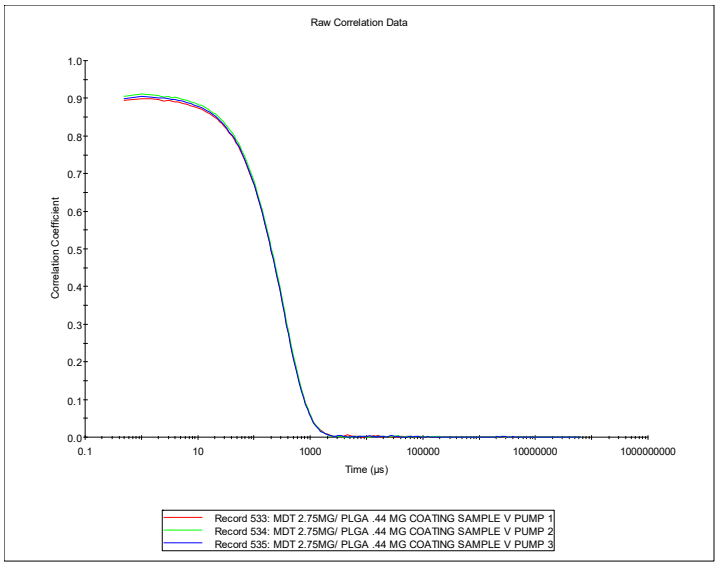
Size by volume



Size by number

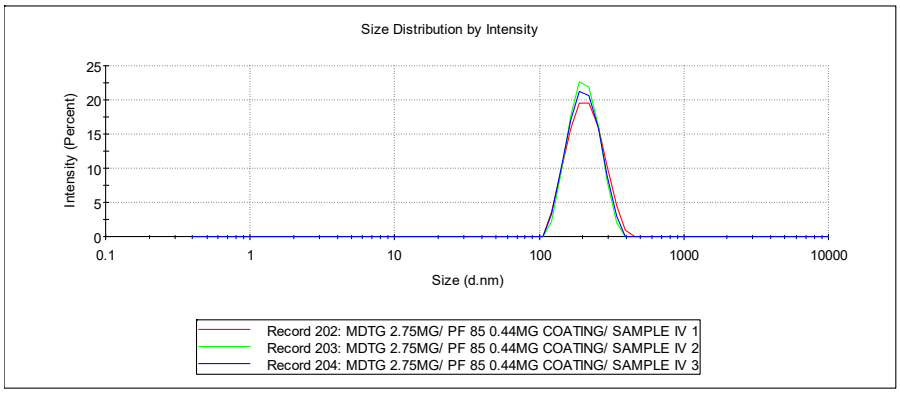


Correlogram

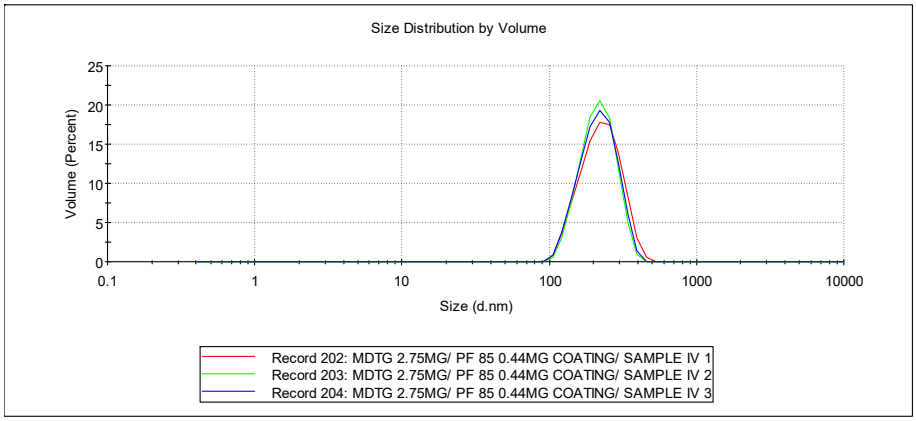


M) P85 0.44mg coating

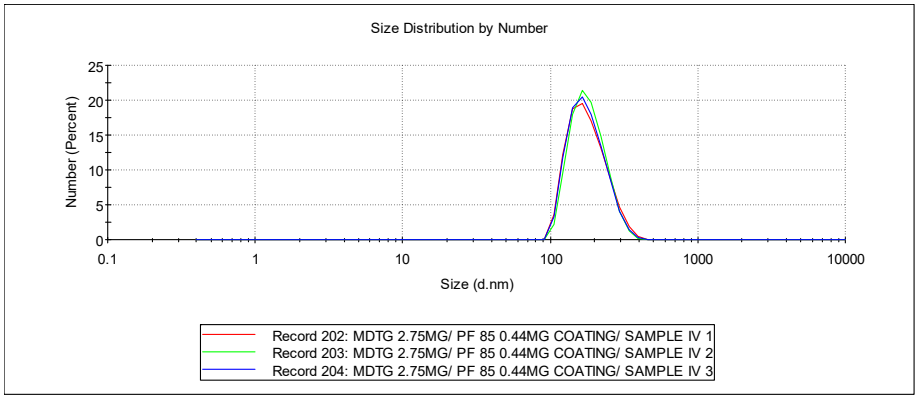
Size by intensity



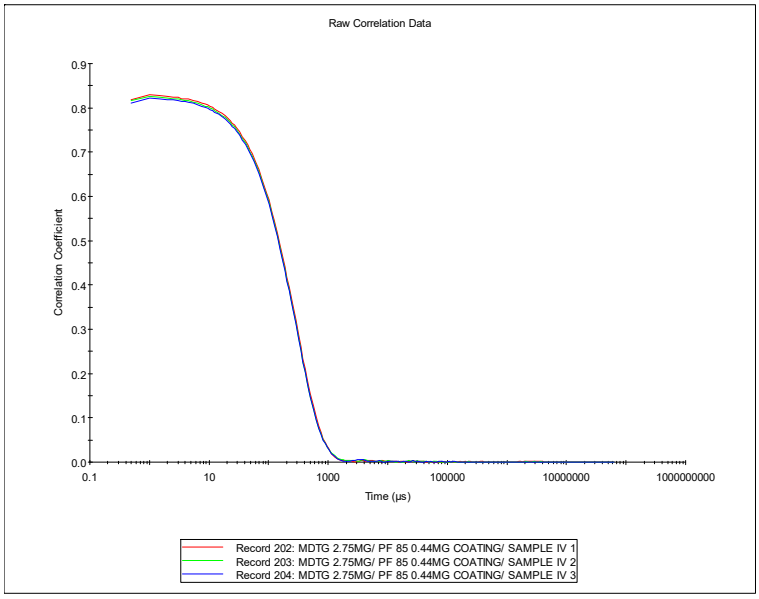
Size by numbers



Size by numbers

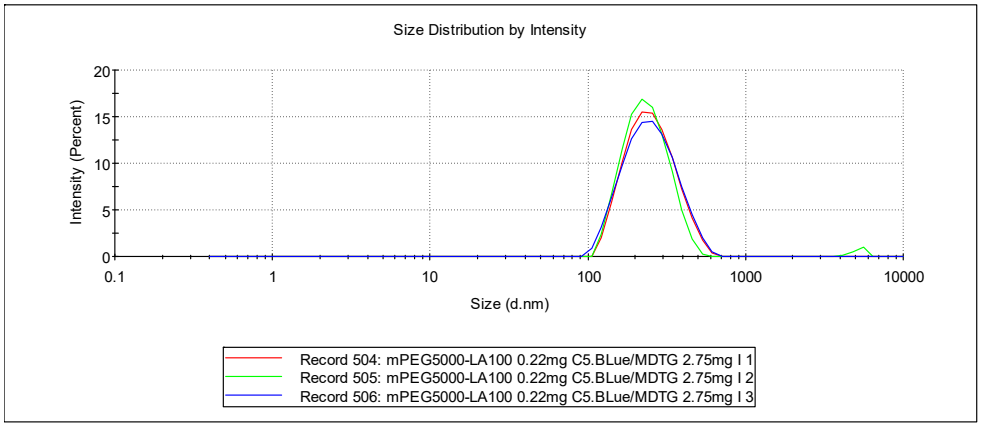


Correlogram

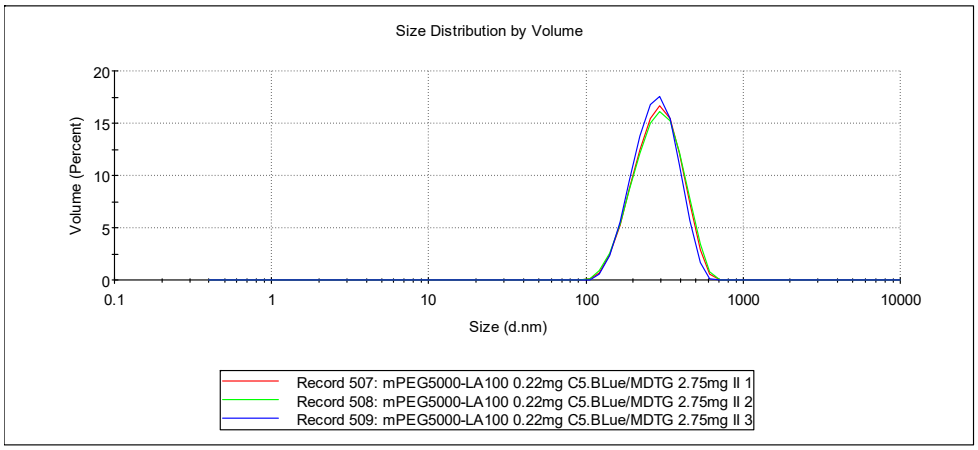


N) mPEG-PLA-Cy5 0.22mg coating

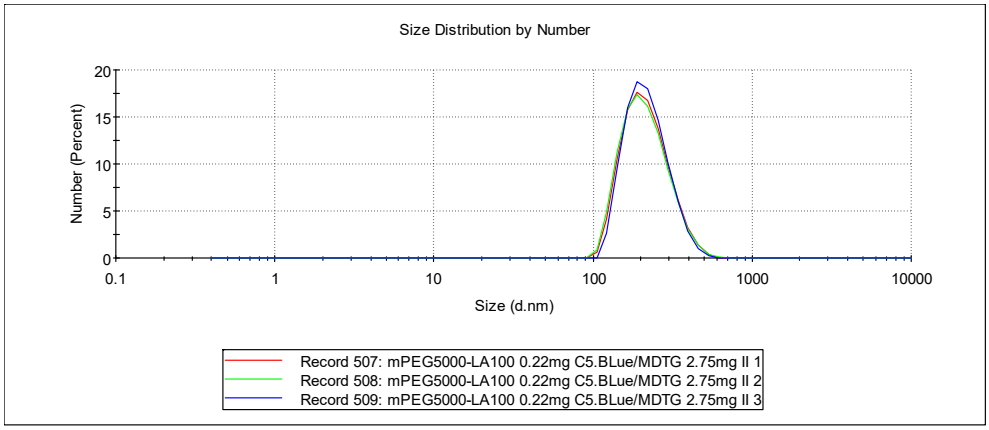
Size by intensity



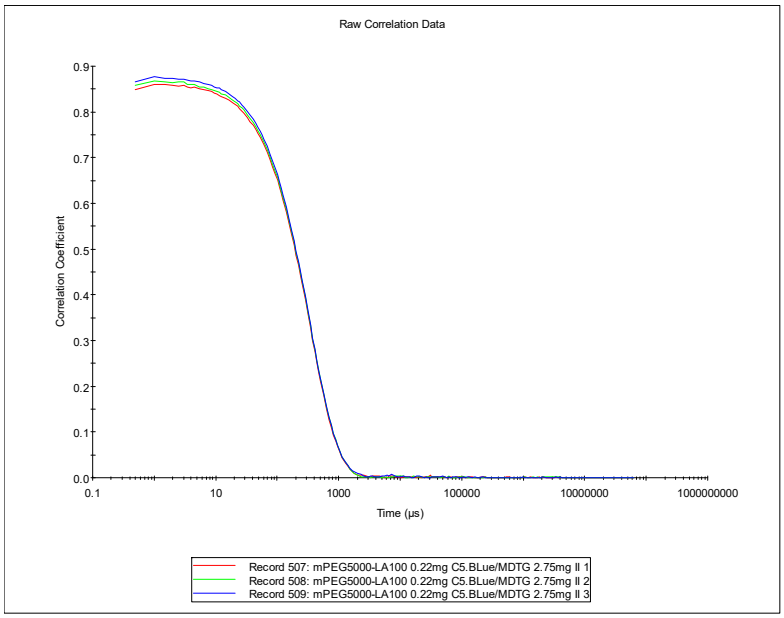
Size by volume



Size by number

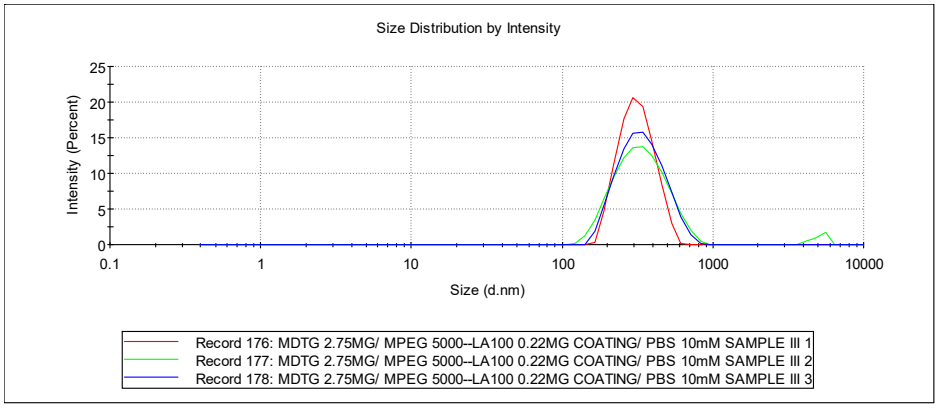


Correlogram

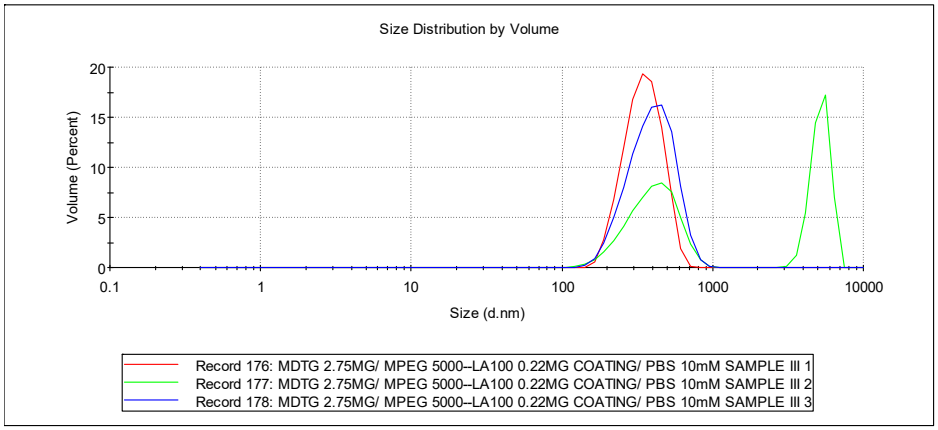


O) mPEG-PLA 0.22mg coating in 100mM PBS

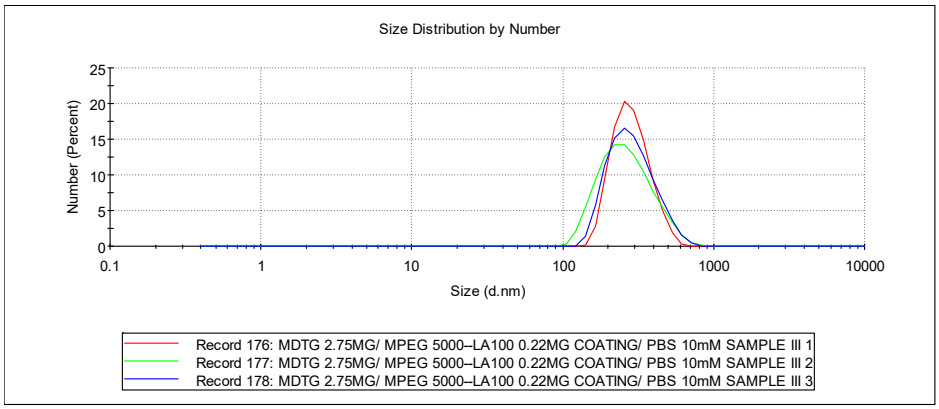
Size by intensity



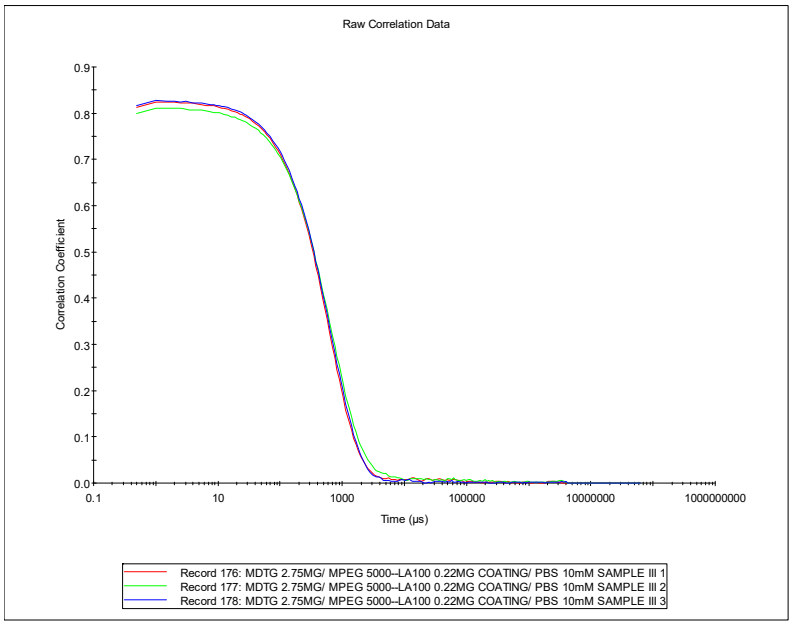
Size by volume



Size by numbers

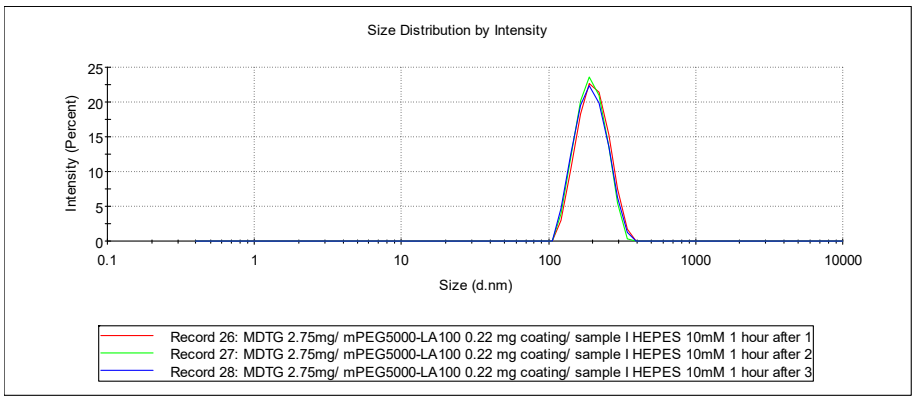


Correlogram

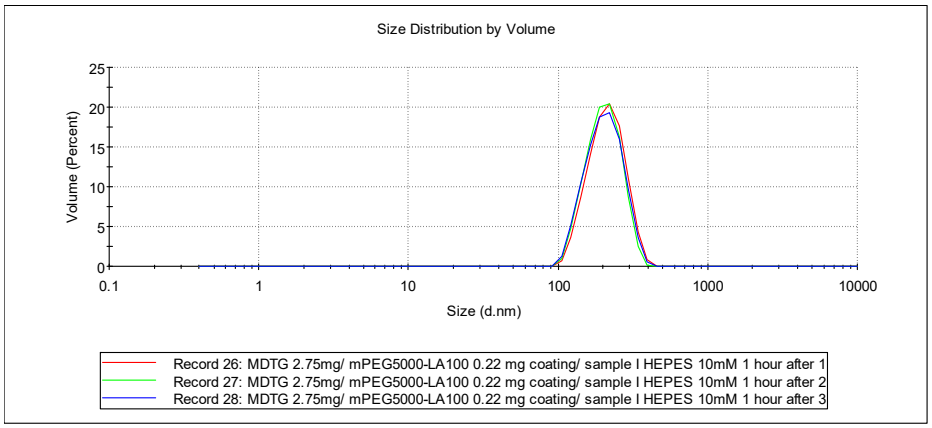


P) mPEG-PLA 0.22mg coating after 1 hour in 10mM HEPES buffer

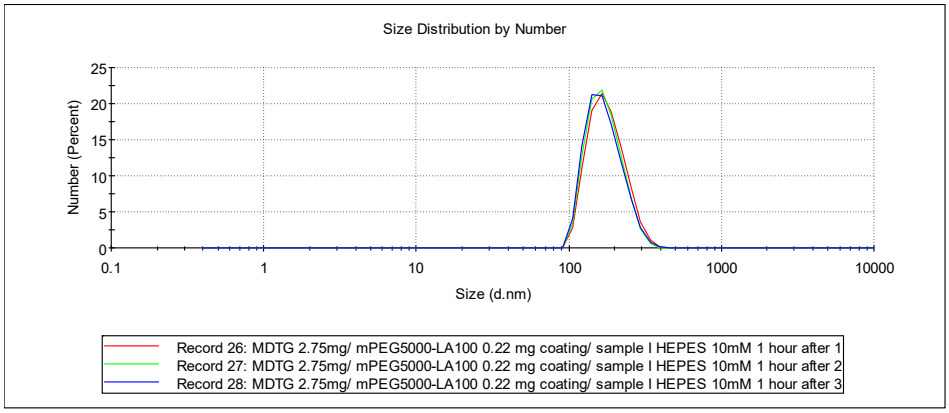
Size by intensity



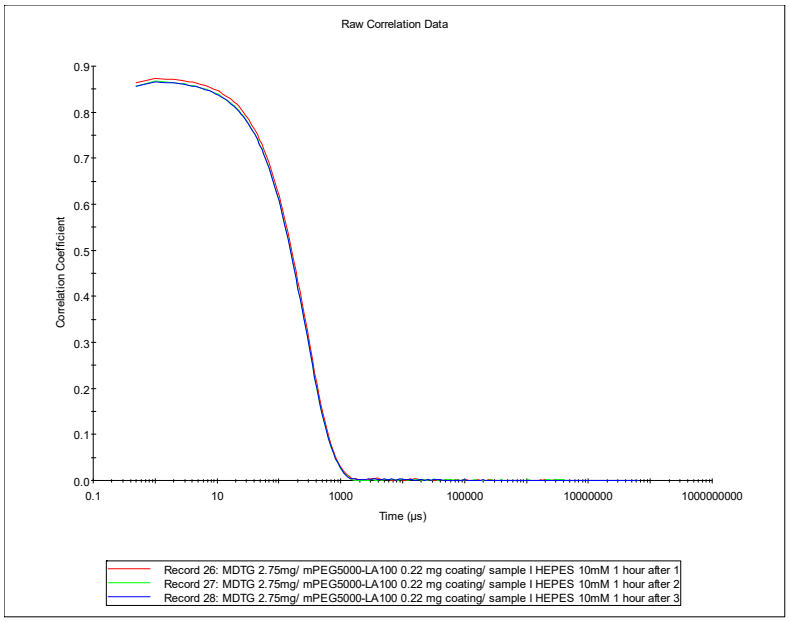
Size by volume



Size by numbers

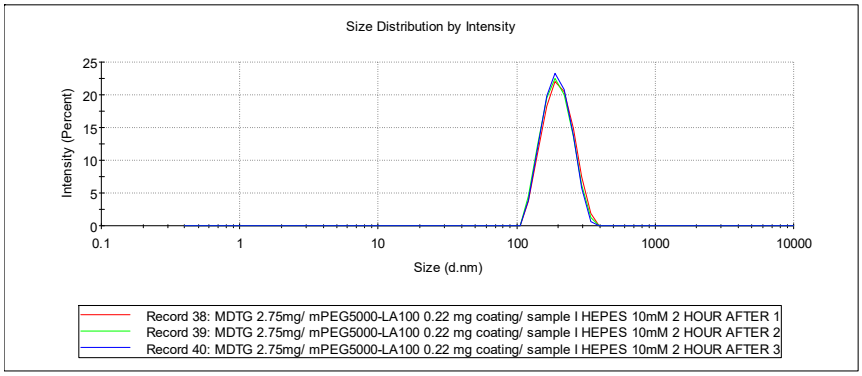


Correlogram

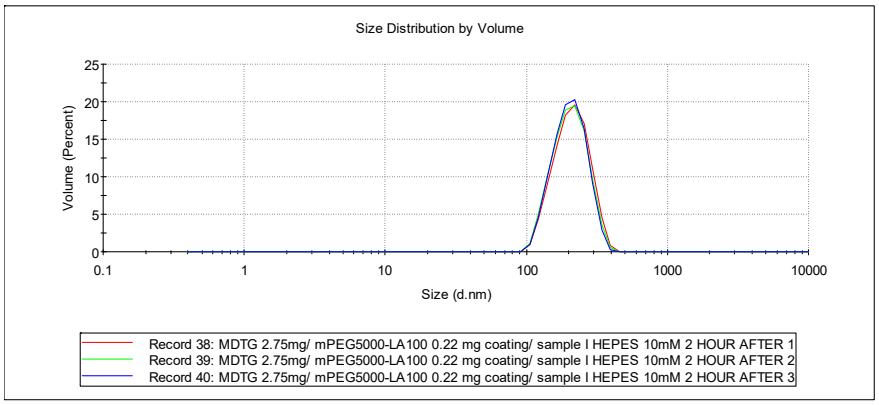


Q) mPEG-PLA 0.22mg coating after 2 hours in 10mM HEPES buffer

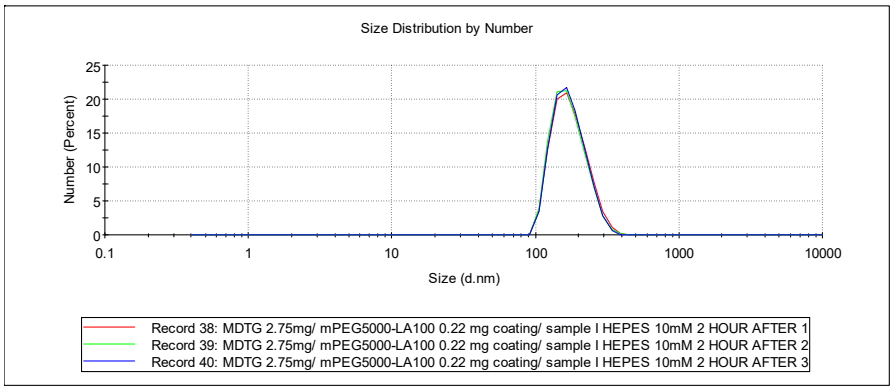
Size by intensity



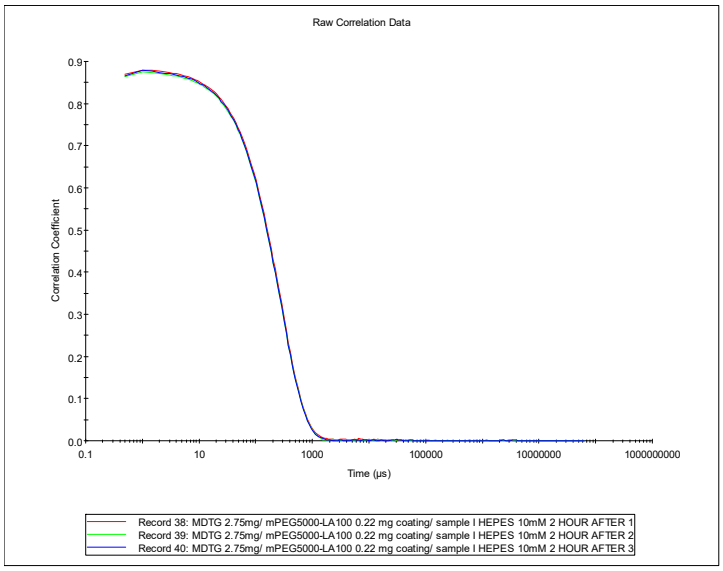
Size by volume



Size by numbers

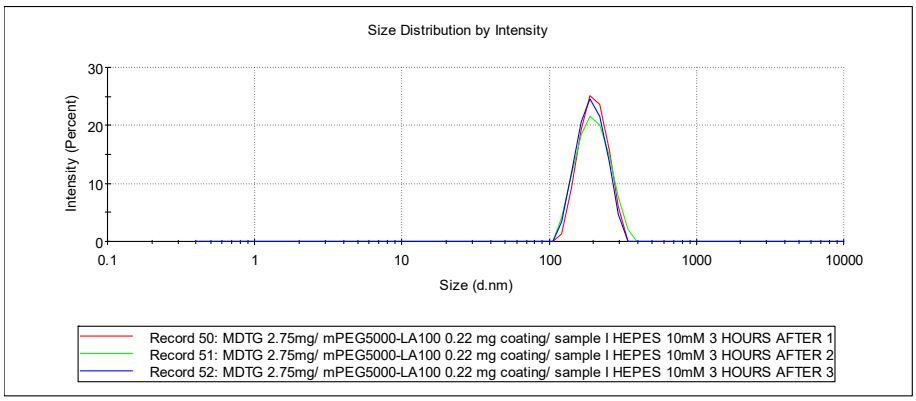


Correlogram

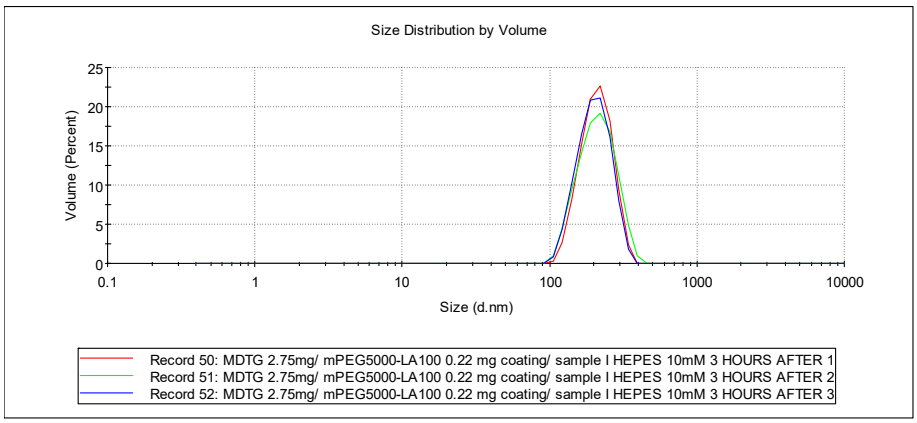


R) mPEG-PLA 0.22mg coating after 3 hours in 10mM HEPES buffer

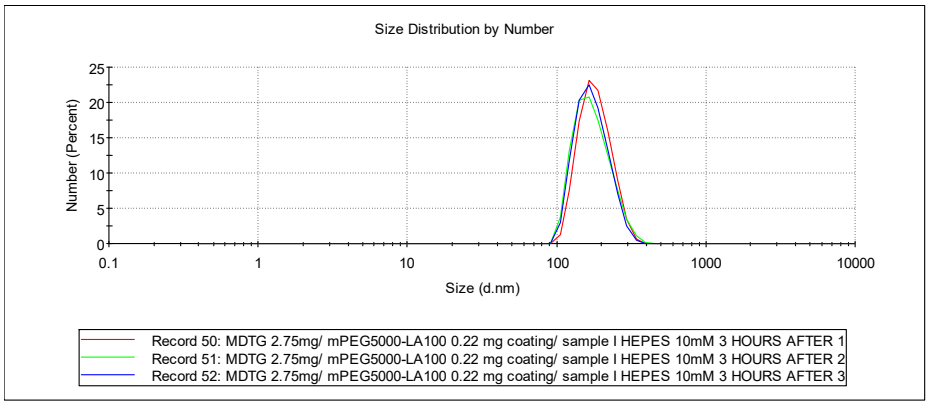
Size by intensity



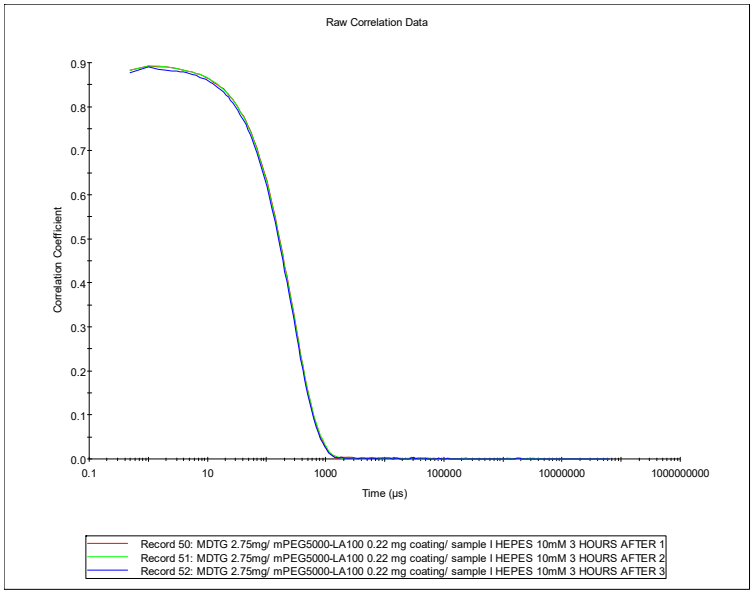
Size by volume



Size by numbers

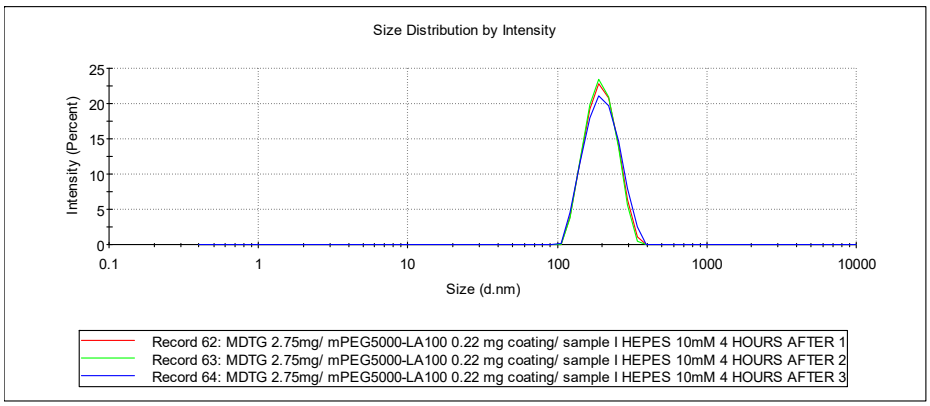


Correlogram

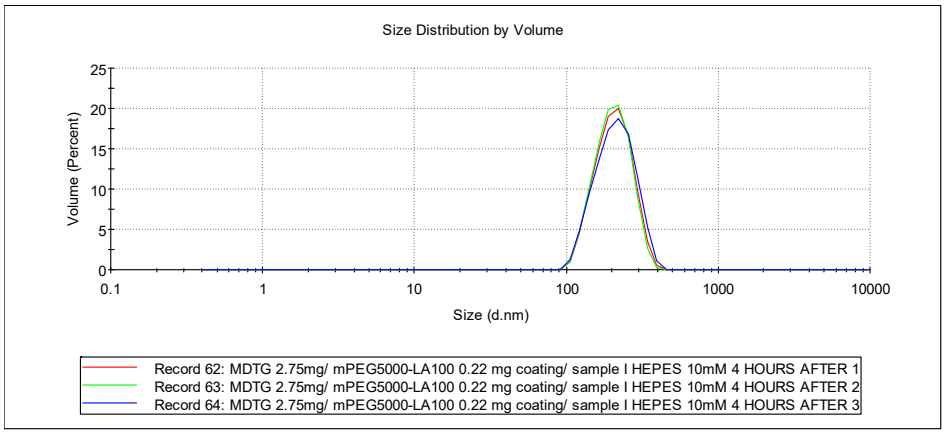


S) mPEG-PLA 0.22mg coating after 4 hours in 10mM HEPES buffer

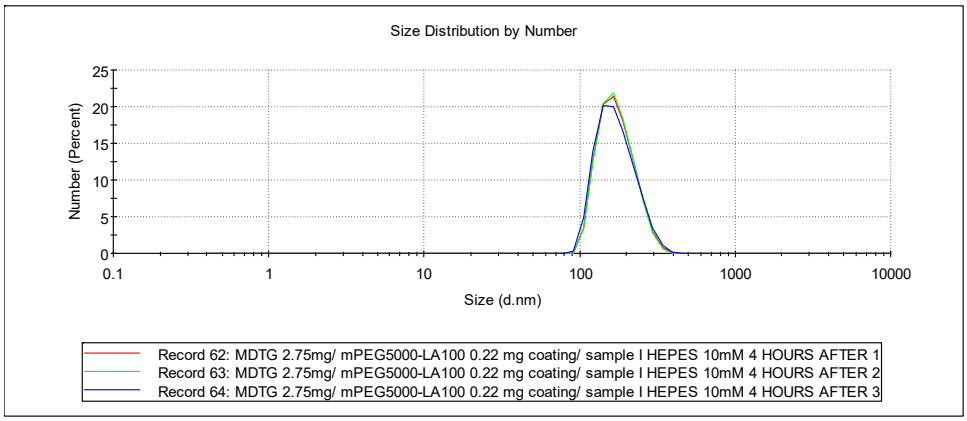
Size by intensity



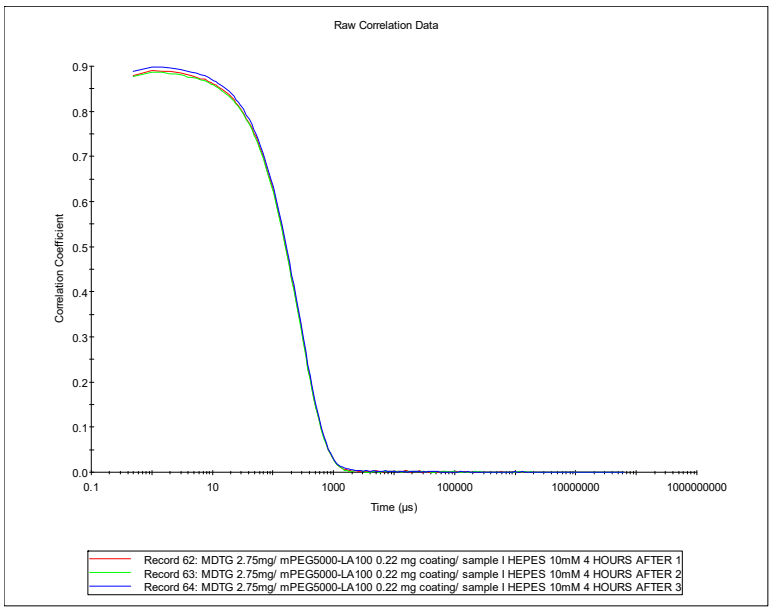
Size by volume



Size by numbers

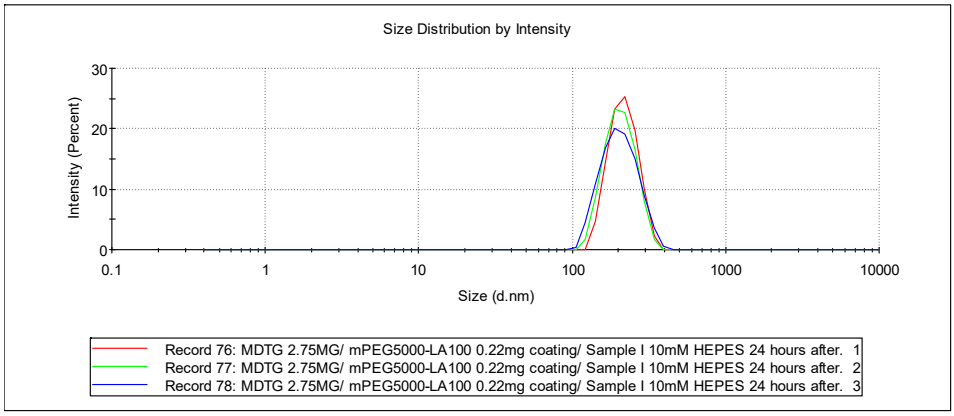


Correlogram

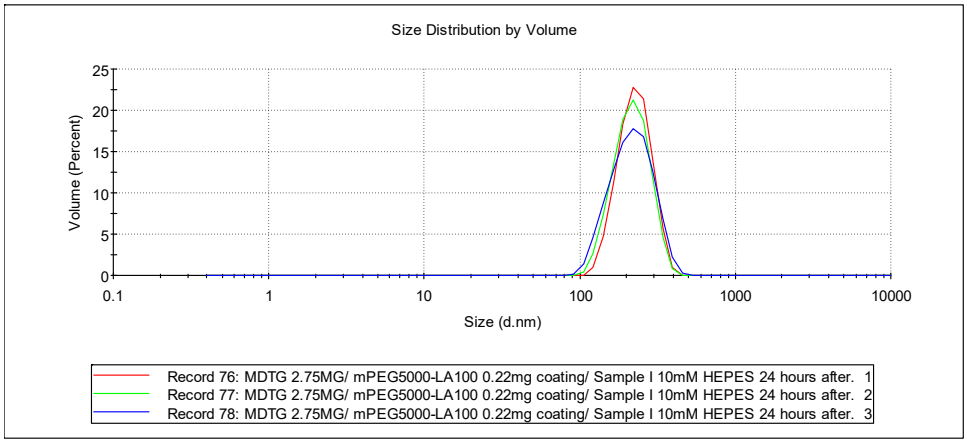


T) mPEG-PLA 0.22mg coating after 24 hours in 10mM HEPES buffer

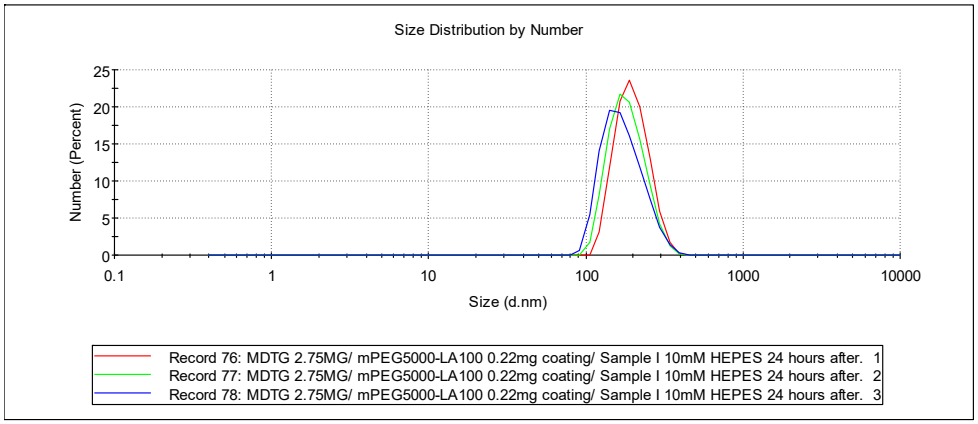
Size by intensity



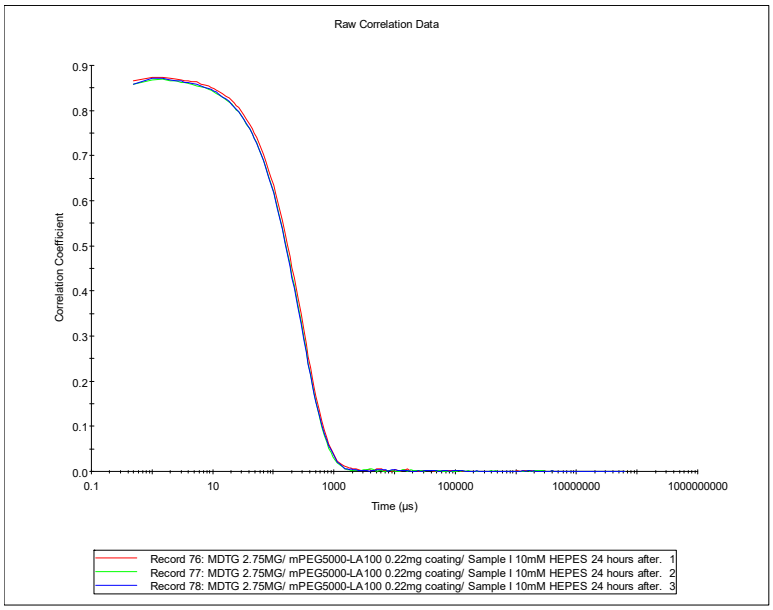
Size by volume



Size by number

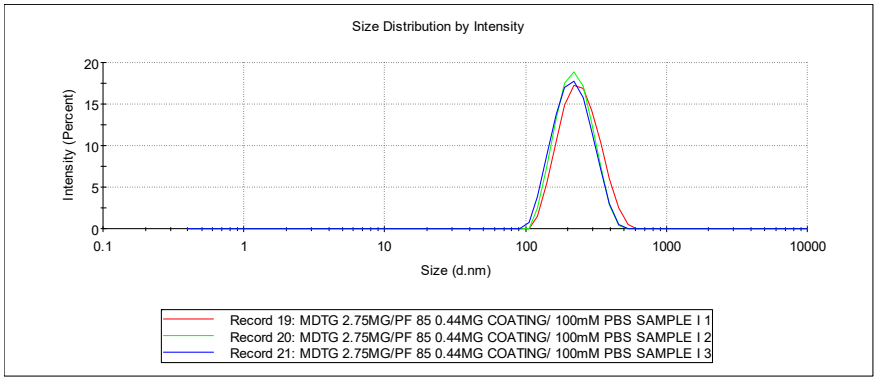


Correlogram

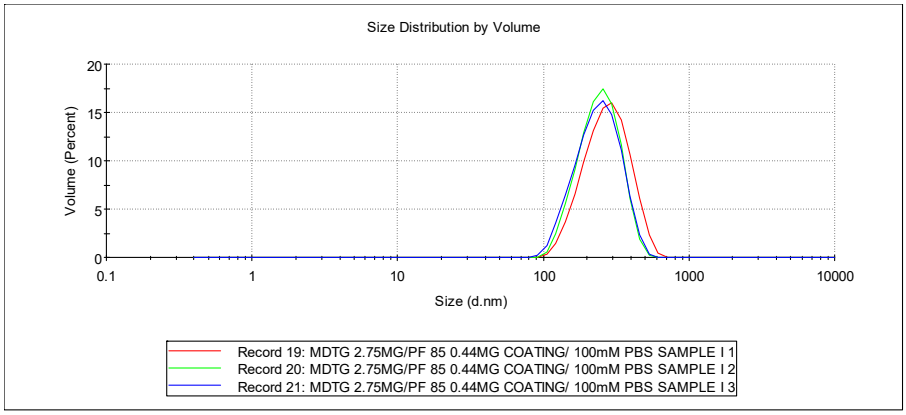


U) P85 0.44mg coating in PBS 100mM

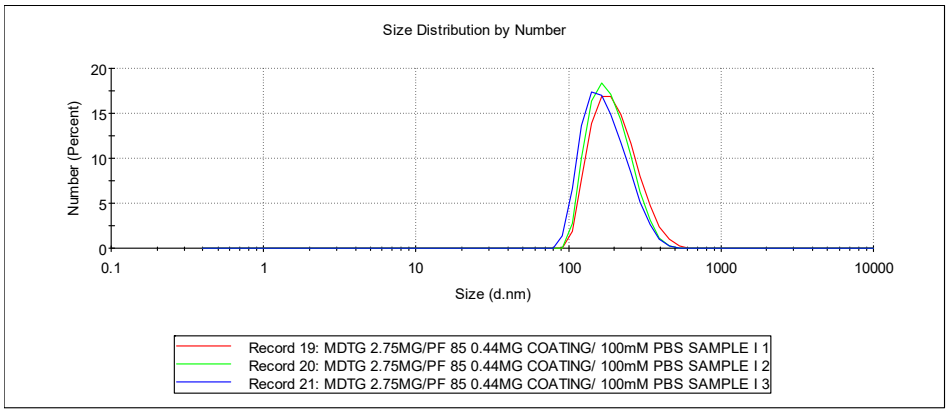
Size by intensity



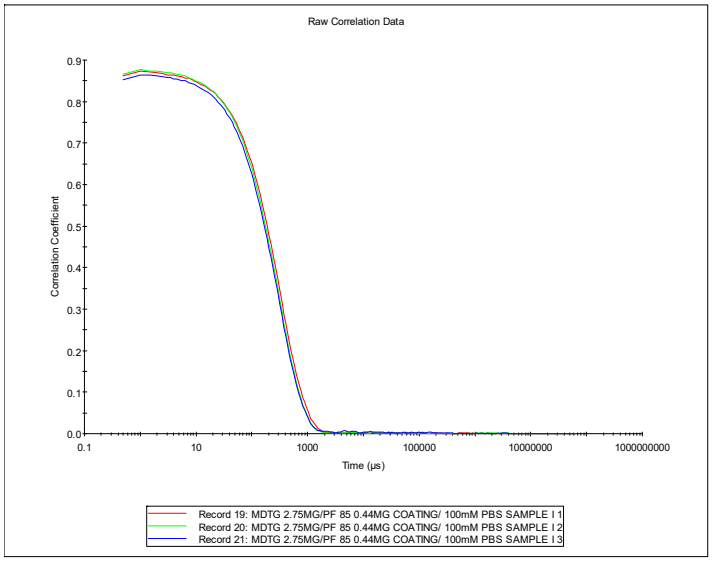
Size by volume



Size by numbers

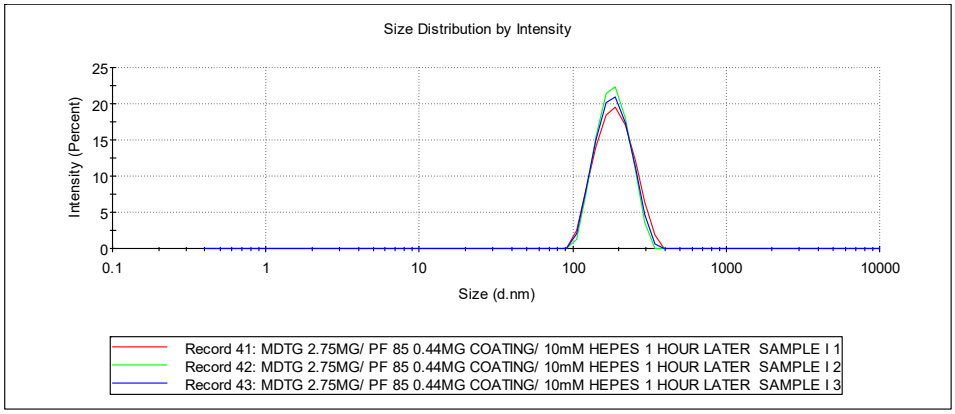


Correlogram

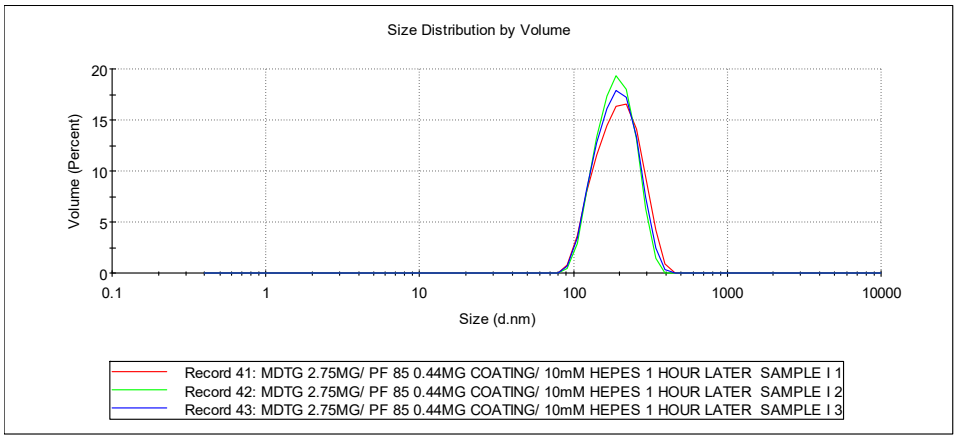


V) P85 0.44mg coating after 1 hour in 10mM HEPES buffer

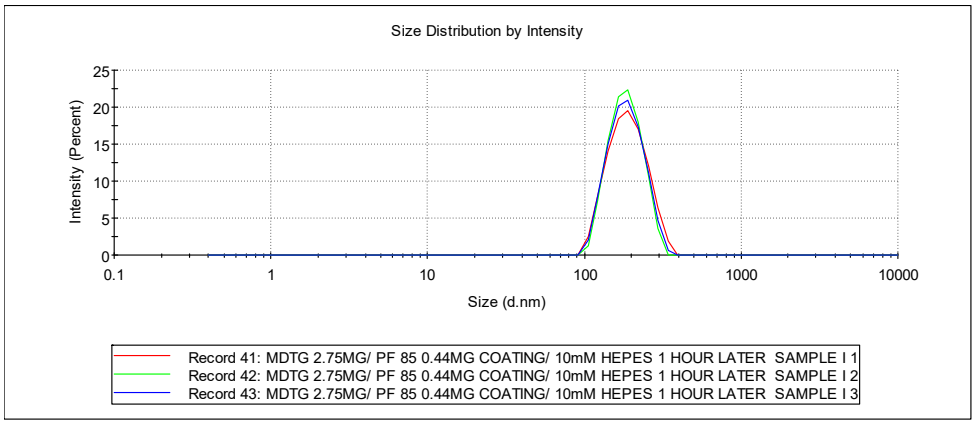
Size by intensity



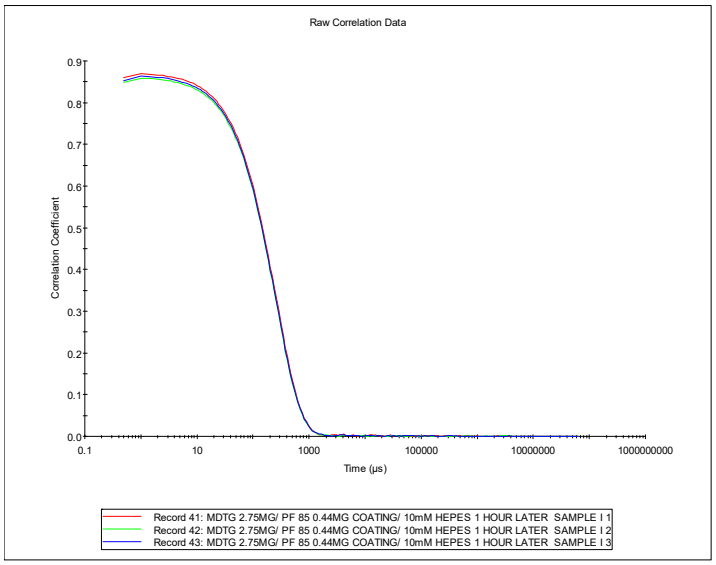
Size by volume



Size by numbers

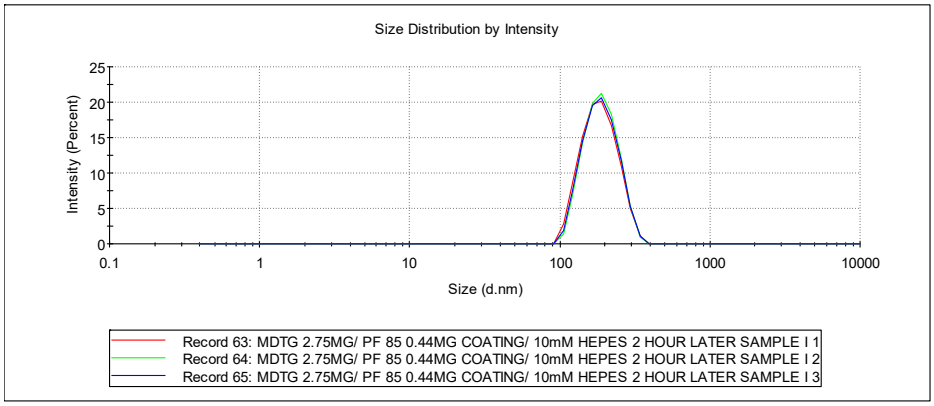


Correlelogram

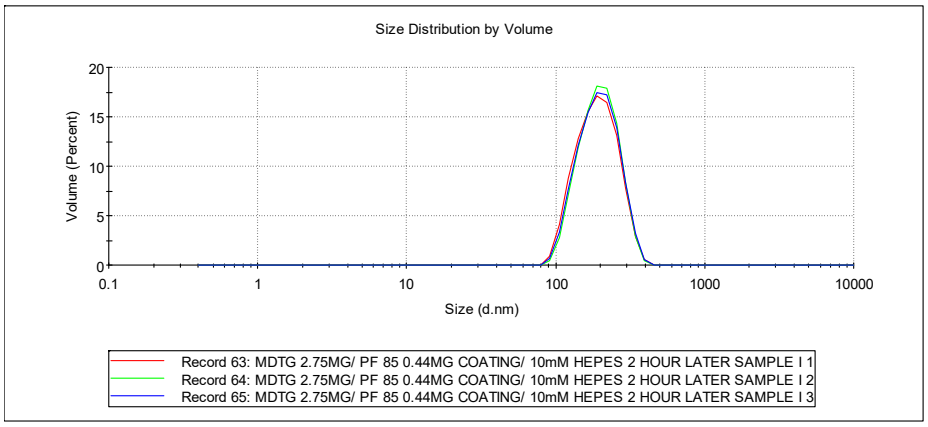


W) P85 0.44mg coating after 2 hour in 10mM HEPES buffer

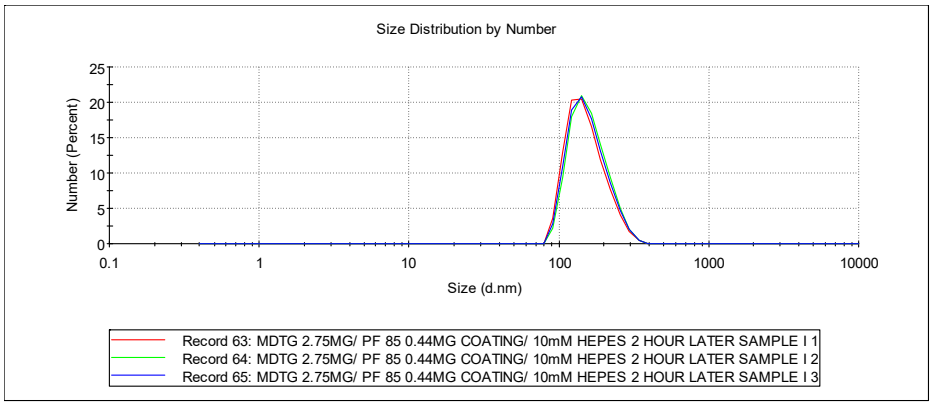
Size by intensity



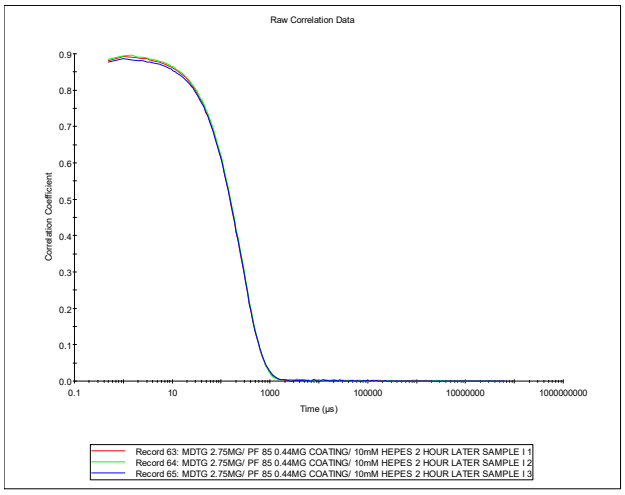
Size by volume



Size by numbers

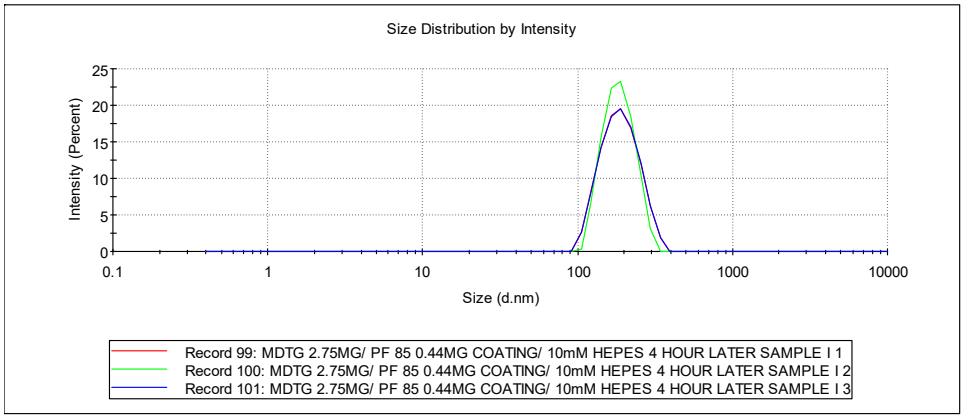


Correlogram

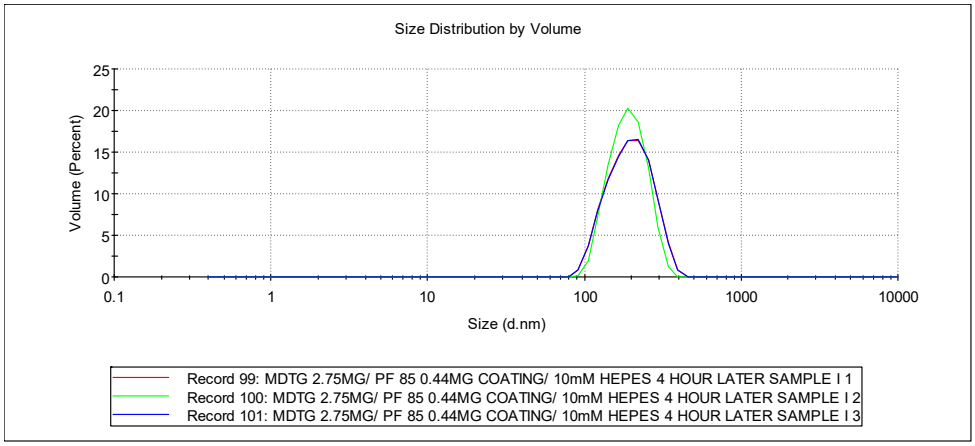


X) P85 0.44mg coating after 3 hour in 10mM HEPES buffer

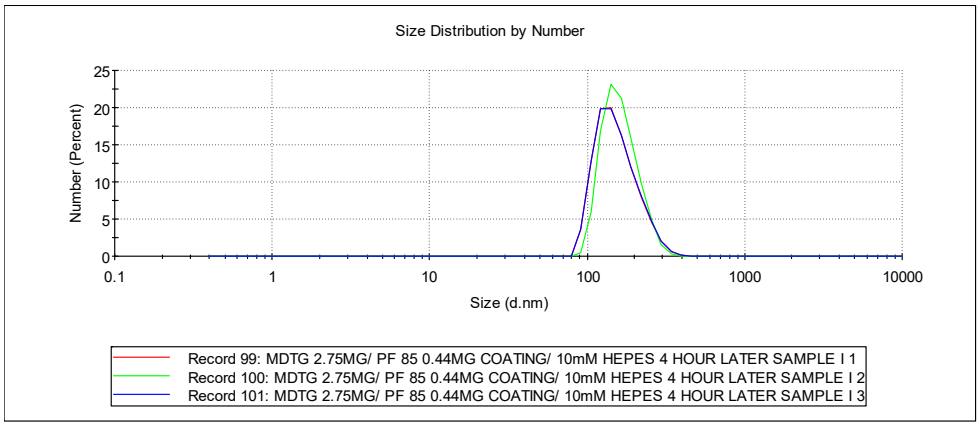
Size by intensity



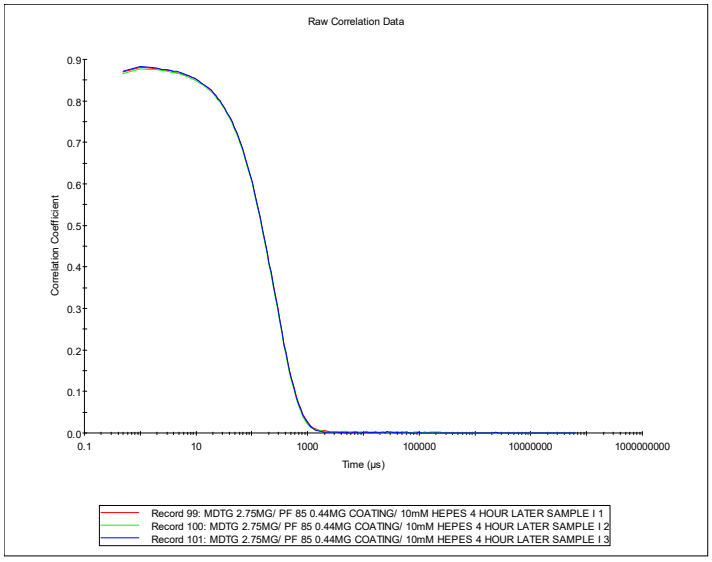
Size by volume



Size by numbers

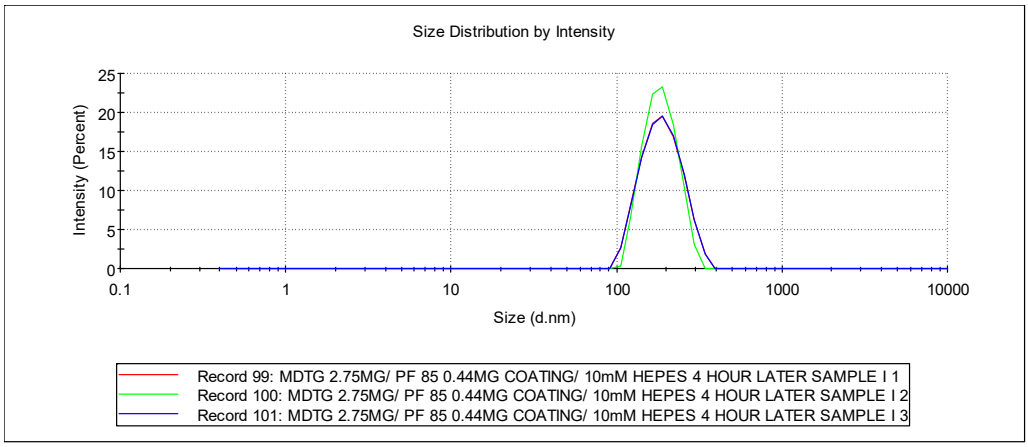


Correlogram

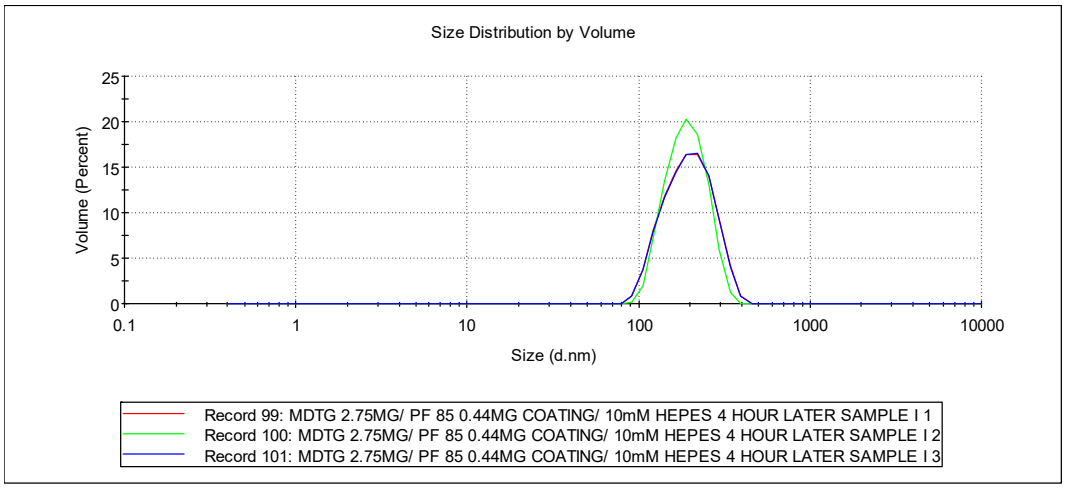


Y) P85 0.44mg coating after 4 hour in 10mM HEPES buffer

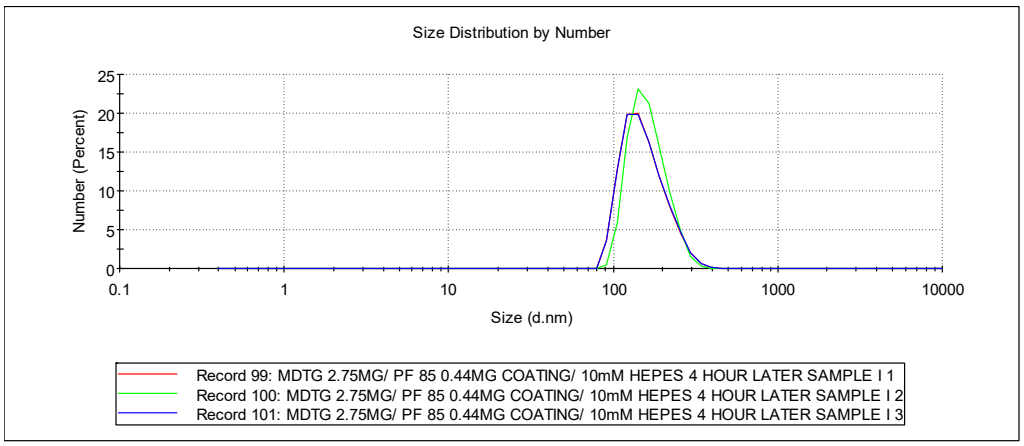
Size by intensity



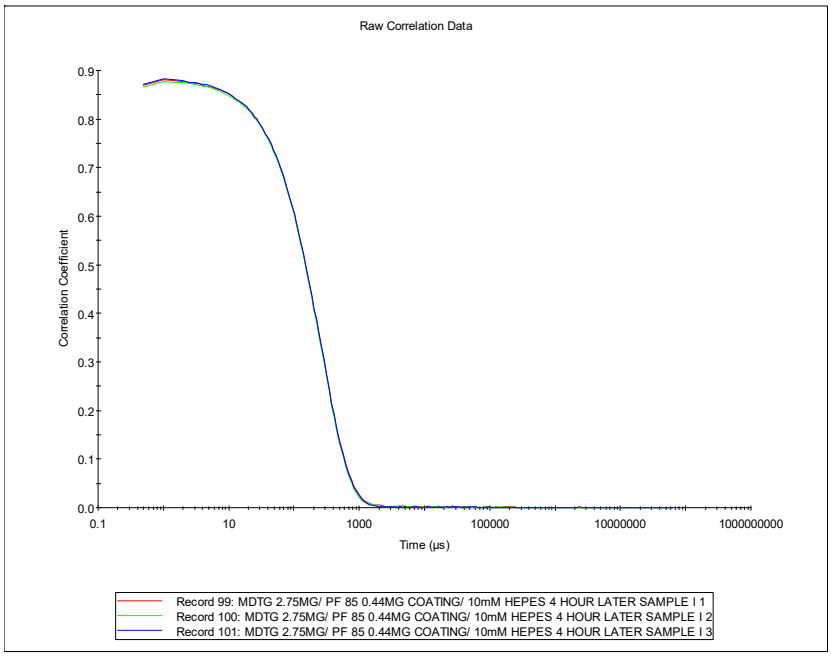
Size by volume



Size by numbers

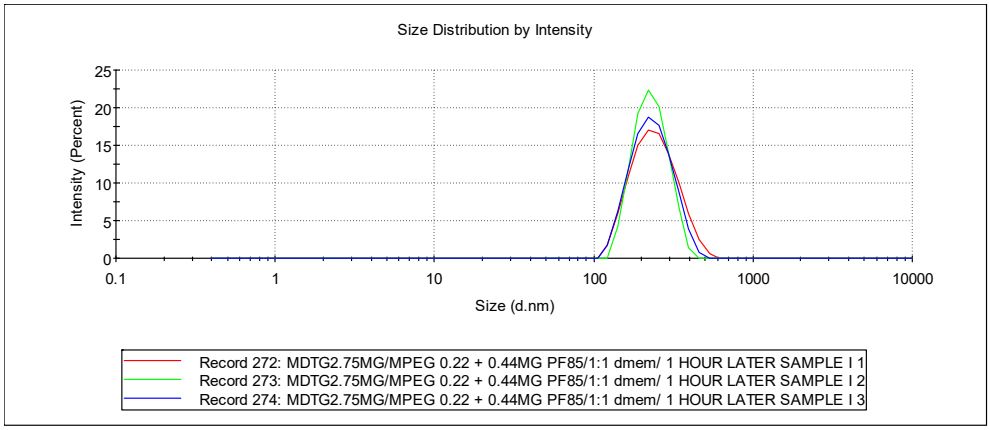


Correlogram

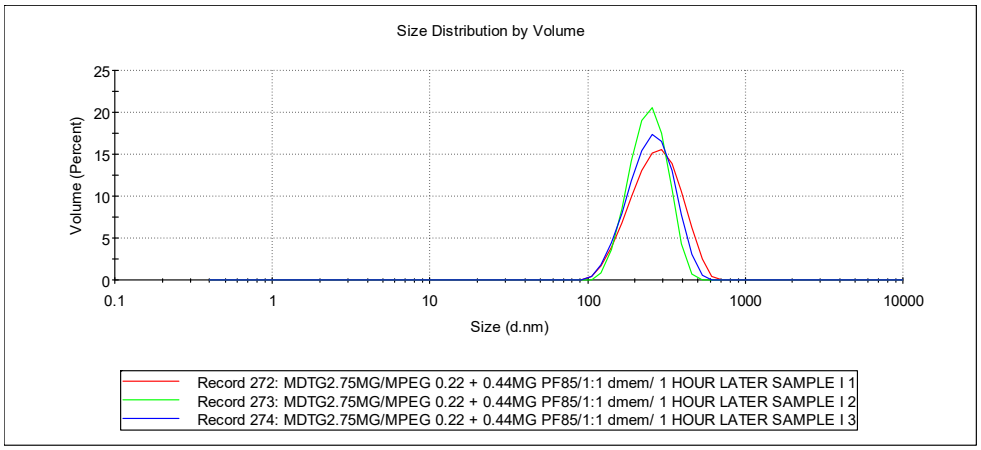


Z) P85 0.44mg coating after 1 hour in DMEM

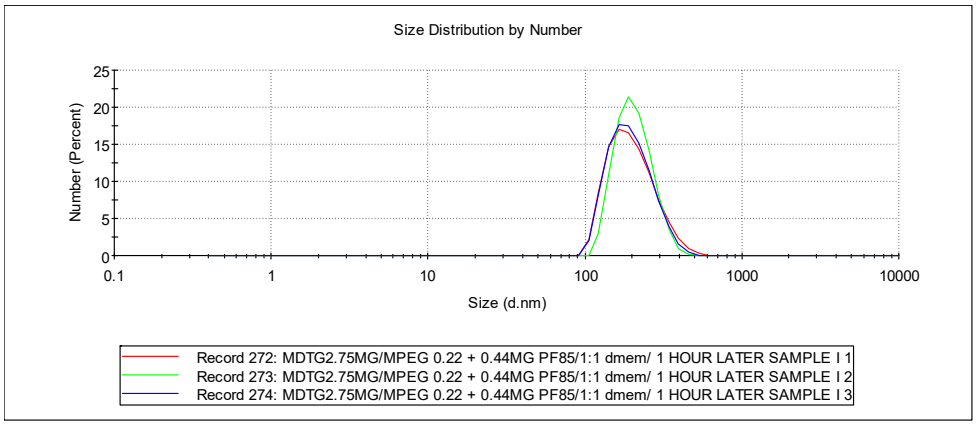
Size by intensity



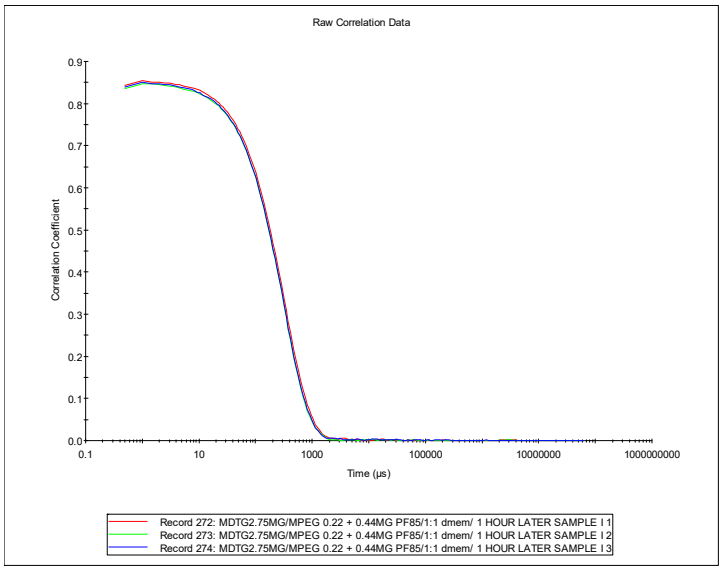
Size by volume



Size by numbers

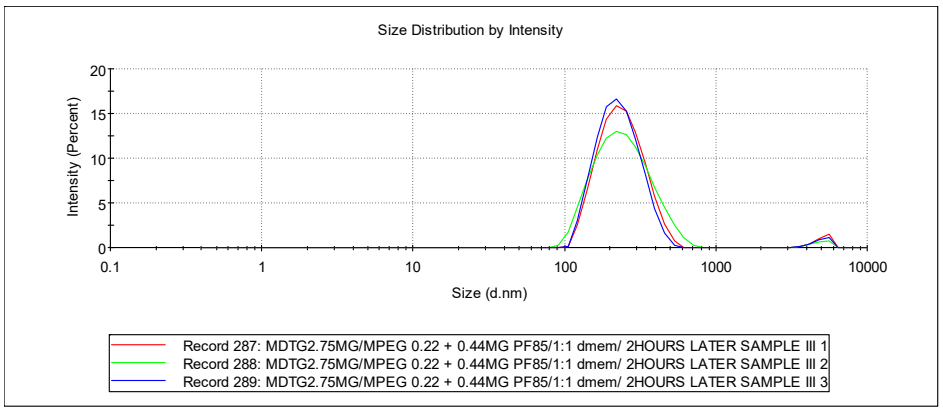


Correlogram

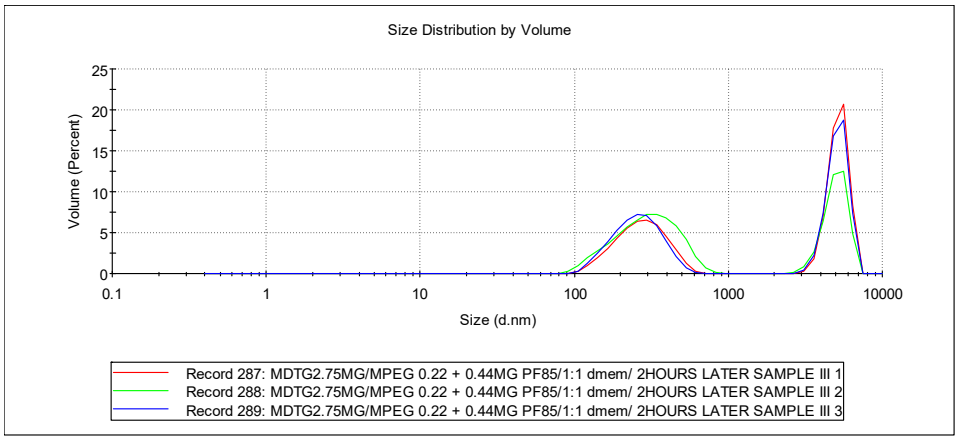


AA) P85 0.44mg coating after 2 hour in DMEM

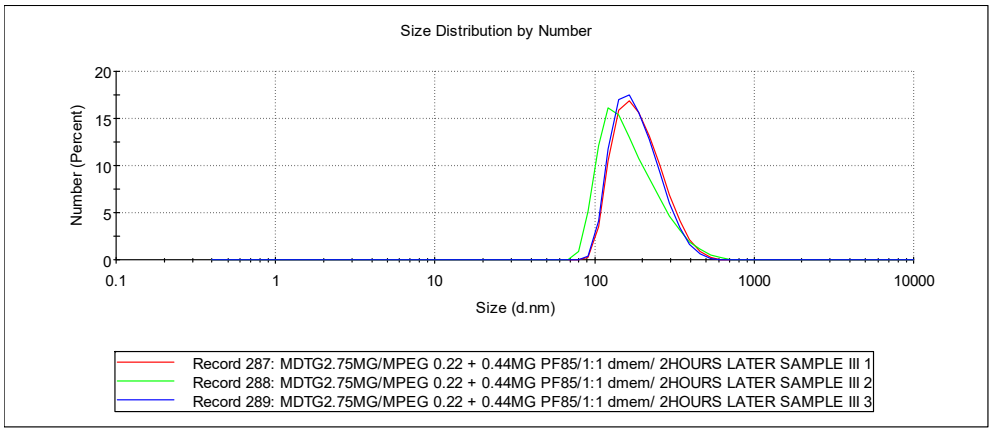
Size by intensity



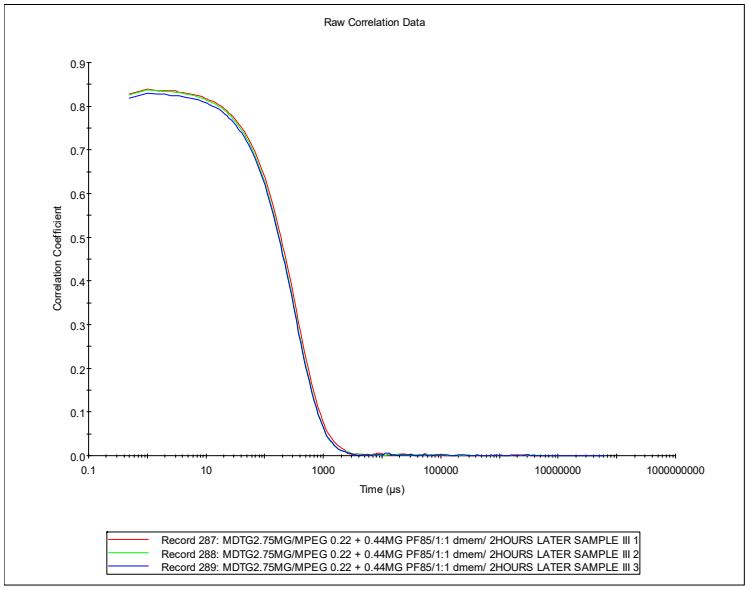
Size by volume



Size by numbers

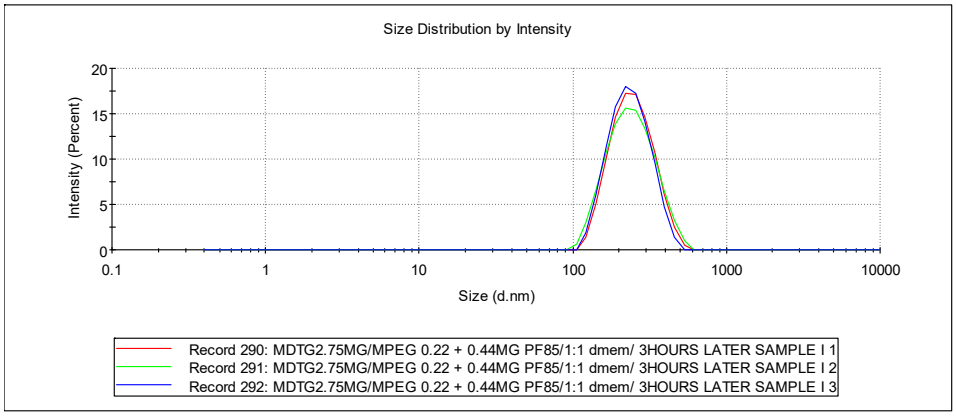


Correlogram

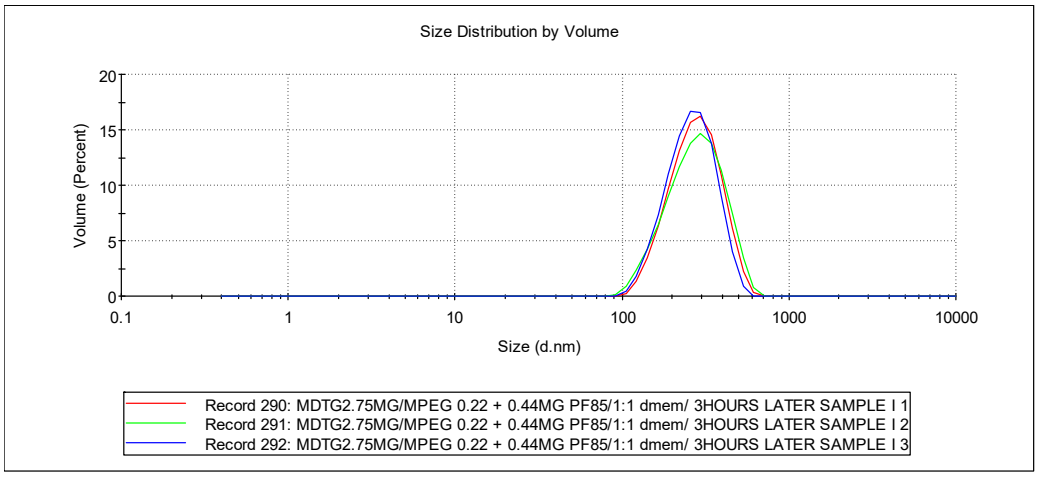


BB) P85 0.44mg coating after 3 hour in DMEM

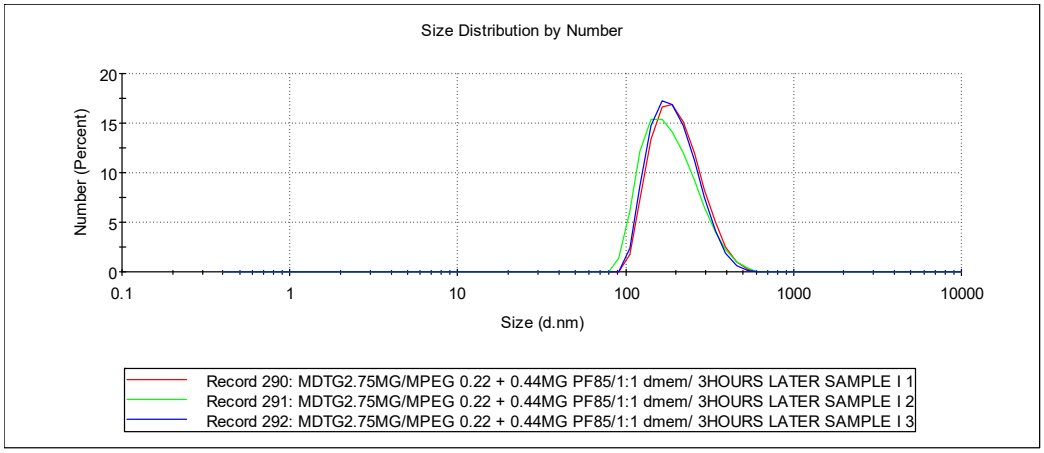
Size by intensity



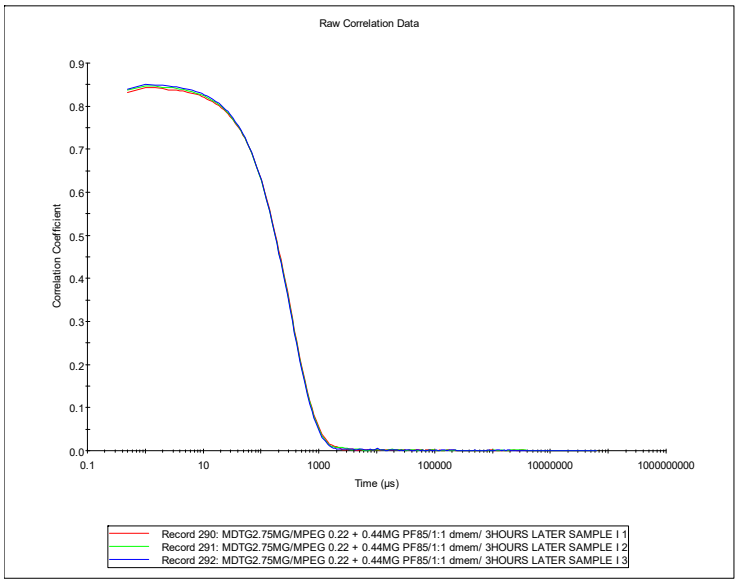
Size by volume



Size by numbers

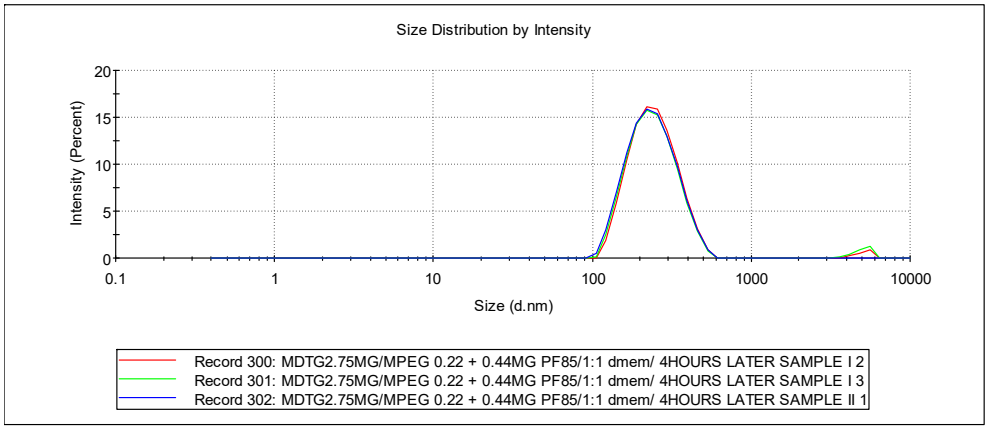


Correlelogram

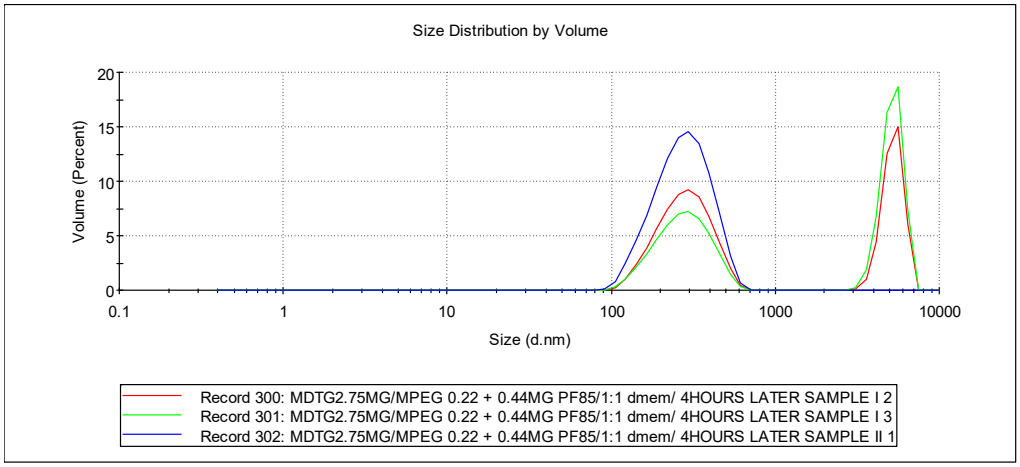


CC) P85 0.44mg coating after 4 hour in DMEM

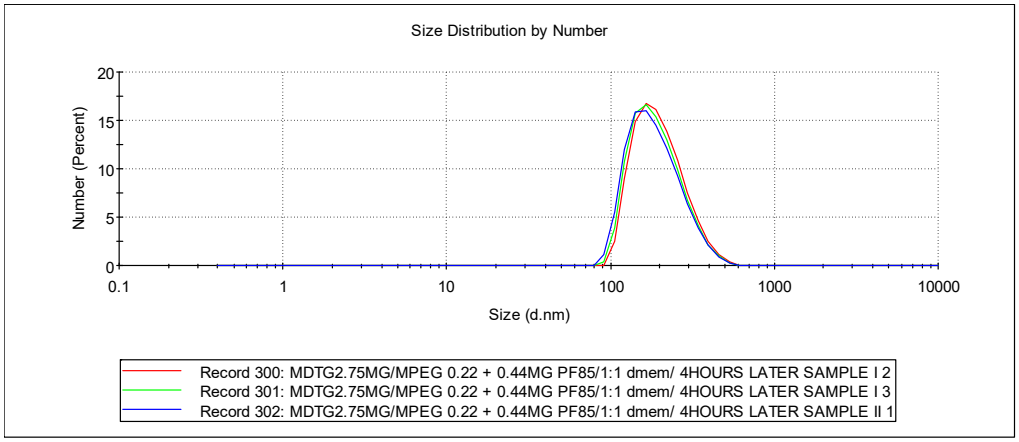
Size by intensity



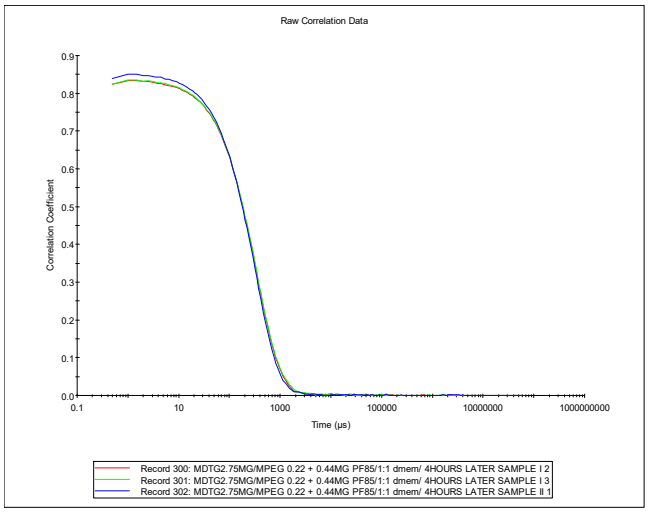
Size by volume



Size by numbers

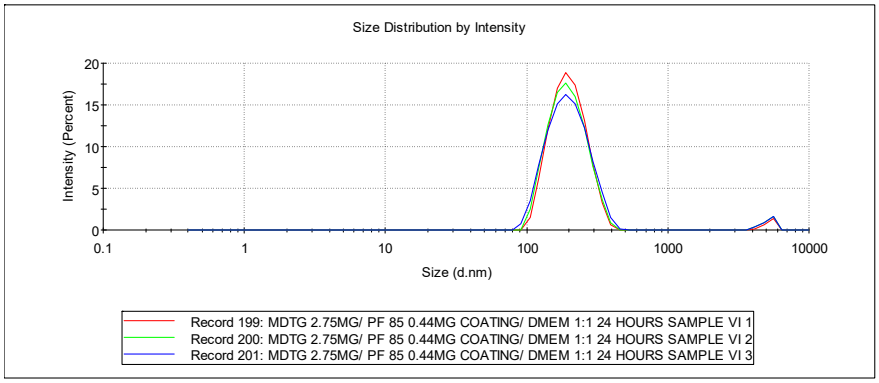


Correlelogram

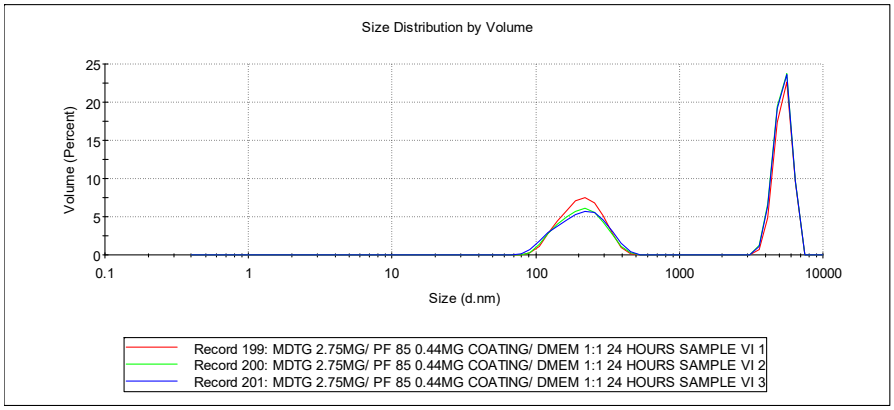


DD) P85 0.44mg coating after 4 hour in DMEM

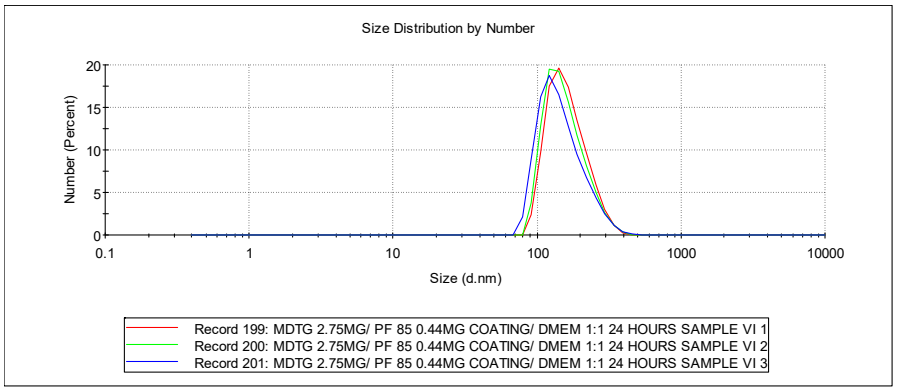
Size by intensity



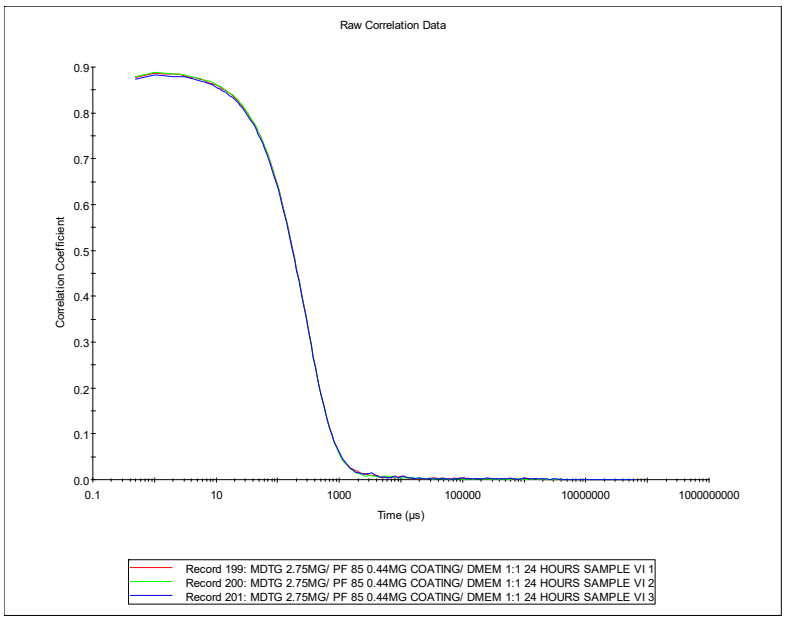
Size by volume



Size by numbers



Correlelogram



References

1. UNAIDS, Nations JU, HIV/AIDS Po. GLOBAL AIDS UPDATE 2020. Seizing the moment. Tackling entrenched inequalities to end epidemics.

. In: Nations JU, HIV/AIDS Po, editors. Switzerland: UNAIDS; 2020.

2. (UNAIDS) JUNPoHA. Oral Pre-Exposure Prophylaxis: Questions and Answers. 2016.

3. Bor J, Fischer C, Modi M, Richman B, Kinker C, King R, et al. Changing Knowledge and Attitudes Towards HIV Treatment-as-Prevention and "Undetectable = Untransmittable": A Systematic Review. AIDS Behav. 2021:1-16.

4. Avoka C, Adzordor P, Bawontuo V, Akila DA, Kuupiel D. Mapping evidence on HIV status awareness among key and vulnerable populations in sub-Sahara Africa: a scoping review protocol. Systematic Reviews. 2020;9(1):266.

5. Oldenburg CE. Integrated HIV prevention and care for key populations. The Lancet HIV. 2019;6(5):e270-e1.

6. UNAIDS, Nations JU, HIV/AIDS Po. Prevailing against pandemics by putting people at the centre. In: UNAIDS, editor. Switzerland: UNAIDS; 2020.

7. UNAIDS, Nations JU, HIV/AIDS Po. 90-90-90 An ambitious treatment target to help end the AIDS epidemic. UNAIDS; 2014.

8. Cohen MS, McCauley M, Gamble TR. HIV treatment as prevention and HPTN 052. Curr Opin HIV AIDS. 2012;7(2):99-105.

9. Grinsztejn B, Hosseinipour MC, Ribaudo HJ, Swindells S, Eron J, Chen YQ, et al. Effects of early versus delayed initiation of antiretroviral treatment on clinical outcomes of HIV-1 infection: results from the phase 3 HPTN 052 randomised controlled trial. The Lancet Infectious Diseases. 2014;14(4):281-90.

10. Bavinton BR, Jin F, Prestage G, Zablotska I, Koelsch KK, Phanuphak N, et al. The Opposites Attract Study of viral load, HIV treatment and HIV transmission in serodiscordant homosexual male couples: design and methods. BMC Public Health. 2014;14(1):917.

11. Rodger AJ, Cambiano V, Bruun T, Vernazza P, Collins S, Degen O, et al. Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): final results of a multicentre, prospective, observational study. The Lancet. 2019;393(10189):2428-38.

12. Walensky RP, Ross EL, Kumarasamy N, Wood R, Noubary F, Paltiel AD, et al. Cost-Effectiveness of HIV Treatment as Prevention in Serodiscordant Couples. New England Journal of Medicine. 2013;369(18):1715-25.

13. Hooper E, Hamilton WD. 1959 Manchester case of syndrome resembling AIDS. The Lancet. 1996;348(9038):1363-5.

14. Corbitt G, Bailey A, Williams G. HIV infection in Manchester, 1959. The Lancet. 1990;336(8706):51.

15. Zhu T, Ho DD. Was HIV present in 1959? Nature. 1995;374(6522):503-4.

16. Worobey M, Gemmel M, Teuwen DE, Haselkorn T, Kunstman K, Bunce M, et al. Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. Nature. 2008;455(7213):661-4.

17. Pneumocystis Pneumonia — Los Angeles. Morbidity and Mortality Weekly Report. 1981;30(21):250-2.

18. Hofmann BO, Kryger P, Pedersen NS, Nielsen JO, Oehlenschlager J, Koerner EA, et al. Sexually Transmitted Diseases, Antibodies to Human Immunodeficiency Virus, and Subsequent Development of Acquired Immunodeficiency Syndrome: Visitors of Homosexual Sauna Clubs in Copenhagen, 7 982–7 983. Sexually Transmitted Diseases. 1988;15(1).

19. Broder S, Gallo RC. A pathogenic retrovirus (HTLV-III) linked to AIDS. N Engl J Med. 1984;311(20):1292-7.
20. Barré-Sinoussi F. HIV: A Discovery Opening the Road to Novel Scientific Knowledge and Global Health Improvement (Nobel Lecture). Angewandte Chemie International Edition. 2009;48(32):5809-14.
21. Barrh -Sinoussi F, Chermann J, Rey F, Nugeyre M, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 1983;220 4599:868-71.
22. Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science. 1984;224(4648):500.
23. Deeks SG, Overbaugh J, Phillips A, Buchbinder S. HIV infection. Nature Reviews Disease Primers. 2015;1(1):15035.
24. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011;1(1):a006841-a.
25. Sauter D, Kirchhoff F. Key Viral Adaptations Preceding the AIDS Pandemic. Cell Host & Microbe. 2019;25(1):27-38.
26. Gottlieb GS, Raugi DN, Smith RA. 90-90-90 for HIV-2? Ending the HIV-2 epidemic by enhancing care and clinical management of patients infected with HIV-2. Lancet HIV. 2018;5(7):e390-e9.
27. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 Infection. New England Journal of Medicine. 2011;364(20):1943-54.
28. Månsson F, Medstrand P, Jansson M, Fenyo E-M, Esbjörnsson J. Transmission HIV-2: Origin, Epidemiology, and Phenotype. 2014.
29. Sáez-Cirión A, Manel N. Immune Responses to Retroviruses. Annual Review of Immunology. 2018;36(1):193-220.
30. Spira AI, Marx P, Patterson B, Mahoney J, Koup RA, Wolinsky S, et al. Cellular Targets of Infection and Route of Viral Dissemination after an Intravaginal Inoculation of Simian Immunodeficiency Virus into Rhesus Macaques. The Journal of experimental medicine. 1996;183:215-25.
31. Emau P, Jiang Y, Agy MB, Tian B, Bekele G, Tsai CC. Post-exposure prophylaxis for SIV revisited: animal model for HIV prevention. AIDS Res Ther. 2006;3:29.
32. Baggaley RF, Boily M-C, White RG, Alary M. Risk of HIV-1 transmission for parenteral exposure and blood transfusion: a systematic review and meta-analysis. AIDS. 2006;20(6):805-12.
33. Wilson DP, Law MG, Grulich AE, Cooper DA, Kaldor JM. Relation between HIV viral load and infectiousness: a model-based analysis. The Lancet. 2008;372(9635):314-20.
34. Thomson KA, Hughes J, Baeten JM, John-Stewart G, Celum C, Cohen CR, et al. Increased Risk of HIV Acquisition Among Women Throughout Pregnancy and During the Postpartum Period: A Prospective Per-Coital-Act Analysis Among Women With HIV-Infected Partners. The Journal of Infectious Diseases. 2018;218(1):16-25.
35. Chen X, Wang Z-X, Pan X-M. HIV-1 tropism prediction by the XGboost and HMM methods. Scientific Reports. 2019;9(1):9997.
36. J. Gordon Betts KAY, James A. Wise, Eddie Johnson, Brandon Poe, Dean H. Kruse, Oksana Korol, Jody E. Johnson, Mark Womble, Peter DeSaix. The Lymphatic and Immune system Houston, Texas: OpenStax; 2013 [Available from:

https://openstax.org/books/anatomy-and-physiology/pages/21-1-anatomy-of-the-lymphatic-and-immune-systems.
37. Scholz EMB, Kashuba ADM. The Lymph Node Reservoir: Physiology, HIV Infection, and Antiretroviral Therapy. Clin Pharmacol Ther. 2021;109(4):918-27.
38. Willard-Mack CL. Normal Structure, Function, and Histology of Lymph Nodes. Toxicologic Pathology. 2006;34(5):409-24.
39. Baroni CD, Uccini S. Lymph nodes in HIV-positive drug abusers with persistent generalized lymphadenopathy: histology, immunohistochemistry, and pathogenetic correlations. Prog AIDS Pathol. 1990;2:33-50.
40. Scholz EMB, Kashuba ADM. The Lymph Node Reservoir: Physiology, HIV Infection, and Antiretroviral Therapy. Clinical Pharmacology & Therapeutics. 2021;109(4):918-27.
41. Vago L, Antonacci MC, Cristina S, Parravicini C, Lazzarin A, Moroni M, et al. Morphogenesis, evolution and prognostic significance of lymphatic tissue lesions in HIV infection. Appl Pathol. 1989;7(5):298-309.
42. Wong JK, Yukl SA. Tissue reservoirs of HIV. Curr Opin HIV AIDS. 2016;11(4):362-70.
43. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep. 1992;41(Rr-17):1-19.
44. Simon B, Grabmeier-Pfistershammer K, Rieger A, Sarcletti M, Schmied B, Puchhammer-Stöckl E. HIV coreceptor tropism in antiretroviral treatment-naive patients newly diagnosed at a late stage of HIV infection. AIDS. 2010;24(13).
45. Kirchhoff F. HIV Life Cycle: Overview. In: Hope TJ, Stevenson M, Richman D, editors. Encyclopedia of AIDS. New York, NY: Springer New York; 2021. p. 1-9.
46. Langtry HD, Campoli-Richards DM. Zidovudine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs. 1989;37(4):408-50.
47. Lauring AS, Andino R. Quasispecies Theory and the Behavior of RNA Viruses. PLOS Pathogens. 2010;6(7):e1001005.
48. Yu F, Wen Y, Wang J, Gong Y, Feng K, Ye R, et al. The Transmission and Evolution of HIV-1 Quasispecies within One Couple: a Follow-up Study based on Next-Generation Sequencing. Scientific Reports. 2018;8(1):1404.
49. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. The Lancet. 2014;384(9939):258-71.
50. Kitahata MM, Koepsell TD, Deyo RA, Maxwell CL, Dodge WT, Wagner EH. Physicians' experience with the acquired immunodeficiency syndrome as a factor in patients' survival. N Engl J Med. 1996;334(11):701-6.
51. Shao J, Kraft JC, Li B, Yu J, Freeling J, Koehn J, et al. Nanodrug formulations to enhance HIV drug exposure in lymphoid tissues and cells: clinical significance and potential impact on treatment and eradication of HIV/AIDS. Nanomedicine (Lond). 2016;11(5):545-64.
52. Rackal JM, Tynan AM, Handford CD, Rzeznikiewiz D, Agha A, Glazier R. Provider training and experience for people living with HIV/AIDS. Cochrane Database Syst Rev. 2011(6):Cd003938.
53. Blankson JN, Persaud D, Siliciano RF. The Challenge of Viral Reservoirs in HIV-1 Infection. Annual Review of Medicine. 2002;53(1):557-93.
54. Wong JK, Günthard HF, Havlir DV, Zhang Z, Haase AT, Ignacio CC, et al. Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. Proc Natl Acad Sci U S A. 1997;94 23:12574-9.

55. Lafeuillade A, Chollet L, Hittinger G, Profizi N, xe, rina, et al. Residual Human Immunodeficiency Virus Type 1 RNA in Lymphoid Tissue of Patients with Sustained Plasma RNA of <200 Copies/mL. The Journal of Infectious Diseases. 1998;177(1):235-8.
56. Harrigan PR, Whaley M, Montaner JSG. Rate of HIV-1 RNA rebound upon stopping antiretroviral therapy. AIDS. 1999;13 8:F59-62.
57. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med. 2003;9(6):727-8.
58. Wong J, Hezareh M, Günthard H, Havlir D, Ignacio C, Spina C, et al. Recovery of Replication-Competent HIV Despite Prolonged Suppression of Plasma Viremia. Science (New York, NY). 1997;278:1291-5.
59. Zhang Y, Benet LZ. The Gut as a Barrier to Drug Absorption. Clinical Pharmacokinetics. 2001;40(3):159-68.
60. Trainor GL. The importance of plasma protein binding in drug discovery. Expert Opin Drug Discov. 2007;2(1):51-64.
61. Fletcher CV, Podany AT. Antiretroviral Drug Penetration into Lymphoid Tissue. In: Hope TJ, Stevenson M, Richman D, editors. Encyclopedia of AIDS. New York, NY: Springer New York; 2014. p. 1-9.
62. Cesar C, Shepherd BE, Jenkins CA, Ghidinelli M, Castro JL, Veloso VG, et al. Use of third line antiretroviral therapy in Latin America. PLoS One. 2014;9(9):e106887.
63. Paredes R, Tzou PL, van Zyl G, Barrow G, Camacho R, Carmona S, et al. Collaborative update of a rule-based expert system for HIV-1 genotypic resistance test interpretation. PloS one [Internet]. 2017 2017; 12(7):[e0181357 p.]. Available from: http://europepmc.org/abstract/MED/28753637

https://doi.org/10.1371/journal.pone.0181357

https://europepmc.org/articles/PMC5533429

https://europepmc.org/articles/PMC5533429?pdf=render.
64. Wainberg MA, Han Y-S. Will drug resistance against dolutegravir in initial therapy ever occur? Frontiers in Pharmacology. 2015;6(90).
65. Wainberg MA, Han Y-S, Mesplède T. Might dolutegravir be part of a functional cure for HIV? Canadian Journal of Microbiology. 2016;62(5):375-82.
66. Brenner BG, Wainberg MA. Clinical benefit of dolutegravir in HIV-1 management related to the high genetic barrier to drug resistance. Virus Research. 2017;239:1-9.
67. Anstett K, Brenner B, Mesplede T, Wainberg MA. HIV drug resistance against strand transfer integrase inhibitors. Retrovirology. 2017;14(1):36.
68. Orkin C, Arasteh K, Górgolas Hernández-Mora M, Pokrovsky V, Overton ET, Girard P-M, et al. Long-Acting Cabotegravir and Rilpivirine after Oral Induction for HIV-1 Infection. New England Journal of Medicine. 2020;382(12):1124-35.
69. Flexner C, Owen A, Siccardi M, Swindells S. Long-acting drugs and formulations for the treatment and prevention of HIV infection. International Journal of Antimicrobial Agents. 2021;57(1):106220.
70. Bares SH, Scarsi KK. A new paradigm for antiretroviral delivery: long-acting cabotegravir and rilpivirine for the treatment and prevention of HIV. Curr Opin HIV AIDS. 2022;17(1):22-31.
71. Berkhout B, Eggink D, Sanders RW. Is there a future for antiviral fusion inhibitors? Current Opinion in Virology. 2012;2(1):50-9.

72. Claborn KR, Meier E, Miller MB, Leffingwell TR. A systematic review of treatment fatigue among HIV-infected patients prescribed antiretroviral therapy. Psychol Health Med. 2015;20(3):255-65.
73. Markham A. Cabotegravir Plus Rilpivirine: First Approval. Drugs. 2020;80(9):915-22.
74. Fernandez C, van Halsema CL. Evaluating cabotegravir/rilpivirine long-acting, injectable in the treatment of HIV infection: emerging data and therapeutic potential. HIV AIDS (Auckl). 2019;11:179-92.
75. Thornhill J, Orkin C. Long-acting injectable HIV therapies: the next frontier. Curr Opin Infect Dis. 2021;34(1):8-15.
76. Kline C, Ndjomou J, Franks T, Kiser R, Coalter V, Smedley J, et al. Persistence of viral reservoirs in multiple tissues after antiretroviral therapy suppression in a macaque RT-SHIV model. PloS one. 2013;8(12):e84275-e.
77. Bourry O, Mannioui A, Sellier P, Roucairol C, Durand-Gasselin L, Dereuddre-Bosquet N, et al. Effect of a short-term HAART on SIV load in macaque tissues is dependent on time of initiation and antiviral diffusion. Retrovirology. 2010;7:78-.
78. Leyre L, Kroon E, Vandergeeten C, Sacdalan C, Colby DJ, Buranapraditkun S, et al. Abundant HIV-infected cells in blood and tissues are rapidly cleared upon ART initiation during acute HIV infection. Sci Transl Med. 2020;12(533).
79. Vibholm LK, Lorenzi JCC, Pai JA, Cohen YZ, Oliveira TY, Barton JP, et al. Characterization of Intact Proviruses in Blood and Lymph Node from HIV-Infected Individuals Undergoing Analytical Treatment Interruption. J Virol. 2019;93(8):e01920-18.
80. McManus WR, Bale MJ, Spindler J, Wiegand A, Musick A, Patro SC, et al. HIV-1 in lymph nodes is maintained by cellular proliferation during antiretroviral therapy. The Journal of Clinical Investigation. 2019;129(11):4629-42.
81. Rose R, Lamers SL, Nolan DJ, Maidji E, Faria NR, Pybus OG, et al. HIV Maintains an Evolving and Dispersed Population in Multiple Tissues during Suppressive Combined Antiretroviral Therapy in Individuals with Cancer. J Virol. 2016;90(20):8984-93.
82. Günthard HF, Calvez V, Paredes R, Pillay D, Shafer RW, Wensing AM, et al. Human Immunodeficiency Virus Drug Resistance: 2018 Recommendations of the International Antiviral Society–USA Panel. Clinical Infectious Diseases. 2018;68(2):177-87.
83. Günthard HF, Havlir DV, Fiscus S, Zhang Z-Q, Eron J, Mellors J, et al. Residual Human Immunodeficiency Virus (HIV) Type 1 RNA and DNA in Lymph Nodes and HIV RNA in Genital Secretions and in Cerebrospinal Fluid after Suppression of Viremia for 2 Years. The Journal of Infectious Diseases. 2001;183(9):1318-27.
84. Lorenzo-Redondo R, Fryer HR, Bedford T, Kim E-Y, Archer J, Kosakovsky Pond SL, et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature. 2016;530(7588):51-6.
85. Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, et al. DrugBank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res. 2008;36(Database issue):D901-6.
86. Trevaskis NL, Kaminskas LM, Porter CJH. From sewer to saviour — targeting the lymphatic system to promote drug exposure and activity. Nature Reviews Drug Discovery. 2015;14(11):781-803.
87. Golombek SK, May JN, Theek B, Appold L, Drude N, Kiessling F, et al. Tumor targeting via EPR: Strategies to enhance patient responses. Adv Drug Deliv Rev. 2018;130:17-38.

88. Casado JL, Monsalvo M, Rojo AM, Fontecha M, Rodriguez-Sagrado MA. Dolutegravir and rilpivirine for the maintenance treatment of virologically suppressed HIV-1 infection. Expert Rev Clin Pharmacol. 2018;11(6):561-70.
89. Adolescents PoAGfAa. Guidelines for the use of Antiretroviral agents in Adults and Adolescents with HIV 2021 (Accessed on May 5th 2021).
90. Patel P, Borkowf CB, Brooks JT, Lasry A, Lansky A, Mermin J. Estimating per-act HIV transmission risk: a systematic review. AIDS. 2014;28(10).
91. Cornelisse VJ, Fairley CK, Stoove M, Asselin J, Chow EPF, Price B, et al. Evaluation of Preexposure (PrEP) Eligibility Criteria, Using Sexually Transmissible Infections as Markers of Human Immunodeficiency Virus (HIV) Risk at Enrollment in PrEPX, a Large Australian HIV PrEP Trial. Clinical Infectious Diseases. 2018;67(12):1847-52.
92. Gibas KM, van den Berg P, Powell VE, Krakower DS. Drug Resistance During HIV Pre-Exposure Prophylaxis. Drugs. 2019;79(6):609-19.
93. Irvine C, Egan KJ, Shubber Z, Van Rompay KKA, Beanland RL, Ford N. Efficacy of HIV Postexposure Prophylaxis: Systematic Review and Meta-analysis of Nonhuman Primate Studies. Clinical Infectious Diseases. 2015;60(suppl_3):S165-S9.
94. Territo H, Justiz Vaillant AA. HIV Prophylaxis. StatPearls. Treasure Island (FL): StatPearls Publishing

Copyright © 2021, StatPearls Publishing LLC.; 2021.

95. Cresswell F, Waters L, Briggs E, Fox J, Harbottle J, Hawkins D, et al. UK guideline for the use of HIV Post-Exposure Prophylaxis Following Sexual Exposure, 2015. International Journal of STD & AIDS. 2016;27(9):713-38.
96. Dominguez KL, Smith DK, Vasavi T, Crepaz N, Lang K, Heneine W, et al. Updated guidelines for antiretroviral postexposure prophylaxis after sexual, injection drug use, or other nonoccupational exposure to HIV—United States, 2016. 2016.
97. Kuhar DT, Henderson DK, Struble KA, Heneine W, Thomas V, Cheever LW, et al. Updated US Public Health Service Guidelines for the Management of Occupational Exposures to Human Immunodeficiency Virus and Recommendations for Postexposure Prophylaxis. Infection Control & Hospital Epidemiology. 2013;34(9):875-92.
98. John SA, Quinn KG, Pleuhs B, Walsh JL, Petroll AE. HIV Post-Exposure Prophylaxis (PEP) Awareness and Non-Occupational PEP (nPEP) Prescribing History Among U.S. Healthcare Providers. AIDS Behav. 2020;24(11):3124-31.
99. Mirzaei H, McFarland W, Karamouzian M, Sharifi H. COVID-19 Among People Living with HIV: A Systematic Review. AIDS Behav. 2021;25(1):85-92.
100. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. Cell Host & Microbe. 2020;27(3):325-8.
101. Hogan AB, Jewell BL, Sherrard-Smith E, Vesga JF, Watson OJ, Whittaker C, et al. Potential impact of the COVID-19 pandemic on HIV, tuberculosis, and malaria in low-income and middle-income countries: a modelling study. The Lancet Global Health. 2020;8(9):e1132-e41.
102. Callaway E. Could new COVID variants undermine vaccines? Labs scramble to find out. Nature. 2021;589(7841):177-8.
103. Mitchell KM, Dimitrov D, Silhol R, Geidelberg L, Moore M, Liu A, et al. The potential effect of COVID-19-related disruptions on HIV incidence and HIV-related mortality among men who have sex with men in the USA: a modelling study. Lancet HIV. 2021;8(4):e206-e15.

104. Booton RD, Fu G, MacGregor L, Li J, Ong JJ, Tucker JD, et al. The impact of disruptions due to COVID-19 on HIV transmission and control among men who have sex with men in China. Journal of the International AIDS Society. 2021;24(4):e25697.
105. Lesosky M, Myer L. Modelling the impact of COVID-19 on HIV. The Lancet HIV. 2020;7(9):e596-e8.
106. Announcement: Updated Guidelines for Antiretroviral Postexposure Prophylaxis after Sexual, Injection-Drug Use, or Other Nonoccupational Exposure to HIV - United States, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(17):458.
107. Churchill D, Waters L, Ahmed N, Angus B, Boffito M, Bower M, et al. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015. HIV Medicine. 2016;17:s2-s104.
108. Kaplan JE, Dominguez K, Jobarteh K, Spira TJ. PostexposureProphylaxisAgainst human immunodeficiency virus (HIV): New guidelines from the WHO: a perspective. Clin infect dis. 2015;3.
109. Chacko L, Ford N, Sbaiti M, Siddiqui R. Adherence to HIV post-exposure prophylaxis in victims of sexual assault: a systematic review and meta-analysis. Sex Transm Infect. 2012;88(5):335-41.
110. Calzavara L, Burchell AN, Remis RS, Major C, Corey P, Myers T, et al. Delayed Application of Condoms Is a Risk Factor for Human Immunodeficiency Virus Infection among Homosexual and Bisexual Men. American Journal of Epidemiology. 2003;157(3):210-7.
111. Yuen GJ, Weller S, Pakes GE. A review of the pharmacokinetics of abacavir. Clin Pharmacokinet. 2008;47(6):351-71.
112. Tang MW, Shafer RW. HIV-1 Antiretroviral Resistance. Drugs. 2012;72(9):e1-e25.
113. Vrouenraets SM, Wit FW, van Tongeren J, Lange JM. Efavirenz: a review. Expert Opin Pharmacother. 2007;8(6):851-71.
114. Gallant JE. The M184V mutation: what it does, how to prevent it, and what to do with it when it's there. AIDS Read. 2006;16(10):556-9.
115. Kobayashi M, Yoshinaga T, Seki T, Wakasa-Morimoto C, Brown KW, Ferris R, et al. In Vitro Antiretroviral Properties of S/GSK1349572, a Next-Generation HIV Integrase Inhibitor. Antimicrobial Agents and Chemotherapy. 2011;55(2):813-21.
116. Raffi F, Jaeger H, Quiros-Roldan E, Albrecht H, Belonosova E, Gatell JM, et al. Once-daily dolutegravir versus twice-daily raltegravir in antiretroviral-naive adults with HIV-1 infection (SPRING-2 study): 96 week results from a randomised, double-blind, non-inferiority trial. The Lancet Infectious Diseases. 2013;13(11):927-35.
117. Rhee SY, Grant PM, Tzou PL, Barrow G, Harrigan PR, Ioannidis JPA, et al. A systematic review of the genetic mechanisms of dolutegravir resistance. J Antimicrob Chemother. 2019;74(11):3135-49.
118. Tang MW, Shafer RW. HIV-1 Antiretroviral Resistance: Scientific Principles and Clinical Applications. Drugs. 2012;72(9):e1-e25.
119. Wainberg MA, Han Y-S. Will drug resistance against dolutegravir in initial therapy ever occur? Frontiers in Pharmacology. 2015;6:90.
120. Balzarini J, Van Damme L. Microbicide drug candidates to prevent HIV infection. The Lancet. 2007;369(9563):787-97.
121. Baeten JM, Hendrix CW, Hillier SL. Topical Microbicides in HIV Prevention: State of the Promise. Annual Review of Medicine. 2020;71(1):361-77.
122. Stein Z. HIV prevention: an update on the status of methods women can use. Am J Public Health. 1993;83(10):1379-82.

123. Woodrow KA, Bennett KM, Lo DD. Mucosal Vaccine Design and Delivery. Annual Review of Biomedical Engineering. 2012;14:17-46.
124. Ensign LM, Cone R, Hanes J. Nanoparticle-based drug delivery to the vagina: a review. J Control Release. 2014;190:500-14.
125. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, et al. Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women. Science. 2010;329(5996):1168-74.
126. Baxter C, Yende-Zuma N, Tshabalala P, Abdool Karim Q, Abdool Karim SS. Safety of coitally administered tenofovir 1% gel, a vaginal microbicide, in chronic hepatitis B virus carriers: results from the CAPRISA 004 trial. Antiviral Res. 2013;99(3):405-8.
127. Hua S. Physiological and Pharmaceutical Considerations for Rectal Drug Formulations. Frontiers in Pharmacology. 2019;10(1196).
128. Cone RA. Barrier properties of mucus. Adv Drug Deliv Rev. 2009;61(2):75-85.
129. ODEBLAD E. THE FUNCTIONAL STRUCTURE OF HUMAN CERVICAL MUCUS. Acta Obstetricia et Gynecologica Scandinavica. 1968;47(S1):57-79.
130. Katz DF, Dunmire EN. Cervical mucus: Problems and opportunities for drug delivery via the vagina and cervix. Advanced Drug Delivery Reviews. 1993;11(3):385-401.
131. Lai SK, Wang Y-Y, Hida K, Cone R, Hanes J. Nanoparticles reveal that human cervicovaginal mucus is riddled with pores larger than viruses. Proc Natl Acad Sci U S A. 2010;107(2):598-603.
132. Acartürk F. Mucoadhesive vaginal drug delivery systems. Recent Pat Drug Deliv Formul. 2009;3(3):193-205.
133. Moench TR, Mumper RJ, Hoen TE, Sun M, Cone RA. Microbicide excipients can greatly increase susceptibility to genital herpes transmission in the mouse. BMC Infectious Diseases. 2010;10(1):331.
134. Kumamoto Y, Iwasaki A. Unique features of antiviral immune system of the vaginal mucosa. Curr Opin Immunol. 2012;24(4):411-6.
135. Wira CR, Fahey JV, Ghosh M, Patel MV, Hickey DK, Ochiel DO. REVIEW ARTICLE: Sex Hormone Regulation of Innate Immunity in the Female Reproductive Tract: The Role of Epithelial Cells in Balancing Reproductive Potential with Protection against Sexually Transmitted Pathogens. American Journal of Reproductive Immunology. 2010;63(6):544-65.
136. Dezzutti CS, Brown ER, Moncla B, Russo J, Cost M, Wang L, et al. Is Wetter Better? An Evaluation of Over-the-Counter Personal Lubricants for Safety and Anti-HIV-1 Activity. PLOS ONE. 2012;7(11):e48328.
137. Hel Z, Stringer E, Mestecky J. Sex steroid hormones, hormonal contraception, and the immunobiology of human immunodeficiency virus-1 infection. Endocr Rev. 2010;31(1):79-97.
138. Baeten JM, Palanee-Phillips T, Brown ER, Schwartz K, Soto-Torres LE, Govender V, et al. Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women. New England Journal of Medicine. 2016;375(22):2121-32.
139. Marrazzo JM, Ramjee G, Richardson BA, Gomez K, Mgodi N, Nair G, et al. Tenofovir-Based Preexposure Prophylaxis for HIV Infection Among African Women. Obstetrical & Gynecological Survey. 2015;70(7):444-6.
140. Delany-Moretlwe S, Lombard C, Baron D, Bekker L-G, Nkala B, Ahmed K, et al. Tenofovir 1% vaginal gel for prevention of HIV-1 infection in women in South Africa (FACTS-001): a phase 3, randomised, double-blind, placebo-controlled trial. The Lancet Infectious Diseases. 2018;18(11):1241-50.

141. Nel A, van Niekerk N, Kapiga S, Bekker L-G, Gama C, Gill K, et al. Safety and Efficacy of a Dapivirine Vaginal Ring for HIV Prevention in Women. New England Journal of Medicine. 2016;375(22):2133-43.
142. Riddler SA, Balkus JE, Parikh UM, Mellors JW, Akello C, Dadabhai S, et al. Clinical and Virologic Outcomes Following Initiation of Antiretroviral Therapy Among Seroconverters in the Microbicide Trials Network-020 Phase III Trial of the Dapivirine Vaginal Ring. Clinical Infectious Diseases. 2018;69(3):523-9.
143. Gichane MW, Katz AWK, Ngure K, Scheckter R, Woeber K, Reddy K, et al. Impact of Women's Home Environment on Use of the Dapivirine Vaginal Ring for HIV Prevention in Sub-Saharan Africa. AIDS and Behavior. 2021.
144. Shafik A, Mostafa RM, Shafik I, Ei-Sibai O, Shafik AA. Functional activity of the rectum: A conduit organ or a storage organ or both? World J Gastroenterol. 2006;12(28):4549-52.
145. Leppik IE, Patel SI. Intramuscular and rectal therapies of acute seizures. Epilepsy Behav. 2015;49:307-12.
146. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology. 2008;134(2):577-94.
147. de Boer AG, Moolenaar F, de Leede LG, Breimer DD. Rectal drug administration: clinical pharmacokinetic considerations. Clin Pharmacokinet. 1982;7(4):285-311.
148. De Boer AG, De Leede LG, Breimer DD. Drug absorption by sublingual and rectal routes. Br J Anaesth. 1984;56(1):69-82.
149. Macfarlane GT, Macfarlane S. Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics. J Clin Gastroenterol. 2011;45 Suppl:S120-7.
150. van Hoogdalem EJ, de Boer AG, Breimer DD. Pharmacokinetics of Rectal Drug Administration, Part I. Clinical Pharmacokinetics. 1991;21(1):11-26.
151. Nunes R, Araújo F, Barreiros L, Bártolo I, Segundo MA, Taveira N, et al. Noncovalent PEG Coating of Nanoparticle Drug Carriers Improves the Local Pharmacokinetics of Rectal Anti-HIV Microbicides. ACS Applied Materials & Interfaces. 2018;10(41):34942-53.
152. Hirano I, Brenner D. Gastrointestinal motility. Gastrointestinal Anatomy and Physiology2014. p. 33-45.
153. Sheikh SZ, Plevy SE. Gastrointestinal immunology and ecology. Gastrointestinal Anatomy and Physiology2014. p. 46-57.
154. Panneerselvam D, Budh DP. Peyer Patches. StatPearls. Treasure Island (FL): StatPearls Publishing

Copyright © 2021, StatPearls Publishing LLC.; 2021.

155. Bonnardel J, Da Silva C, Henri S, Tamoutounour S, Chasson L, Montañana-Sanchis F, et al. Innate and adaptive immune functions of peyer's patch monocyte-derived cells. Cell Rep. 2015;11(5):770-84.
156. Mabbott NA, Donaldson DS, Ohno H, Williams IR, Mahajan A. Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. Mucosal Immunol. 2013;6(4):666-77.
157. Bujoreanu I, Gupta V. Anatomy, Lymph Nodes. StatPearls. Treasure Island (FL): StatPearls Publishing

Copyright © 2021, StatPearls Publishing LLC.; 2021.

158. Mikhael M, Khan YS. Anatomy, Abdomen and Pelvis, Lymphatic Drainage. StatPearls. Treasure Island (FL): StatPearls Publishing

Copyright © 2021, StatPearls Publishing LLC.; 2021.
159. Smedley J, Turkbey B, Bernardo ML, Del Prete GQ, Estes JD, Griffiths GL, et al. Tracking the luminal exposure and lymphatic drainage pathways of intravaginal and intrarectal inocula used in nonhuman primate models of HIV transmission. PLoS One. 2014;9(3):e92830.
160. Purohit TJ, Hanning SM, Wu Z. Advances in rectal drug delivery systems. Pharmaceutical Development and Technology. 2018;23(10):942-52.
161. Jannin V, Lemagnen G, Gueroult P, Larrouture D, Tuleu C. Rectal route in the 21st Century to treat children. Adv Drug Deliv Rev. 2014;73:34-49.
162. Mahler G, Shuler M, Glahn R. Characterization of Caco-2 and HT29-MTX cocultures in an in vitro digestion/cell culture model used to predict iron bioavailability. The Journal of nutritional biochemistry. 2008;20:494-502.
163. Crater JS, Carrier RL. Barrier Properties of Gastrointestinal Mucus to Nanoparticle Transport. Macromolecular Bioscience. 2010;10(12):1473-83.
164. Schimpel C, Teubl B, Absenger M, Meindl C, Fröhlich E, Leitinger G, et al. Development of an advanced intestinal in vitro triple culture permeability model to study transport of nanoparticles. Mol Pharm. 2014;11(3):808-18.
165. Cranston RD, Lama JR, Richardson BA, Carballo-Diéguez A, Kunjara Na Ayudhya RP, Liu K, et al. MTN-017: A Rectal Phase 2 Extended Safety and Acceptability Study of Tenofovir Reduced-Glycerin 1% Gel. Clin Infect Dis. 2017;64(5):614-20.
166. Carballo-Diéguez A, Balán IC, Brown W, III, Giguere R, Dolezal C, Leu C-S, et al. High levels of adherence to a rectal microbicide gel and to oral Pre-Exposure Prophylaxis (PrEP) achieved in MTN-017 among men who have sex with men (MSM) and transgender women. PLOS ONE. 2017;12(7):e0181607.
167. Fletcher CV, Staskus K, Wietgrefe SW, Rothenberger M, Reilly C, Chipman JG, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proceedings of the National Academy of Sciences. 2014;111(6):2307-12.
168. Mandal S, Belshan M, Holec A, Zhou Y, Destache CJ. An Enhanced Emtricitabine-Loaded Long-Acting Nanoformulation for Prevention or Treatment of HIV Infection. Antimicrobial Agents and Chemotherapy. 2017;61(1):e01475-16.
169. Sillman B, Bade AN, Dash PK, Bhargavan B, Kocher T, Mathews S, et al. Creation of a long-acting nanoformulated dolutegravir. Nature Communications. 2018;9(1):443.
170. Murray JM, Kelleher AD, Cooper DA. Timing of the components of the HIV life cycle in productively infected CD4+ T cells in a population of HIV-infected individuals. J Virol. 2011;85(20):10798-805.
171. Parienti J-J. On-demand PrEP efficacy: forgiveness or timely dosing. The Lancet HIV. 2020;7(2):e79-e80.
172. Tan DH-S. PrEP on demand or every day? The Lancet HIV. 2017;4(9):e379-e80.
173. Siedner MJ, Tumarkin E, Bogoch II. HIV post-exposure prophylaxis (PEP). BMJ. 2018;363:k4928.
174. Alghamdi A, Hempel A, Heendeniya A, Clifford-Rashotte M, Tan DHS, Bogoch II. HIV postexposure prophylaxis-in-pocket: long-term follow-up of individuals with low-frequency, high-risk HIV exposures. AIDS. 2020;34(3).
175. Wood BR. Prevention of HIV for persons with low-frequency, high-risk exposures: PrEP (preexposure prophylaxis), PEP (postexposure prophylaxis), or 'PIP' (postexposure prophylaxis in-pocket). Aids. 2020;34(3):481-2.
176. Morris BJ, Hankins CA. Effect of male circumcision on risk of sexually transmitted infections and cervical cancer in women. The Lancet Global Health. 2017;5(11):e1054-e5.

177. Tobian AAR, Kacker S, Quinn TC. Male Circumcision: A Globally Relevant but Under-Utilized Method for the Prevention of HIV and Other Sexually Transmitted Infections. Annual Review of Medicine. 2014;65(1):293-306.
178. Gray RH, Kigozi G, Serwadda D, Makumbi F, Watya S, Nalugoda F, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. Lancet. 2007;369(9562):657-66.
179. Bailey RC, Moses S, Parker CB, Agot K, Maclean I, Krieger JN, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. Lancet. 2007;369(9562):643-56.
180. Millett GA, Flores SA, Marks G, Reed JB, Herbst JH. Circumcision status and risk of HIV and sexually transmitted infections among men who have sex with men: a meta-analysis. Jama. 2008;300(14):1674-84.
181. Smith DK, Herbst JH, Zhang X, Rose CE. Condom effectiveness for HIV prevention by consistency of use among men who have sex with men in the United States. J Acquir Immune Defic Syndr. 2015;68(3):337-44.
182. Weller S, Davis K. Condom effectiveness in reducing heterosexual HIV transmission. Cochrane Database Syst Rev. 2001(3):Cd003255.
183. Weir SS, Roddy RE, Zekeng L, Feldblum PJ. Nonoxynol-9 use, genital ulcers, and HIV infection in a cohort of sex workers. Genitourin Med. 1995;71(2):78-81.
184. Update: barrier protection against HIV infection and other sexually transmitted diseases. MMWR Morb Mortal Wkly Rep. 1993;42(30):589-91, 97.
185. Cook RL, Rosenberg MJ. Do spermicides containing nonoxynol-9 prevent sexually transmitted infections? A meta-analysis. Sex Transm Dis. 1998;25(3):144-50.
186. Feldblum PJ, Morrison CS, Roddy RE, Cates W, Jr. The effectiveness of barrier methods of contraception in preventing the spread of HIV. Aids. 1995;9 Suppl A:S85-93.
187. Wiyeh AB, Mome RKB, Mahasha PW, Kongnyuy EJ, Wiysonge CS. Effectiveness of the female condom in preventing HIV and sexually transmitted infections: a systematic review and meta-analysis. BMC Public Health. 2020;20(1):319.
188. Gallo MF, Kilbourne-Brook M, Coffey PS. A review of the effectiveness and acceptability of the female condom for dual protection. Sex Health. 2012;9(1):18-26.
189. Moore L, Beksinska M, Nisbett A, Festin M, Gollub E. Knowledge, attitudes, practices and behaviors associated with female condoms in developing countries: a scoping review. Open Access Journal of Contraception. 2015;2015:125-42.
190. Giannou FK, Tsiara CG, Nikolopoulos GK, Talias M, Benetou V, Kantzanou M, et al. Condom effectiveness in reducing heterosexual HIV transmission: a systematic review and meta-analysis of studies on HIV serodiscordant couples. Expert Rev Pharmacoecon Outcomes Res. 2016;16(4):489-99.
191. Johnson WD, O'Leary A, Flores SA. Per-partner condom effectiveness against HIV for men who have sex with men. AIDS. 2018;32(11).
192. Sanders SA, Yarber WL, Kaufman EL, Crosby RA, Graham CA, Milhausen RR. Condom use errors and problems: a global view. Sex Health. 2012;9(1):81-95.
193. Smith DK, Herbst JH, Zhang X, Rose CE. Condom Effectiveness for HIV Prevention by Consistency of Use Among Men Who Have Sex With Men in the United States. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2015;68(3).
194. Crosby R, Shrier LA, Charnigo RJ, Weathers C, Sanders SA, Graham CA, et al. A prospective event-level analysis of condom use experiences following STI testing among patients in three US cities. Sexually transmitted diseases. 2012;39(10):756-60.

195. Choi SYP, Chen KL, Jiang ZQ. Client-perpetuated violence and condom failure among female sex workers in southwestern China. Sexually transmitted diseases. 2008;35(2):141-6.
196. Tounkara FK, Diabaté S, Guédou FA, Ahoussinou C, Kintin F, Zannou DM, et al. Violence, condom breakage, and HIV infection among female sex workers in Benin, West Africa. Sexually transmitted diseases. 2014;41(5):312-8.
197. Johnson AM, Wadsworth J, Wellings K, Bradshaw S, Field J. Sexual lifestyles and HIV risk. Nature. 1992;360(6403):410-2.
198. McGarrigle CA, Mercer CH, Fenton KA, Copas AJ, Wellings K, Erens B, et al. Investigating the relationship between HIV testing and risk behaviour in Britain: National Survey of Sexual Attitudes and Lifestyles 2000. AIDS. 2005;19(1).
199. Clifton S, Nardone A, Field N, Mercer CH, Tanton C, Macdowall W, et al. HIV testing, risk perception, and behaviour in the British population. AIDS (London, England). 2016;30(6):943-52.
200. Ammi Shah SS, Alison Brown, Sophie Nash, Ross Harris, Peter Kirwan, , Paul Birrell DDA, Anne Presanis, Cuong Chau, Jameel Khawam, Adamma , Aghaizu ANL, Meaghan Kall, Josephine Shonaike, Hamish , Mohammed NR, Gary Murphy, Gwenda Hughes, Ann. K. Sullivan, Nicky Connor , Delpech. aV. Trends in HIV testing, new diagnoses and people receiving HIV-related care in the United Kingdom: data to the end of December 2019. In: Public Health England L, editor. London: Public Health England; 2020.
201. Lodi S, Phillips A, Touloumi G, Geskus R, Meyer L, Thiébaut R, et al. Time from human immunodeficiency virus seroconversion to reaching CD4+ cell count thresholds <200, <350, and <500 Cells/mm³: assessment of need following changes in treatment guidelines. Clin Infect Dis. 2011;53(8):817-25.
202. Chadborn TR, Delpech VC, Sabin CA, Sinka K, Evans BG. The late diagnosis and consequent short-term mortality of HIV-infected heterosexuals (England and Wales, 2000-2004). Aids. 2006;20(18):2371-9.
203. Committee TNA. Thailand National Operational Plan Accelerating Ending AIDS, 2015 - 2019. In: Department of Disease Control MoPHT, editor. Bangkok, Thailand: Department of Disease Control, Ministry of Public Health Thailand; 2014.
204. Palfreeman A, Sullivan A, Rayment M, Waters L, Buckley A, Burns F, et al. British HIV Association/British Association for Sexual Health and HIV/British Infection Association adult HIV testing guidelines 2020. HIV Medicine. 2020;21(S6):1-26.
205. Pankam T, Saensiriphan S, Areeyolwattana S, Barisri N, Pengnonyang S, Sirivichayakul S, et al. The validation and evaluation of anti-HIV testing algorithm used in mobile clinic setting for men who have sex with men in metropolitan Bangkok, Thailand. Asian Pac J Allergy Immunol. 2018;36(1):42-50.
206. Online Surveys platform. In: [JISC] JISC, editor. Online Surveys platform. UK: Joint Information Systems Committee [JISC]; 2021.
207. (2020). RCT. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. .
208. Bozdogan H. Model selection and Akaike's Information Criterion (AIC): The general theory and its analytical extensions. Psychometrika. 1987;52(3):345-70.
209. Trussell J, Warner L, Hatcher R. Condom Slippage and Breakage Rates. Family planning perspectives. 1992;24:20-3.
210. Bradley J, Rajaram SP, Moses S, Boily MC, Ramesh BM, Isac S, et al. Why do condoms break? A study of female sex workers in Bangalore, south India. Sexually Transmitted Infections. 2012;88(3):163.

211. Lindberg LD, Sonenstein FL, Ku L, Levine G. Young Men's Experience with Condom Breakage. Family Planning Perspectives. 1997;29(3):128-40.
212. Lytle DC, Routson LB, Seaborn GB, Dixon LG, Bushar HF, Cyr HW. An In Vitro Evaluation of Condoms as Barriers to a Small Virus. Sexually Transmitted Diseases. 1997;24(3).
213. Gordon R. A critical review of the physics and statistics of condoms and their role in individual versus societal survival of the AIDS epidemic. J Sex Marital Ther. 1989;15(1):5-30.
214. Russell-Brown P, Piedrahita C, Foldesy R, Steiner M, Townsend J. Comparison of condom breakage during human use with performance in laboratory testing. Contraception. 1992;45(5):429-37.
215. Rugpao S, Beyrer C, Tovanabutra S, Natpratan C, Nelson KE, Celentano DD, et al. Multiple Condom Use and Decreased Condom Breakage and Slippage in Thailand. JAIDS Journal of Acquired Immune Deficiency Syndromes. 1997;14(2):169-73.
216. Copen C. Condom Use During Sexual Intercourse Among Women and Men Aged 15-44 in the United States: 2011-2015 National Survey of Family Growth. National Health Statistics Reports. 2017;2017.
217. Spruyt A, Steiner MJ, Joanis C, Glover LH, Piedrahita C, Alvarado G, et al. Identifying condom users at risk for breakage and slippage: findings from three international sites. Am J Public Health. 1998;88(2):239-44.
218. Mercer CH, Jones KG, Johnson AM, Lewis R, Mitchell KR, Gravningen K, et al. How can we objectively categorise partnership type? A novel classification of population survey data to inform epidemiological research and clinical practice. Sexually Transmitted Infections. 2017;93(2):129.
219. Nasrullah M, Oraka E, Chavez PR, Johnson CH, DiNenno E. Factors Associated With Condom Use Among Sexually Active US Adults, National Survey of Family Growth, 2006-2010 and 2011-2013. J Sex Med. 2017;14(4):541-50.
220. Wegner R, Lewis MA, Davis KC, Neilson EC, Norris J. Tactics Young Women Use to Resist Condom Use When a Partner Wants to Use a Condom. J Sex Res. 2018;55(7):817-23.
221. Measor L. Condom use: a culture of resistance. Sex Education. 2006;6(4):393-402.
222. Davis KC, Hendershot CS, George WH, Norris J, Heiman JR. Alcohol's effects on sexual decision making: an integration of alcohol myopia and individual differences. J Stud Alcohol Drugs. 2007;68(6):843-51.
223. Stoner SA, Norris J, George WH, Morrison DM, Zawacki T, Davis KC, et al. Women's condom use assertiveness and sexual risk-taking: effects of alcohol intoxication and adult victimization. Addict Behav. 2008;33(9):1167-76.
224. Mercer CH, Prah P, Field N, Tanton C, Macdowall W, Clifton S, et al. The health and well-being of men who have sex with men (MSM) in Britain: Evidence from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). BMC Public Health. 2016;16(1):525.
225. Tanton C, Geary RS, Clifton S, Field N, Heap KL, Mapp F, et al. Sexual health clinic attendance and non-attendance in Britain: findings from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). Sexually Transmitted Infections. 2018;94(4):268.
226. O'Halloran C SS, Nash S, Brown A, Croxford S, Connor N, Sullivan AK, Delpech V, , ON. G. HIV in the United Kingdom: Towards Zero 2030. 2019 report. In: Public Health England L, editor. London: Public Health England; 2019.
227. Rojanapithayakorn W. The 100% Condom Use Programme in Asia. Reproductive Health Matters. 2006;14(28):41-52.

228. Allen DR, Carey JW, Manopaiboon C, Jenkins RA, Uthaivoravit W, Kilmarx PH, et al. Sexual Health Risks Among Young Thai Women: Implications for HIV/STD Prevention and Contraception. AIDS and Behavior. 2003;7(1):9-21.
229. Bond KC, Valente TW, Kendall C. Social network influences on reproductive health behaviors in urban northern Thailand. Social Science & Medicine. 1999;49(12):1599-614.
230. Xu F, Kilmarx PH, Supawitkul S, Yanpaisarn S, Limpakarnjanarat K, Manopaiboon C, et al. HIV-1 seroprevalence, risk factors, and preventive behaviors among women in northern Thailand. JAIDS, Journal of Acquired Immune Deficiency Syndromes. 2000;25(4):353-9.
231. Lertpiriyasuwat C, Plipat T, Jenkins RA. A survey of sexual risk behavior for HIV infection in Nakhonsawan, Thailand, 2001. AIDS. 2003;17(13).
232. Tian X, Angioletti-Uberti S, Battaglia G. On the design of precision nanomedicines. Science Advances. 2020;6(4):eaat0919.
233. Ehrlich P. Croonian lecture.—On immunity with special reference to cell life. Proceedings of the Royal Society of London. 1900;66(424-433):424-48.
234. Vella S, Schwartländer B, Sow SP, Eholie SP, Murphy RL. The history of antiretroviral therapy and of its implementation in resource-limited areas of the world. AIDS. 2012;26(10).
235. Kandel CE, Walmsley SL. Dolutegravir - a review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug Des Devel Ther. 2015;9:3547-55.
236. Curtis L, Nichols G, Stainsby C, Lim J, Aylott A, Wynne B, et al. Dolutegravir: Clinical and Laboratory Safety in Integrase Inhibitor–Naive Patients. HIV Clinical Trials. 2014;15(5):199-208.
237. Cahn P, Pozniak AL, Mingrone H, Shuldyakov A, Brites C, Andrade-Villanueva JF, et al. Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study. Lancet. 2013;382(9893):700-8.
238. Stellbrink HJ, Reynes J, Lazzarin A, Voronin E, Pulido F, Felizarta F, et al. Dolutegravir in antiretroviral-naive adults with HIV-1: 96-week results from a randomized dose-ranging study. Aids. 2013;27(11):1771-8.
239. Clotet B, Feinberg J, van Lunzen J, Khuong-Josses M-A, Antinori A, Dumitru I, et al. Once-daily dolutegravir versus darunavir plus ritonavir in antiretroviral-naive adults with HIV-1 infection (FLAMINGO): 48 week results from the randomised open-label phase 3b study. The Lancet. 2014;383(9936):2222-31.
240. Scheper H, van Holten N, Hovens J, de Boer M. Severe depression as a neuropsychiatric side effect induced by dolutegravir. HIV Med. 2018;19(4):e58-e9.
241. de Boer MGJ, van den Berk GEL, van Holten N, Oryszcyn JE, Dorama W, Moha Da, et al. Intolerance of dolutegravir-containing combination antiretroviral therapy regimens in real-life clinical practice. AIDS. 2016;30(18).
242. Dri DA, Marianecci C, Carafa M, Gaucci E, Gramaglia D. Surfactants, Nanomedicines and Nanocarriers: A Critical Evaluation on Clinical Trials. Pharmaceutics. 2021;13(3).
243. Gao Y, Kraft JC, Yu D, Ho RJY. Recent developments of nanotherapeutics for targeted and long-acting, combination HIV chemotherapy. European Journal of Pharmaceutics and Biopharmaceutics. 2019;138:75-91.
244. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. The FASEB Journal. 2005;19(3):311-30.
245. Aguilera-Correa JJ, Esteban J, Vallet-Regí M. Inorganic and Polymeric Nanoparticles for Human Viral and Bacterial Infections Prevention and Treatment. Nanomaterials (Basel). 2021;11(1):137.

246. Hua S, Wu SY. Editorial: Advances and Challenges in Nanomedicine. Front Pharmacol. 2018;9:1397.
247. Cheng Z, Al Zaki A, Hui JZ, Muzykantov VR, Tsourkas A. Multifunctional Nanoparticles: Cost Versus Benefit of Adding Targeting and Imaging Capabilities. Science. 2012;338(6109):903.
248. Barenholz Y. Doxil®--the first FDA-approved nano-drug: lessons learned. J Control Release. 2012;160(2):117-34.
249. Zazo H, Colino CI, Lanao JM. Current applications of nanoparticles in infectious diseases. J Control Release. 2016;224:86-102.
250. Lee NY, Ko WC, Hsueh PR. Nanoparticles in the Treatment of Infections Caused by Multidrug-Resistant Organisms. Front Pharmacol. 2019;10:1153.
251. Martinez ZS, Castro E, Seong C-S, Cerón MR, Echegoyen L, Llano M. Fullerene Derivatives Strongly Inhibit HIV-1 Replication by Affecting Virus Maturation without Impairing Protease Activity. Antimicrobial agents and chemotherapy. 2016;60(10):5731-41.
252. Mora-Huertas CE, Fessi H, Elaissari A. Polymer-based nanocapsules for drug delivery. International Journal of Pharmaceutics. 2010;385(1):113-42.
253. Miladi K, Sfar S, Fessi H, Elaissari A. Nanoprecipitation Process: From Particle Preparation to In Vivo Applications. In: Vauthier C, Ponchel G, editors. Polymer Nanoparticles for Nanomedicines: A Guide for their Design, Preparation and Development. Cham: Springer International Publishing; 2016. p. 17-53.
254. Malavia NK, Zurakowski D, Schroeder A, Princiotto AM, Laury AR, Barash HE, et al. Liposomes for HIV prophylaxis. Biomaterials. 2011;32(33):8663-8.
255. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013;8(1):102.
256. Jeevanandam J, Chan YS, Danquah MK. Nano-formulations of drugs: Recent developments, impact and challenges. Biochimie. 2016;128-129:99-112.
257. Badilli U, Gumustas M, Uslu B, Ozkan SA. Chapter 9 - Lipid-based nanoparticles for dermal drug delivery. In: Grumezescu AM, editor. Organic Materials as Smart Nanocarriers for Drug Delivery: William Andrew Publishing; 2018. p. 369-413.
258. Estanqueiro M, Conceição J, Amaral MH, Sousa Lobo JM. Chapter 12 - The role of liposomes and lipid nanoparticles in the skin hydration. In: Grumezescu AM, editor. Nanobiomaterials in Galenic Formulations and Cosmetics: William Andrew Publishing; 2016. p. 297-326.
259. Gaur PK, Mishra S, Bajpai M, Mishra A. Enhanced Oral Bioavailability of Efavirenz by Solid Lipid Nanoparticles: In Vitro Drug Release and Pharmacokinetics Studies. BioMed Research International. 2014;2014:363404.
260. Guerrero-Beltran C, Rodriguez-Izquierdo I, Serramia MJ, Araya-Durán I, Márquez-Miranda V, Gomez R, et al. Anionic Carbosilane Dendrimers Destabilize the GP120-CD4 Complex Blocking HIV-1 Entry and Cell to Cell Fusion. Bioconjugate Chemistry. 2018;29(5):1584-94.
261. García-Broncano P, Ceña-Diez R, de la Mata FJ, Gómez R, Resino S, Muñoz-Fernández M. Efficacy of carbosilane dendrimers with an antiretroviral combination against HIV-1 in the presence of semen-derived enhancer of viral infection. Eur J Pharmacol. 2017;811:155-63.
262. Patel V, Rajani C, Paul D, Borisa P, Rajpoot K, Youngren-Ortiz SR, et al. Chapter 8 - Dendrimers as novel drug-delivery system and its applications. In: Tekade RK, editor. Drug Delivery Systems: Academic Press; 2020. p. 333-92.

263. Martínez Rivas CJ, Tarhini M, Badri W, Miladi K, Greige-Gerges H, Nazari QA, et al. Nanoprecipitation process: From encapsulation to drug delivery. International Journal of Pharmaceutics. 2017;532(1):66-81.
264. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. International Journal of Pharmaceutics. 1989;55(1):R1-R4.
265. Lassalle V, Ferreira ML. PLA Nano- and Microparticles for Drug Delivery: An Overview of the Methods of Preparation. Macromolecular Bioscience. 2007;7(6):767-83.
266. Bilati U, Allémann E, Doelker E. Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. European Journal of Pharmaceutical Sciences. 2005;24(1):67-75.
267. Lince F, Marchisio DL, Barresi AA. Strategies to control the particle size distribution of poly-ε-caprolactone nanoparticles for pharmaceutical applications. Journal of Colloid and Interface Science. 2008;322(2):505-15.
268. Anton N, Benoit J-P, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates—A review. Journal of Controlled Release. 2008;128(3):185-99.
269. Dong Y, Feng S-S. Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs. Biomaterials. 2004;25(14):2843-9.
270. Galindo-Rodriguez SA, Allemann E, Fessi H, Doelker E. Polymeric nanoparticles for oral delivery of drugs and vaccines: a critical evaluation of in vivo studies. Crit Rev Ther Drug Carrier Syst. 2005;22(5):419-64.
271. Mandal S, Kang G, Prathipati PK, Zhou Y, Fan W, Li Q, et al. Nanoencapsulation introduces long-acting phenomenon to tenofovir alafenamide and emtricitabine drug combination: A comparative pre-exposure prophylaxis efficacy study against HIV-1 vaginal transmission. J Control Release. 2019;294:216-25.
272. Para MF, Glidden DV, Coombs RW, Collier AC, Condra JH, Craig C, et al. Baseline human immunodeficiency virus type 1 phenotype, genotype, and RNA response after switching from long-term hard-capsule saquinavir to indinavir or soft-gel-capsule saquinavir in AIDS clinical trials group protocol 333. J Infect Dis. 2000;182(3):733-43.
273. Spinner CD, Boesecke C, Zink A, Jessen H, Stellbrink H-J, Rockstroh JK, et al. HIV pre-exposure prophylaxis (PrEP): a review of current knowledge of oral systemic HIV PrEP in humans. Infection. 2016;44(2):151-8.
274. Prathipati PK, Mandal S, Pon G, Vivekanandan R, Destache CJ. Pharmacokinetic and Tissue Distribution Profile of Long Acting Tenofovir Alafenamide and Elvitegravir Loaded Nanoparticles in Humanized Mice Model. Pharm Res. 2017;34(12):2749-55.
275. Huttunen KM, Raunio H, Rautio J. Prodrugs--from serendipity to rational design. Pharmacol Rev. 2011;63(3):750-71.
276. Rautio J, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Järvinen T, et al. Prodrugs: design and clinical applications. Nature Reviews Drug Discovery. 2008;7(3):255-70.
277. Anastasi C, Quéléver G, Burlet S, Garino C, Souard F, Kraus J-L. New antiviral nucleoside prodrugs await application. Curr Med Chem. 2003;10(18):1825-43.
278. Ray AS, Fordyce MW, Hitchcock MJ. Tenofovir alafenamide: A novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus. Antiviral Res. 2016;125:63-70.
279. Palombo MS, Singh Y, Sinko PJ. Prodrug and conjugate drug delivery strategies for improving HIV/AIDS therapy. J Drug Deliv Sci Technol. 2009;19(1):3-14.

280. Agarwal HK, Chhikara BS, Bhavaraju S, Mandal D, Doncel GF, Parang K. Emtricitabine Prodrugs with Improved Anti-HIV Activity and Cellular Uptake. Molecular Pharmaceutics. 2013;10(2):467-76.
281. Singh D, McMillan J, Hilaire J, Gautam N, Palandri D, Alnouti Y, et al. Development and characterization of a long-acting nanoformulated abacavir prodrug. Nanomedicine (Lond). 2016;11(15):1913-27.
282. Smith N, Bade AN, Soni D, Gautam N, Alnouti Y, Herskovitz J, et al. A long acting nanoformulated lamivudine ProTide. Biomaterials. 2019;223:119476.
283. Kobayashi M, Yoshinaga T, Seki T, Wakasa-Morimoto C, Brown KW, Ferris R, et al. In Vitro antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. Antimicrobial agents and chemotherapy. 2011;55(2):813-21.
284. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US) NCfBI-. PubChem Compound Summary for CID 54726191, Dolutegravir; [cited 2022 Jan. 6]. 2022 [cited 2022 January 6, 2022]. Available from: Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Dolutegravir.
285. Pommier Y, Johnson AA, Marchand C. Integrase inhibitors to treat HIV/Aids. Nature Reviews Drug Discovery. 2005;4(3):236-48.
286. Marinello J, Marchand C, Mott BT, Bain A, Thomas CJ, Pommier Y. Comparison of raltegravir and elvitegravir on HIV-1 integrase catalytic reactions and on a series of drug-resistant integrase mutants. Biochemistry. 2008;47(36):9345-54.
287. Healthcare V. Summary of product characteristics. Dolutegravir European Medicines Agency2021 [updated September 8th 2021. Available from: https://www.ema.europa.eu/en/documents/product-information/tivicay-epar-product-information_en.pdf.
288. Thailand MoPH. Mytegra Summary of Product Characteristics 2021 [updated 2021. Available from: https://www.fda.moph.go.th/sites/oss/Drug%20Registration/MyTegra%201C%2015051-64%20(NG)/MYTEGRA_SmPC%20(18-6-2021).pdf.
289. Mohammad IS, Hu H, Yin L, He W. Drug nanocrystals: Fabrication methods and promising therapeutic applications. International Journal of Pharmaceutics. 2019;562:187-202.
290. Edagwa B, McMillan J, Sillman B, Gendelman HE. Long-acting slow effective release antiretroviral therapy. Expert Opinion on Drug Delivery. 2017;14(11):1281-91.
291. Shafiu Kamba A, Ismail M, Tengku Ibrahim TA, Zakaria ZAB. A pH-sensitive, biobased calcium carbonate aragonite nanocrystal as a novel anticancer delivery system. BioMed research international. 2013;2013:587451.
292. Müller RH, Keck CM. Second generation of drug nanocrystals for delivery of poorly soluble drugs: smartCrystal technology. European Journal of Pharmaceutical Sciences. 2008;34(1, Supplement):S20-S1.
293. de Waard H, Frijlink HW, Hinrichs WLJ. Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharm Res. 2011;28(5):1220-3.
294. Styliari ID, Taresco V, Theophilus A, Alexander C, Garnett M, Laughton C. Nanoformulation-by-design: an experimental and molecular dynamics study for polymer coated drug nanoparticles. RSC Advances. 2020;10(33):19521-33.
295. McCrorie P, Mistry J, Taresco V, Lovato T, Fay M, Ward I, et al. Etoposide and olaparib polymer-coated nanoparticles within a bioadhesive sprayable hydrogel for post-surgical

localised delivery to brain tumours. European Journal of Pharmaceutics and Biopharmaceutics. 2020;157.
296. Simamora P, Chern W. Poly-L-lactic acid: an overview. J Drugs Dermatol. 2006;5(5):436-40.
297. Zhao R, Zhu M, Zhou S, Feng W, Chen H. Rapamycin-Loaded mPEG-PLGA Nanoparticles Ameliorate Hepatic Steatosis and Liver Injury in Non-alcoholic Fatty Liver Disease. Frontiers in Chemistry. 2020;8:407.
298. Pitto-Barry A, Barry NPE. Pluronic® block-copolymers in medicine: from chemical and biological versatility to rationalisation and clinical advances. Polymer Chemistry. 2014;5(10):3291-7.
299. Ghasemi R, Abdollahi M, Emamgholi Zadeh E, Khodabakhshi K, Badeli A, Bagheri H, et al. mPEG-PLA and PLA-PEG-PLA nanoparticles as new carriers for delivery of recombinant human Growth Hormone (rhGH). Scientific Reports. 2018;8(1):9854.
300. Behrens I, Pena AIV, Alonso MJ, Kissel T. Comparative Uptake Studies of Bioadhesive and Non-Bioadhesive Nanoparticles in Human Intestinal Cell Lines and Rats: The Effect of Mucus on Particle Adsorption and Transport. Pharm Res. 2002;19(8):1185-93.
301. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials. 2000;21(23):2475-90.
302. Sankar V, Nareshkumar PN, Ajitkumar GN, Penmetsa SD, Hariharan S. Comparative studies of lamivudine-zidovudine nanoparticles for the selective uptake by macrophages. Curr Drug Deliv. 2012;9(5):506-14.
303. Gaymalov ZZ, Yang Z, Pisarev VM, Alakhov VY, Kabanov AV. The effect of the nonionic block copolymer pluronic P85 on gene expression in mouse muscle and antigen-presenting cells. Biomaterials. 2009;30(6):1232-45.
304. Jain-Gupta N, Contreras-Rodriguez A, Vemulapalli R, Witonsky SG, Boyle SM, Sriranganathan N. Pluronic P85 enhances the efficacy of outer membrane vesicles as a subunit vaccine against Brucella melitensis challenge in mice. FEMS Immunol Med Microbiol. 2012;66(3):436-44.
305. Neudeck BL, Alford TD, Faith NG, Czuprynski CJ. The Poloxamer P85 Is Protective Against Listeria monocytogenes Invasion. Foodborne Pathogens and Disease. 2008;5(6):859-65.
306. Phan H, Minut RI, McCrorie P, Vasey C, Larder RR, Krumins E, et al. Role of self-assembly conditions and amphiphilic balance on nanoparticle formation of PEG-PDLLA copolymers in aqueous environments. Journal of Polymer Science Part A: Polymer Chemistry. 2019;57(17):1801-10.
307. McCrorie P, Vasey CE, Smith SJ, Marlow M, Alexander C, Rahman R. Biomedical engineering approaches to enhance therapeutic delivery for malignant glioma. J Control Release. 2020;328:917-31.
308. Shen Z, Reznikoff G, Dranoff G, Rock KL. Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. J Immunol. 1997;158(6):2723-30.
309. Adachi A, Koenig S, Gendelman HE, Daugherty D, Gattoni-Celli S, Fauci AS, et al. Productive, persistent infection of human colorectal cell lines with human immunodeficiency virus. J Virol. 1987;61(1):209-13.
310. Wachsmann P, Lamprecht A. Chapter nineteen - Polymeric Nanoparticles for the Selective Therapy of Inflammatory Bowel Disease. In: Düzgüneş N, editor. Methods in Enzymology. 508: Academic Press; 2012. p. 377-97.

311. Taresco V, Abelha TF, Cavanagh RJ, Vasey CE, Anane-Adjei AB, Pearce AK, et al. Functionalized Block Co-Polymer Pro-Drug Nanoparticles with Anti-Cancer Efficacy in 3D Spheroids and in an Orthotopic Triple Negative Breast Cancer Model. Advanced Therapeutics. 2021;4(1):2000103.
312. Qin C, Feng W, Chu Y, Lee JB, Berton M, Bettonte S, et al. Development and validation of a cost-effective and sensitive bioanalytical HPLC-UV method for determination of lopinavir in rat and human plasma. Biomed Chromatogr. 2020;34(11):e4934.
313. Scientific F. Corning® Filtration Guide

Innovative Products for Filtration and Ultrafiltration 2022 [Available from: https://assets.fishersci.com/TFS-Assets/CCG/EU/Corning/Product-Information/COR011_EN%20CORNING%20FILTRATION%20GUIDE.pdf.
314. Zlomke C, Barth M, Mäder K. Polymer degradation induced drug precipitation in PLGA implants – Why less is sometimes more. European Journal of Pharmaceutics and Biopharmaceutics. 2019;139:142-52.
315. Crystalline and Amorphous Solids. 2020.
316. Malkoch M, Malmström E, Nyström AM. 6.04 - Dendrimers: Properties and Applications. In: Matyjaszewski K, Möller M, editors. Polymer Science: A Comprehensive Reference. Amsterdam: Elsevier; 2012. p. 113-76.
317. Cornes JS. Number, size, and distribution of Peyer's patches in the human small intestine: Part I The development of Peyer's patches. Gut. 1965;6(3):225-9.
318. Patel RP, Shah P, Barve K, Patel N, Gandhi J. Peyer's Patch: Targeted Drug Delivery for Therapeutics Benefits. In: Misra A, Shahiwala A, editors. Novel Drug Delivery Technologies: Innovative Strategies for Drug Re-positioning. Singapore: Springer Singapore; 2019. p. 121-49.
319. Gendelman HE, Orenstein JM, Martin MA, Ferrua C, Mitra R, Phipps T, et al. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. The Journal of experimental medicine. 1988;167(4):1428-41.
320. Govender T, Stolnik S, Garnett MC, Illum L, Davis SS. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. Journal of Controlled Release. 1999;57(2):171-85.
321. Mansour HM, Sohn M, Al-Ghananeem A, Deluca PP. Materials for pharmaceutical dosage forms: molecular pharmaceutics and controlled release drug delivery aspects. Int J Mol Sci. 2010;11(9):3298-322.
322. Abushrida A, Elhuni I, Taresco V, Marciani L, Stolnik S, Garnett MC. A simple and efficient method for polymer coating of iron oxide nanoparticles. Journal of Drug Delivery Science and Technology. 2020;55:101460.
323. Dong Y, Feng S-S. In vitro and in vivo evaluation of methoxy polyethylene glycol–polylactide (MPEG–PLA) nanoparticles for small-molecule drug chemotherapy. Biomaterials. 2007;28(28):4154-60.
324. Dong Y, Feng S-S. Nanoparticles of poly(D,L-lactide)/methoxy poly(ethylene glycol)-poly(D,L-lactide) blends for controlled release of paclitaxel. Journal of Biomedical Materials Research Part A. 2006;78A(1):12-9.
325. Louissaint NA, Fuchs EJ, Bakshi RP, Nimmagadda S, Du Y, Macura KJ, et al. Distribution of Cell-Free and Cell-Associated HIV Surrogates in the Female Genital Tract After Simulated Vaginal Intercourse. The Journal of Infectious Diseases. 2012;205(5):725-32.

326. Louissaint NA, Nimmagadda S, Fuchs EJ, Bakshi RP, Cao Y-J, Lee LA, et al. Distribution of Cell-Free and Cell-Associated HIV Surrogates in the Colon After Simulated Receptive Anal Intercourse in Men Who Have Sex With Men. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2012;59(1).
327. Raj A, Reed E, Santana MC, Walley AY, Welles SL, Horsburgh CR, et al. The associations of binge alcohol use with HIV/STI risk and diagnosis among heterosexual African American men. Drug Alcohol Depend. 2009;101(1-2):101-6.
328. Gantner P, Allavena C, Duvivier C, Cabie A, Reynes J, Makinson A, et al. Post-exposure prophylaxis completion and condom use in the context of potential sexual exposure to HIV. HIV Med. 2020;21(7):463-9.
329. Nwokolo N, Whitlock G, McOwan A. Not just PrEP: other reasons for London's HIV decline. The Lancet HIV. 2017;4(4):e153.
330. Fagan J, Frye V, Calixte R, Jain S, Molla L, Lawal A, et al. "It's Like Plan B but for HIV!" Design and Evaluation of a Media Campaign to Drive Demand for PEP. AIDS Behav. 2020;24(12):3337-45.
331. Leshin D, Olshtain-Pops K, Moses A, Elinav H. Limited awareness of the effective timing of HIV post-exposure prophylaxis among people with high-risk exposure to HIV. Eur J Clin Microbiol Infect Dis. 2019;38(4):779-84.
332. Hornberger J, Holodniy M, Robertus K, Winnike M, Gibson E, Verhulst E. A Systematic Review of Cost-Utility Analyses in HIV/AIDS: Implications for Public Policy. Medical Decision Making. 2007;27(6):789-821.
333. Shiferaw Y, Alemu A, Assefa A, Tesfaye B, Gibermedhin E, Amare M. Perception of risk of HIV and sexual risk behaviors among University students: implication for planning interventions. BMC Res Notes. 2014;7:162.
334. Kann L, McManus T, Harris WA, Shanklin SL, Flint KH, Queen B, et al. Youth Risk Behavior Surveillance - United States, 2017. MMWR Surveill Summ. 2018;67(8):1-114.
335. Beltzer N, Saboni L, Sauvage C, Lydié N, Semaille C, Warszawski J, et al. An 18-year follow-up of HIV knowledge, risk perception, and practices in young adults. AIDS. 2013;27(6).
336. The Lancet HIV. End resistance to dolutegravir roll-out. The Lancet HIV. 2020;7(9):e593.
337. Abrantes CG, Duarte D, Reis CP. An Overview of Pharmaceutical Excipients: Safe or Not Safe? Journal of Pharmaceutical Sciences. 2016;105(7):2019-26.
338. Ramot Y, Haim-Zada M, Domb AJ, Nyska A. Biocompatibility and safety of PLA and its copolymers. Adv Drug Deliv Rev. 2016;107:153-62.
339. D'Souza A A, Shegokar R. Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications. Expert Opin Drug Deliv. 2016;13(9):1257-75.
340. Bodratti AM, Alexandridis P. Amphiphilic block copolymers in drug delivery: advances in formulation structure and performance. Expert Opinion on Drug Delivery. 2018;15(11):1085-104.
341. Alakhova DY, Rapoport NY, Batrakova EV, Timoshin AA, Li S, Nicholls D, et al. Differential metabolic responses to pluronic in MDR and non-MDR cells: a novel pathway for chemosensitization of drug resistant cancers. J Control Release. 2010;142(1):89-100.
342. Arranja A, Ivashchenko O, Denkova AG, Morawska K, van Vlierberghe S, Dubruel P, et al. SPECT/CT Imaging of Pluronic Nanocarriers with Varying Poly(ethylene oxide) Block Length and Aggregation State. Molecular Pharmaceutics. 2016;13(3):1158-65.
343. Chu B. Structure and Dynamics of Block Copolymer Colloids. Langmuir. 1995;11(2):414-21.

344. Schimpel C, Teubl B, Absenger M, Meindl C, Fröhlich E, Leitinger G, et al. Development of an Advanced Intestinal in Vitro Triple Culture Permeability Model To Study Transport of Nanoparticles. Molecular Pharmaceutics. 2014;11(3):808-18.
345. Rieux Ad, Ragnarsson EGE, Gullberg E, Préat V, Schneider Y-J, Artursson P. Transport of nanoparticles across an in vitro model of the human intestinal follicle associated epithelium. European Journal of Pharmaceutical Sciences. 2005;25(4):455-65.
346. Bewick V, Cheek L, Ball J. Statistics review 7: Correlation and regression. Crit Care. 2003;7(6):451-9.
347. Tolles J, Meurer WJ. Logistic Regression: Relating Patient Characteristics to Outcomes. JAMA. 2016;316(5):533-4.