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Predicting physical stability of pharmaceuticals and investigating use of Raman spectroscopy to interrogate molecular relaxation events

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Abstract

Poor water solubility and slow dissolution are common issues in pharmaceuticals. The pharmaceutical industry widely employs amorphous solid dispersion to enhance dissolution, accelerate it, and promote bioavailability.

Glass, unlike crystalline solids, lacks long-range order. The glass transition temperature (T_g) marks its shift from a glassy to a rubbery state, distinct from the melting point where solid turns to liquid. Understanding drug molecules in their amorphous state under varied conditions is crucial, warranting further investigation.

The widely held belief that amorphous drug stability is ensured below $T_g - 50^\circ\text{C}$ is questioned. Previous studies show crystal nucleation well below this temperature, such as in indomethacin at $T_g - 55\text{ K}$, 3,3'-dimethoxy-4,4'-bis(2,2-diphenylvinyl) biphenyl at $T_g - 175\text{ K}$, and amorphous naproxen at $T_g - 89\text{ K}$, challenging the reliability of $T_g - 50$ rule.

Amorphous compounds exhibit two types of molecular mobility: global mobility (primary alpha, α -relaxation) responsible for glass transition, and local mobility (secondary beta, β -relaxation). A specific form of β -relaxation, involving intermolecular rotations of whole molecules, is known as Johari-Goldstein (JG) beta secondary relaxation, serving as a precursor to global α -relaxation mobility.

Previous studies often empathized macroscopic properties of the materials such as alpha relaxation, mechanical or dielectric changes. The novelty of our study lies in using Raman spectroscopy and directly interrogating the molecular structure, conformation and inter-molecular bonding during temperature variation around alpha and beta transitions.

We conducted an in-depth Raman spectroscopy study of alpha and beta relaxation processes. In Chapter 3, paracetamol served as the model drug to explore spectral changes around alpha and beta transition temperatures for reproducibility. Chapters 4 and 5 continued this investigation with nifedipine and felodipine as model drugs, chosen due to their similar T_g values but contrasting crystallization tendencies.

In our study, no changes were detected near the glass transition temperature, contrary to literature. Unlike previous studies on polymers, we focused on small molecules, potentially explaining the lack of observed beta relaxation changes. Different relaxation time scales between polymers and small molecules might be a factor. Fast cooling in polymer systems may lead to excess enthalpy seen in DSC at the glass transition temperature. Previous DSC research showed annealing enables beta-relaxations. For future studies, a heat-cool-anneal-heat approach could explore sample enthalpic relaxation, diverging from our heat-cool-heat method.

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List of abbreviations

ATR	Attenuated total reflection
BCS	Biopharmaceutical Classification System
CCD	Charged couple device
CM	Coupling model
DCS	Developability Classification System
DMA	Dynamic mechanical analysis
DRS	Dielectric relaxation spectroscopy
DSC	Differential scanning calorimetry
DTA	Differential thermal analysis
FTIR	Fourier transform infrared
GFA	Glass forming ability
IAS	Anti-Stokes Raman scattering strength
IMC	Isothermal microcalorimetry
IR	Infrared
IRI	Integrated Raman Intensity
IS	Stokes Raman scattering strength
JG	Johari-Goldstein
MAS	Magic-angle spinning
MCT	Mode Coupling Theory
MDSC	Modulated Differential Scanning Calorimetry
NMR	Nuclear Magnetic Resonance

XRD	X-ray diffraction
RPH	Raman Peak Height
SD	Solid Dispersion
SEDD	Self-Emulsifying Drug Delivery System
SLAD	Solubility Limited Absorbable Dose
THz-TDS	Terahertz Time-Domain Spectroscopy
T_g	Glass transition temperature
T_{gα}	alpha transition temperature
T_{gβ}	beta transition temperature
T_k	Kauzmann temperature
T_m	Melting point
TMA	Thermomechanical Analysis
TSDC	Thermally Stimulated Depolarization Current
ULF	Ultra-Low Frequency
VFTH	Vogel-Fulcher-Tammann-Hesse
VTF	Vogel-Tammann-Fulcher
XRD	X-Ray Diffraction

Chapter 1 Introduction

1.1 Poorly soluble drugs

Oral drug delivery has many advantages in that it induces high patient compliance and is relatively low in cost. Approximately 40 to 70% of pharmaceutically relevant chemicals and marketed oral drugs are poorly water soluble (Williams et al, 2013). Despite their advantages, many oral drugs face challenges of low aqueous solubility and permeability upon administration (Leuner & Dressman, 2000). Poor water solubility can be significantly problematic in oral bioavailability because most oral drug molecules need to be in dissolution media to be able to reach sufficient dissolution level and achieve useful oral bioavailability.

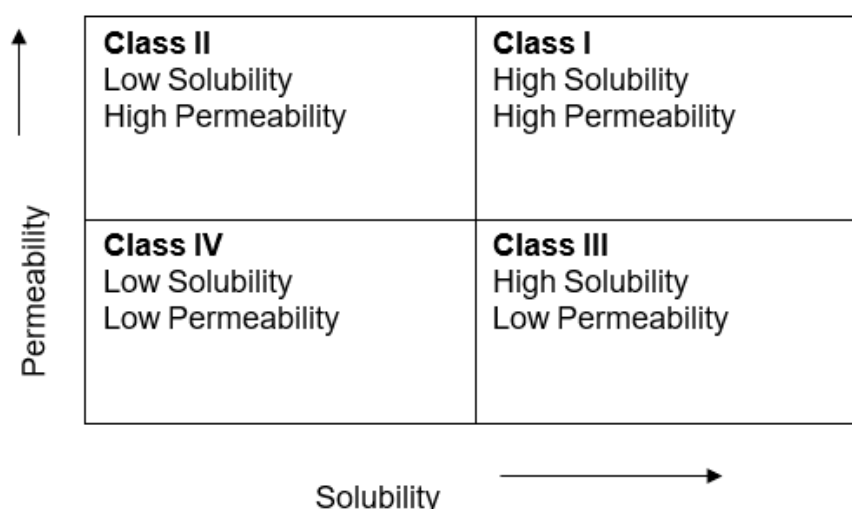


Figure 1.1. Biopharmaceutical Classification System (BCS). Used with permission of John Wiley & Sons - Books, from (Newman and Augustijns, 2015); permission conveyed through Copyright Clearance Center, Inc.

The Biopharmaceutical Classification System (BCS) (Newman & Augustijns, 2015) was established to allow prediction of the bioavailability of oral drugs by categorizing the drug molecules according to their aqueous solubility and permeability relationship. According to BCS (Figure 1.1), class I molecules have both high solubility and high permeability; class II are those

having low solubility and high permeability; class III have high solubility but low permeability; and class IV have both poor solubility and poor permeability. BCS class II and IV drugs show many characteristics that are problematic for effective oral delivery and hence the development of an effective delivery system for these drugs is a challenge. Over the past few decades there has been an increase of BCS class II drugs from about 30% to 50-60% among the marketed and the new pipeline compounds in the pharmaceutical industries (Ku, 2008). As a consequence, there has been increased effort to develop means to improve low solubility in BCS class II, which is the most frequent class for orally active new chemical entities (Takagi et al, 2006).

The Developability Classification System (DCS) is a revised classification system for oral drugs that will more extensively classify drugs according to the factors affecting their oral absorption (Butler & Dressman, 2010). Compared to the previous BCS system, DCS is more focused on tackling problems in product development rather than being a regulatory classification system. DCS aims to incorporate the solubility limited absorbable dose (SLAD) concept and introduce two sub classes, class IIa and class IIb, for class II drugs (Figure 1.2). For the class II drugs, SLAD is represented as a boundary between class IIa and IIb for high permeability drugs and it extends on the idea that permeability and solubility are compensatory. As a result of compensatory influence of high permeability, class IIa compounds can be formulated to achieve complete oral absorption from a standard solid oral dosage form without relying on complex solubilisation technologies, whereas class IIb drugs will be not absorbed unless the drug is formulated in an already solubilized form. Hence, class IIb drugs require reasonable solubilising strategies. Reduction of drug particle size, solid dispersion, and addition of surfactant and polymer into the drug forms have been performed to improve the dissolution rate and solubility of DCS IIb drug (Li et al, 2012; Lohani et al, 2014). A study was performed to improve the solubility and bioavailability of esomeprazole magnesium salt (ESM) by preparing solid dispersions using a supercritical anti-solvent (SAS) process, and as a consequence, transformed ESM from a crystalline to an

amorphous state with a more porous morphology, leading to a high dissolution rate (Pyo et al, 2023).

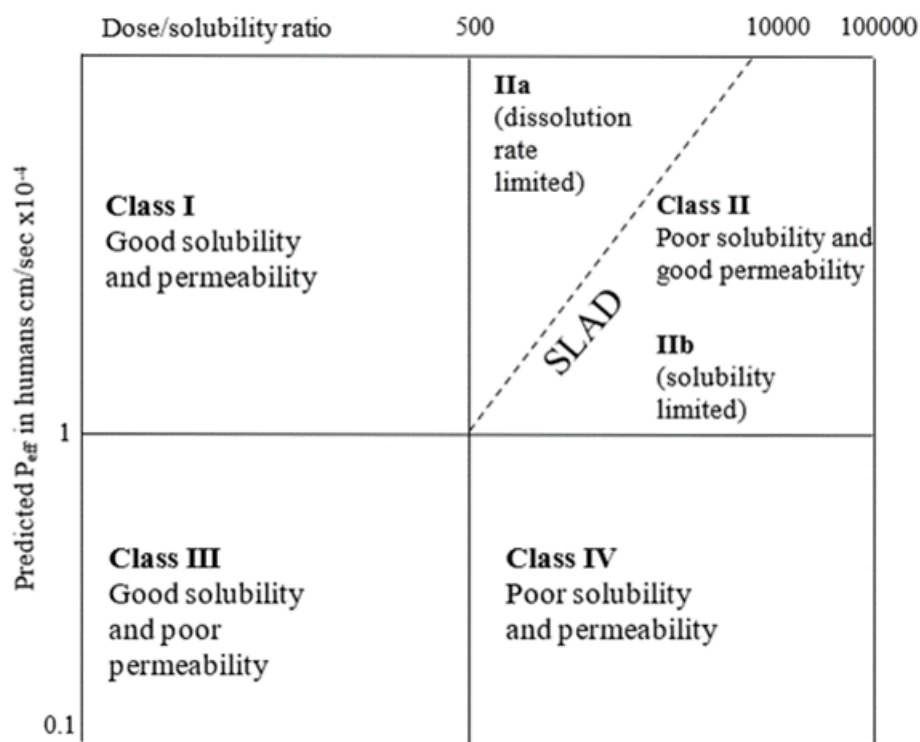


Figure 1.2. Developability Classification System (DCS). Used with permission of Elsevier Science & Technology Journals, from (Butler and Dressman, 2010); permission conveyed through Copyright Clearance Center, Inc.

1.2 Strategies of improving the dissolution properties

Various techniques have been developed to enhance permeability of the poorly permeable drugs and to improve the solubility and dissolution rate of poorly water-soluble drugs, hence generate a supersaturation state of drug in the gastrointestinal fluid to help improve bioavailability (Gurunath et al, 2013). These techniques include: solid dispersion (SD), salt, microemulsion, solid solutions, eutectic mixtures, self-emulsifying drug delivery system (SEDD),

liposome, polymorphs, pseudopolymorphs (including solvates), complexation, hydrotrophy, micelle formation, nano-suspensions, etc (Mishra et al, 2015).

Solid dispersions are one of the strategies to improve the drug release profile of poorly water-soluble drugs. The poorly soluble drugs could form solid dispersions with inert hydrophilic polymer carriers and acquire drug release profile that is affected by the polymer properties (Vasconcelos et al, 2007). Solid dispersions using crystalline drug were observed to be not as effective as using the amorphous form of drugs, which are less thermodynamically stable compared to the crystalline form (Simonelli et al, 1969). Amorphous solid consists of atoms and molecules that are arranged in a disordered manner with no translational symmetry, unlike the crystalline solid structure (Turton, 2000). Due to higher energy state (or higher Gibb's free energy), the solubility of amorphous solid is enhanced over its crystalline counterpart (Hancock & Parks, 2000).

Amorphous character is often desirable in pharmaceutical formulations. Common ways of obtaining amorphous drugs are as follows: mechanical activation of a crystalline mass (e.g. during milling and compaction of crystals); rapid precipitation from solution (e.g. during lyophilisation or spray drying); and hot melt extrusion (Hancock & Zografi, 1997). The amorphous drug would need to be in a highly disordered molecular state and its disordered state should be stabilised so that all molecular motions that cause recrystallization are slowed down for a sufficient pharmaceutical time-scale that equates to its shelf life.

1.3 Molecular mobility in amorphous solids

Amorphous pharmaceuticals show greater molecular mobility and chemical reactivity compared to their corresponding crystalline forms. The increased free energy results in the tendency to crystallize. The molecular processes that cause the recrystallization of amorphous solids are not fully understood and are the subject of intensive research. One of the most

important factors that contribute to the stability of amorphous drugs is the molecular mobility (Hancock et al, 1995). Relaxation process is an effect related to an application of external stress and return into equilibrium. In order to determine molecular mobility, it would be helpful to understand the temperature dependent relaxation phenomenon in the supercooled liquid and amorphous state.

1.3.1 Glass transition temperature

When liquid is cooled down, its molecular motion slows down. The liquid state solidifies to a crystalline solid or stays as supercooled liquid state below the melting point (T_m). If the liquid is cooled down sufficiently quickly, crystallization can be avoided because there would not be enough time for the rearrangement of the molecules into crystalline structure. As the temperature of the supercooled liquid is lowered further it would become increasingly sluggish (Schoenholz, 2018). The supercooled liquid structure would appear 'frozen' below the glass transition temperature (T_g) and solidify to a glass (Ferrer et al, 1998).

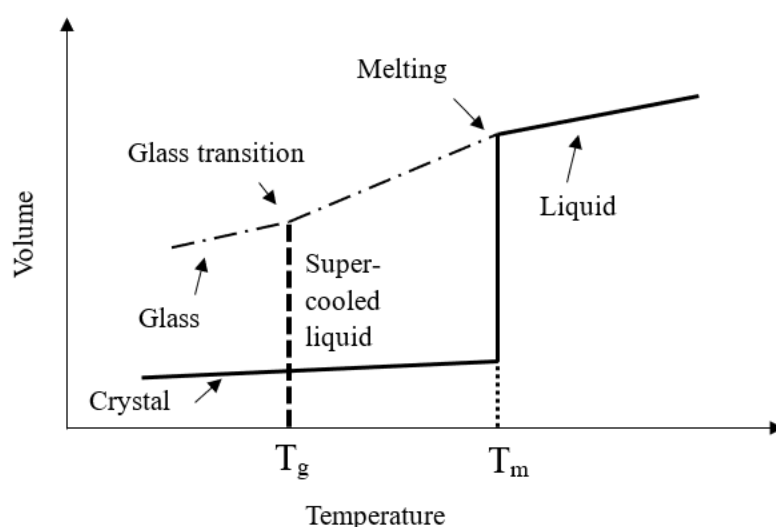


Figure 1.3. Volume of various state of material affected by temperature. Used with permission of John Wiley & Sons - Books, from (Zallen, 1983) ; permission conveyed through Copyright Clearance Center, Inc.

The relationship between glass transition and molecular mobility could be explained in a graph as shown in Figure 1.3. A material is plotted on a volume versus temperature graph $V(T)$ (Zallen, 1983). A change of phase occurs when liquid state solidifies. The phase transition to crystal is marked as a sharp gradient in $V(T)$ with low cooling rate. The $V(T)$ slope of the glass transition is smaller than that of a crystal. Glass transition temperature is significant in that it determines the upper limit temperature for storage of amorphous drugs, and knowledge of it is therefore essential in the pharmaceutical development.

1.3.2 Vogel-Tammann-Fulcher (VTF) equation

Many of essential physical properties of amorphous materials are determined by temperature dependence of molecular mobility. The temperature dependence can often be described by using Vogel-Tammann-Fulcher (VTF) equation (Angell, 1995). Physical properties of amorphous materials and the range of glass transition temperatures can be determined by temperature dependence of molecular motions (Hancock & Zografi, 1997). Temperature dependence of structural relaxation time (τ) for various amorphous materials (Peleg, 1992) can be described by VTF equation (Equation 1), which has been developed for mechanical and electrical relaxation (Williams et al, 1955):

Equation 1

$$\tau = \tau_0 \exp\left(D \frac{T_0}{T} - T_0\right) \quad (1)$$

τ is the mean molecular relaxation time and T is the temperature. T_0 corresponds to the Kauzmann temperature (T_k). τ_0 is related to the relaxation time constant. D is temperature-independent constant. It is possible to classify supercooled liquids as either having “strong” or “fragile” glass forming tendencies. The viscosity of strong glass formers changes gradually as they approach the glass transition temperature, whereas fragile glass formers show viscosity that increases slowly at first, but then changes dramatically as the

transition approaches. For instance, the constant D in the VTF equation indicates if the amorphous system is a strong glass former, with high values (>100) or a fragile glass former when corresponding to low values (<10). When it comes to viscosity, strong glass formers exhibit Arrhenius temperature dependence. On the other hand, the glass formers that are fragile show increase in viscosity much steeper than the Arrhenius law near T_g (Furukawa & Tanaka, 2016). Using VTF equation, temperature dependence of amorphous indomethacin has been measured (Andronis & Zografi, 1997). Viscoelastic measurements were made with a controlled rheometer. The study has indicated that indomethacin has a value of $D = 8.9$ and is a fragile glass former. In reference to the study that suggests fragile glass has a low glass forming ability (GFA) (Kawakami et al, 2015), drugs classified as fragile glass formers, such as indomethacin would have low GFA and possibly would need to be stabilized, possibly by using excipients miscible with the drug (Kawakami et al, 2012). Fragility could be viewed as a parameter that is useful in practical standpoint and could lead to physics-driven design of stable amorphous pharmaceutical formulations.

1.3.3 Kauzmann temperature (T_k)

When a liquid is supercooled, the difference in entropy between the liquid and solid phase decreases. Kauzmann temperature (T_k) is the temperature at which the difference in entropies reaches zero. It is generally considered that roughly 50°C below T_g is the T_k at which the translational and rotational motions of the pharmaceutical cease on pharmaceutically relevant time scales (Sibik & Zeitler, 2016a; Zhou et al, 2002). This is supported by the assumption that when a glass is cooled to the T_k , the molecular activity is so slow that it seems to be non-existent on the time-scale for drug storage. However, it is being observed that even at below T_k there is still sufficient mobility for crystallisation from the beta relaxation, which will be discussed further in the later Section 1.3.5 (Grzybowska et al, 2010; Hikima et al, 1999; Okamoto & Oguni, 1996).

1.3.4 Global and local mobility – primary (α -) and secondary (β -) relaxations

Amorphous materials generally comprise two types of molecular mobility or relaxation called global and local mobility (Bhattacharya & Suryanarayanan, 2009). Mobility that accounts for glass transition temperature is the 'global mobility,' which is also called primary alpha (α -) relaxation (Hancock et al, 1995). The molecular mobility tends to be cooperative with motions that involve translation or rotation of the molecules (Paluch et al, 2003). On the other hand, the local noncooperative mobility is referred as the secondary beta (β -) relaxation (Paluch et al, 2003; Vyazovkin & Dranca, 2005). Beta relaxation involves less molecules moving cooperatively compared to alpha relaxation as illustrated in Figure 1.4. It involves lower activation energy compared to global mobility and therefore occurs below the glass transition temperature called beta transition temperature (T_β). For this reason, the beta relaxation becomes major relaxation mechanism for aging amorphous materials below T_g (Vyazovkin & Dranca, 2006).

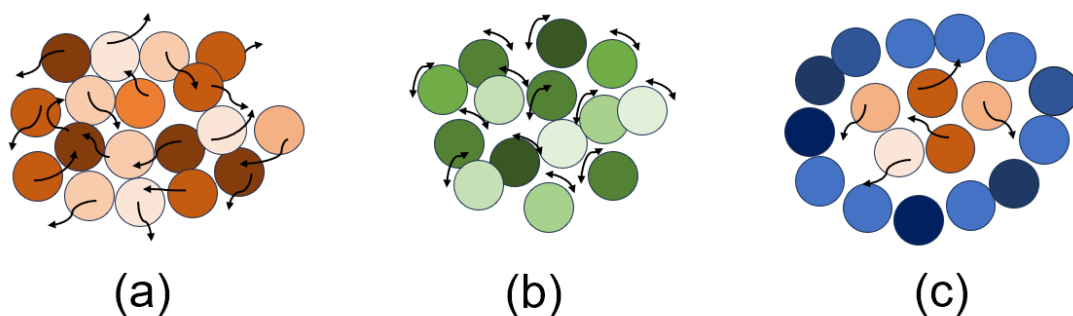


Figure 1.4. Molecular motions - (a) alpha relaxation and two Johari and Goldstein beta relaxation: (b) cooperative vibration model (c) islands of mobility model

Many studies particularly relating pharmaceutical formulation are focused on alpha relaxation and disregard beta relaxation to be significant for glass transition. Johari and Goldstein found that there is beta relaxation of a special class that has similar properties to alpha relaxation - intermolecular motions that involve rotation and/or translation of the entire molecule. This

universal secondary relaxation is referred to as the Johari-Goldstein (JG) beta (β -) secondary relaxation. This is illustrated in Figure 1.4 (b). Many low molecular weight organic molecules exhibit the slowest JG beta relaxation process (Johari & Goldstein, 1970). Studies indicate that JG beta relaxation originates from a movement of the entire molecules and is intermolecular in nature (Grzybowska et al, 2010). Amorphous solids with JG beta relaxation have been regarded as **structurally inhomogeneous** glass structure with “islands of mobility” which are “regions where the structure is relatively loose and molecules can undergo hindered rotations (Johari, 1985)”. This is illustrated in Figure 1.4. (c). JG beta relaxation could be considered as a precursor of the mobility of global alpha relaxation (Grzybowska et al, 2016). However, the exact nature of these relaxations is still being debated. Dielectric relaxation studies carried out on sorbitol, xylitol, and threitol have demonstrated that JG beta relaxation peaks at a frequency coincide with the independent relaxation frequency related to the cooperative alpha relaxation (Ngai & Paluch, 2003). Understanding of the glass transition and role of molecular mobility in the stability of amorphous pharmaceutical requires insight into the behaviour of the JG beta relaxation.

1.3.5 Beta transition temperature and stability

The primary alpha (α -) relaxation is considered crucial for ageing and crystallization of supercooled liquid materials at temperature above T_g . On the other hand, secondary beta (β -) relaxation temperature is important in that it is the lower temperature limit of the thermal energy required to surpass potential energy barriers related to recrystallization (Kissi et al, 2018a). However, alpha relaxation has not been a reliable predictor for stability of some amorphous materials and it could be important to look beyond glass transition and investigate the properties of beta relaxation.

Some studies have found evidence of crystal nucleation at temperatures well below the T_g , for example, in indomethacin where crystallization has been observed at temperature as low as $T_g - 55$ K, or for

3,3'- dimethoxy-4,4'-bis(2,2-diphenylvinyl) biphenyl at T_g –175 K and for amorphous naproxen at T_g – 89 K (Okamoto & Oguni, 1996; Sibik et al, 2015; Vyazovkin & Dranca, 2007). Thus, belief that molecular mobility of amorphous materials is negligible 50°C below T_g , does not account for mobility towards instability at below T_g .

T_g is not always a good predictor of crystallization tendency. Nifedipine and indomethacin both have similar molecular weight and T_g but present differences in their physical stability; the onset of recrystallization for nifedipine is 1 day and that of indomethacin is 30 days stored at chosen condition - at 0% relative humidity, 298 K (Kissi et al, 2018a). Another study also shows that nifedipine and felodipine, which both share almost same T_g , have drastically different crystallization tendencies (Marsac et al, 2006).

In some studies, crystallization rate at temperatures close to T_g was controlled by relaxation time of the beta relaxation and not of the alpha relaxation (Hikima et al, 1995a; Hikima et al, 1995b; Hikima et al, 1998). In the study of triphenylethylene, crystallization growth rate was measured and could only be modelled successfully if the beta relaxation was controlling the growth rate of nucleus rather than the alpha relaxation (Hikima et al, 1999). Hence, there is need to study the role of beta relaxation, in addition to alpha relaxation in studying the stability of amorphous materials.

Furthermore, up to present time, the studies on beta relaxation have been focused on polymers. Table 1.1 shows alpha relaxation and beta relaxation temperatures of some drugs, polymers and polyalcohols reported in the literature (Bērziņš et al, 2022; Kissi et al, 2018a; Kissi et al, 2018b; Ngai et al, 2015; Sibik et al, 2014a). In considering the significance of the amorphous system in pharmaceutical drugs, the attention should be directed to investigating the role of beta relaxation in drug formulations.

Table 1.1 Alpha and beta transition temperatures of some drugs, polymers and polyalcohols reported in the literature (Bērzinš et al, 2022; Kissi et al, 2018a; Kissi et al, 2018b; Ngai et al, 2015; Sibik et al, 2014a)

Material		Alpha relaxation temperature (K)	Beta relaxation temperature (K)
Drug (Bērzinš et al, 2022; Kissi et al, 2018a; Kissi et al, 2018b)	Carvedilol	224.6	327
	Celecoxib	326	190 -210
	Cimetidine	308.2 - 318.2	243.2 - 268.2
	Indomethacin	212.8	333.4
	Zafirlukast	377.2	305.2 - 310.2
Polymer (Ngai et al, 2015)	PMMA	390	250
	PS	373	190
	PVC	350	170
	1,4-PBD	185	109
	cis-PBD	170	120
	BPA-PC	423	190
	PVP	422	316
	PET	345	172.4
	PEMA	338	214
	PVME	250	190
	aPP	259	190
	PIB	200	140
	PES	500	151.5 - 215.7
	PVAc	315	210
Polyalcohol (Sibik et al, 2014)	Sorbitol	269	178
	Xylitol	253	176
	Threitol	216	146

1.3.6 The current analytical methods for structural relaxations

Studying the mobility of amorphous system around alpha and beta transition temperature is a means of characterizing the amorphous material for stability applications. There are various instrumental techniques used to characterise molecular mobility in amorphous state such as dielectric relaxation spectroscopy (DRS), thermally stimulated depolarization current spectroscopy (TSDC), dynamic mechanical analysis (DMA) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and enthalpic recovery experiments in the differential scanning calorimetry (DSC). The analytical techniques used to characterize molecular mobility, operating principle and what the technique measures are summarized in Table 1.2.

Table 1.2. Techniques that characterize relaxation events. Reproduced with permission of Elsevier Science & Technology Journals, from (Bhattacharya & Suryanarayanan, 2009); permission conveyed through Copyright Clearance Center, Inc.

Analytical Techniques	Principle	Subject of measurement	Usefulness
Dielectric relaxation spectroscopy (DRS)	Reorientation of dipoles under the influence of electric field of variable frequency	Relaxation time	Characterises beta relaxations in small and macromolecules using variable frequency or temperature; Direct determination of relaxation times and their distribution
Thermally stimulated current spectroscopy (TSC)	Reorientation of dipoles by electric field followed by quenching to freeze the orientations and subsequent measurement of depolarization current during heating	Relaxation time	Characterises local motions in pharmaceuticals; Great complementary tool to DRS in that DRS can be conveniently used to study mobility above the T _g whereas TSDC is suited to examine mobility below the T _g
Dynamic mechanical analysis Spectroscopy (DMA)	Measurement of oscillatory strain or stress given to the sample, and the resulting strain or stress developed in the sample	Glass transition temperature	Characterises the temperature dependent mobility of amorphous pharmaceuticals and determines alpha and beta transition temperatures
Nuclear magnetic resonance Spectroscopy (NMR)	Measurement of the emission of absorbed radio frequency radiation by nuclei in presence of an external magnetic field	Relaxation time	Studies beta relaxations in pharmaceuticals; Used for determination of origin of molecular motions and, correlation between chemical stability and local motions.
Differential scanning calorimetry (DSC)	Measurement of heat flow associated with thermally induced transitions	Glass transition temperature	Simultaneously characterises alpha and beta relaxations as well as crystallization

1.3.6.1 Dielectric relaxation spectroscopy (DRS)

Dielectric relaxation spectroscopy (DRS) determines electrical properties in materials from observing reorientation of dipoles under the influence of electric field of variable frequency from 10^{-6} to 10^{12} Hz (Kremer, 2002). The frequency range by the DRS has been extended to THz frequencies (10^{11} - 10^{13}) Hz (Masayoshi, 2007). This is called terahertz time-domain spectroscopy (THz-TDS). DRS is the most commonly used method to directly measure the relaxation processes (Sokolov et al, 2001). DRS directly measures the time scale of intramolecular and intermolecular motion of drug molecules. DRS is useful in that it characterises alpha and beta relaxations in variable frequency or temperature. Dielectric relaxation data can be versatile because it can fit in many different models. Mathematical models have been used to describe dielectric relaxation process and obtain information about relaxation times, strength and peak broadening and asymmetry parameters (Kremer & Schönhal, 2003). For instance, extended coupling model (CM) has been used to study the intermolecular local mobility reflected in beta relaxation of celecoxib (Grzybowska et al, 2010).

1.3.6.2 Terahertz time-domain spectroscopy (THz-TDS)

Terahertz time-domain spectroscopy (THz-TDS) is a dielectric spectroscopy that has frequencies from 100 GHz to 3 THz, which is referred as the part of the microwave and infra-red frequencies (Tuononen et al, 2010). Given its frequency range, it can measure fast intermolecular motions (Sibik & Zeitler, 2016b). THz-TDS is very sensitive to the bonds due to match of the energy between the terahertz photons and the hydrogen bonds (Parrott & Zeitler, 2015). It is particularly good for investigation of hydrogen-bonding interactions in organic materials. THz-TDS is one of the most recent analytical methods to study amorphous materials. THz-TDS has been used to study JG beta relaxation process below T_g in amorphous materials - polyalcohols, such as glycerol, threitol, xylitol, and sorbitol (Sibik et al, 2014a).

1.3.6.3 Thermally stimulated depolarization current (TSDC)

Thermally stimulated current (TSC) technique characterizes materials by flowing short-circuit current through the sample during heating (Iwamoto & Taguchi, 2018). Thermally stimulated depolarization current (TSDC) is one of the TSC technique that applies electric field to the sample to create dipolar reorientation. TSDC procedure involves quenching and subsequent reheating of the sample to measure depolarization and to characterize molecular mobility (Bhattacharya & Suryanarayanan, 2009). TSDC measurements give important information on amorphous pharmaceuticals, especially on molecular mobility in amorphous phase (Boutonnet-Fagegaltier et al, 2002). Kinetic parameters associated with different relaxation modes of indomethacin have been obtained using TSDC, which also allowed characterization of the distribution of relaxation times (Correia et al, 2001). The TSDC technique used to obtain the relaxation time measurement below transition temperature captured some of the fast motions not measured by the calorimetric techniques such as isothermal microcalorimetry (IMC) and modulated differential scanning calorimetry (MDSC) (Bhugra et al, 2008). Hence, TSDC is a valuable tool in detecting global mobility compared to the traditional calorimetric techniques. TSDC is considered a complementary tool to DRS because DRS is suited to characterise the molecular mobility above the T_g when TSDC can be used to study the molecular mobility below the T_g (Alie et al, 2004).

1.3.6.4 Dynamic mechanical analysis (DMA) spectroscopy

Dynamic mechanical analysis (DMA) is a material characterization technique that provides measurement where oscillatory strain or stress is given to the sample, and the resulting strain or stress developed in the sample is measured as the output (Hoshino, 2014). The mechanical properties such as viscosity, elasticity and damping parameter can be determined as a function of temperature (Jones et al, 2012). DMA is a more preferred method for glass transition and relaxation events, compared to DSC because the properties measured during transition from crystalline to amorphous phase are more

significant in the mechanical process than in specific heat (Ebnesajjad, 2011). Using DMA, alpha and beta relaxation events in relation to associated transition temperature have been studied for nine amorphous nifedipine, carvedilol, cimetidine, celecoxib, zafirlukast, sodium taurocholate, Zent X and, Zent V. The data obtained from DMA of the sample amorphous drugs are from the damping parameter. Figure 1.5 shows alpha transition temperature plotted against onset of recrystallisation (1/time) and no clear relation between T_g and the physical stability of amorphous drugs were observed. However, clear correlation was observed with the onset of recrystallization of beta transition temperature in Figure 1.6.

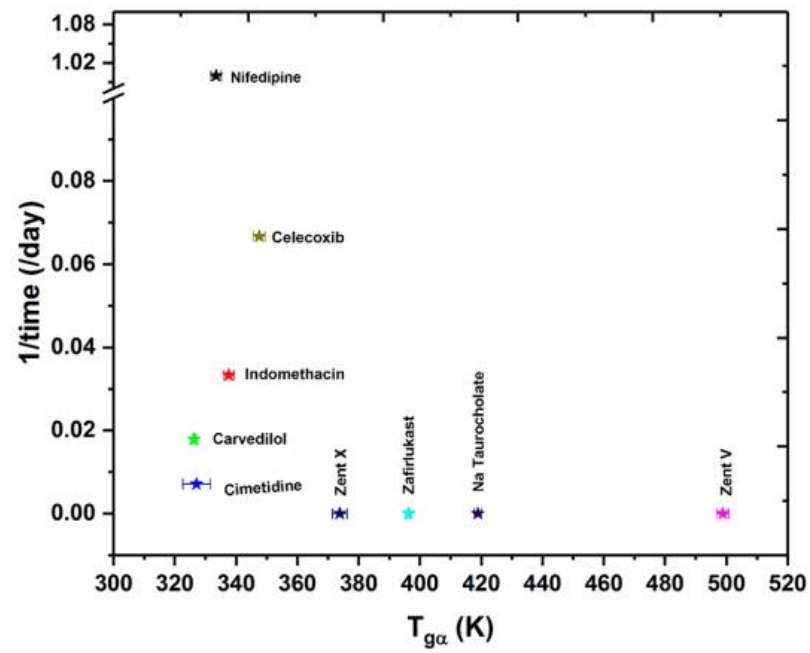


Figure 1.5. Alpha transition temperature ($T_{g\alpha}$) versus the onset of recrystallization (1/time (/day)) (Kissi et al, 2018).

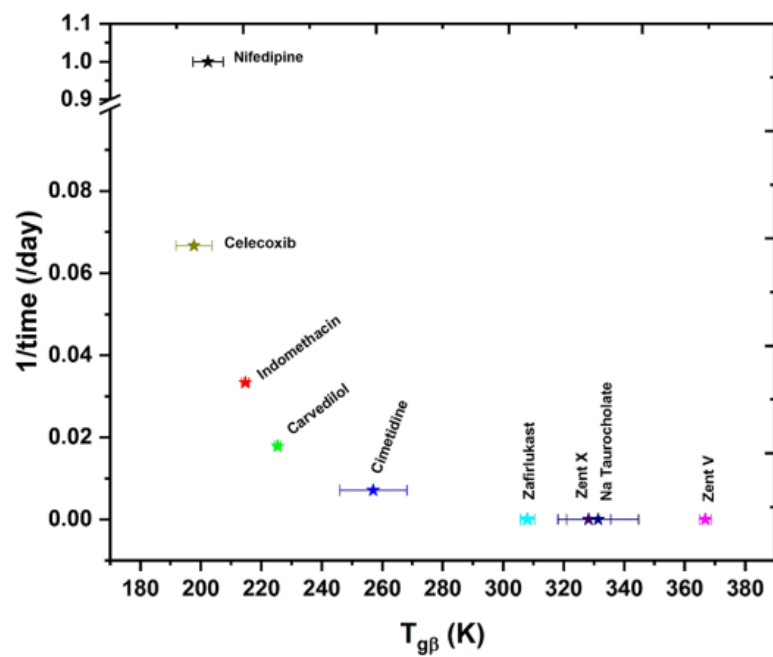


Figure 1.6. Beta transition temperature ($T_{g\beta}$) versus onset of recrystallization (1/time (/day)) (Kissi et al, 2018).

1.3.6.5 Nuclear magnetic resonance (NMR) spectroscopy

Solid-state nuclear magnetic resonance (NMR) spectroscopy is a technique that measures the absorption of radio frequency radiation by NMR-active atomic nuclei of a sample molecule to elucidate its chemical structure, 3D structure and dynamics of solids and semi-solids (Holzgrabe et al, 2008; Reif et al, 2021). Due to orientation dependence of the nuclear spin interactions in the solid state, the majority of high-resolution solid-state NMR spectra are measured under magic-angle spinning (MAS). NMR active nuclei such as ^1H and ^{17}O exhibit relaxation properties that can be analysed to characterize the molecular motions of the sample molecules (Ruan et al, 1999). NMR supports the mode coupling theory (MCT), which proposes that fast beta relaxation plays the role of precursor for the alpha relaxation (Bengtzelius et al, 1984). NMR could study differences in molecular dynamics of different types of amorphous material (Dries et al, 1988). NMR could carry out studies that explain chemical stability observed for amorphous pharmaceuticals in relation to molecular mobility (Yoshioka & Aso, 2007). NMR has been useful in observing beta relaxations in various materials. ^2H NMR has investigated the crossover from viscous process to β -relaxation in the orthoterphenyl and H-bridged glycerol (Schnauss et al, 1992). ^2H NMR was also used to analyse the JG beta relaxations in simple organic molecules (Vogel & Rössler, 2000).

1.3.6.6 Raman spectroscopy

Raman spectroscopy is a technique based on scattering phenomenon of light to probe chemical structure of a material (Šašić, 2008). The Raman effect involves the inelastic scattering rather than absorption of light from a sample. In inelastic scattering, the energy of photons changes upon interaction with a sample. As shown in Figure 1.7, when light is shone to a sample, molecules are promoted to an excited state. When molecules lose energy, they return to the ground state emitting light with the same frequency as the initial energy level (Rayleigh scattering) or with a different frequency (Raman scattering). Raman scattering can be either Stokes or anti-Stokes scattering.

With Stokes scattering molecules are initially in the ground state, whereas with anti-Stokes, molecules are initially in the excited state. In Stokes scattering, some of the energy of the photon is transferred to the molecular vibration, resulting in reduced frequency of the scattered light. On the other hand, in anti-Stokes scattering, the excess energy of the excited molecular vibration is released and thus the frequency of the scattered light is increased.

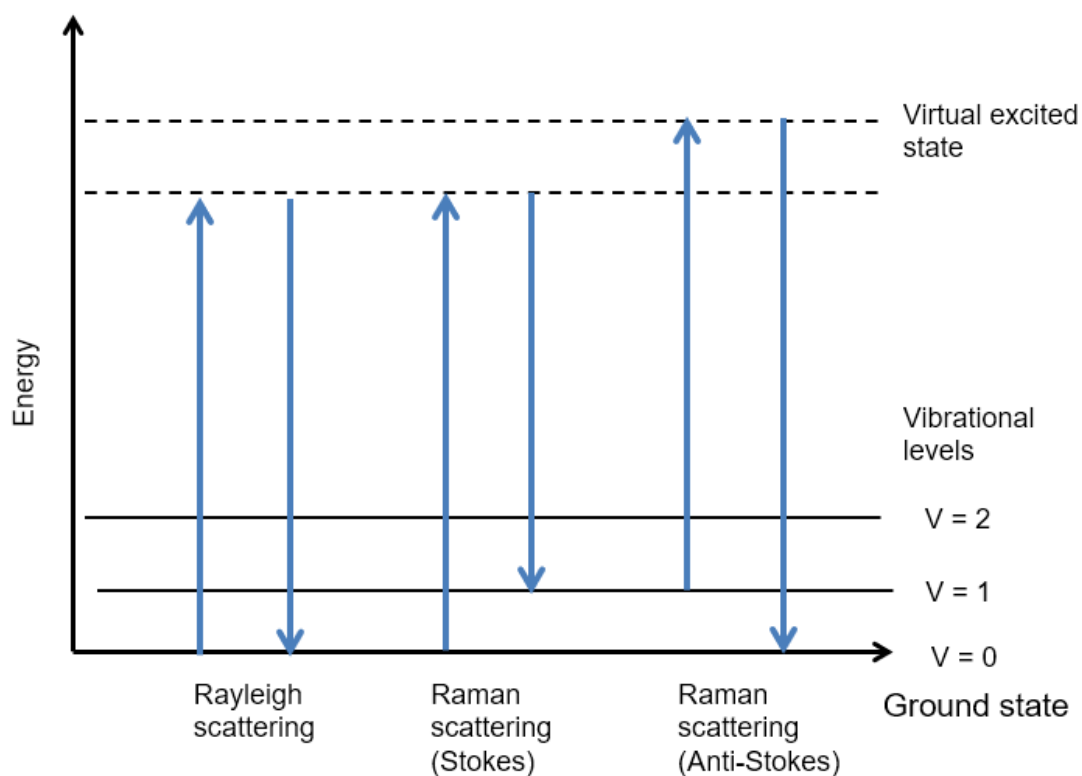


Figure 1.7. Scheme of energy level changes in Rayleigh scattering and Raman scattering

For the work presented in this thesis, Raman spectroscopy was selected because it is possible to collect data from a very wide energy window (with modern filters). Recent advances in Raman technology have allowed facile collection of data down to 30 cm^{-1} and below, whereas with infrared spectroscopy limit is around 400 cm^{-1} before specialised and expensive technology such as Terahertz. Raman spectroscopy therefore offers a convenient way to capture the maximum amount of vibration information about a molecule in the simplest manner possible. The wavelength range and

resolution depend on the gratings and detector parameters. On the other hand, infrared (IR) spectroscopy could measure intra-molecular vibration, however does not cover the frequency range of inter-molecular vibration. In addition, the selection rules for IR transitions differ from Raman. A vibration is IR active when there is a change in dipole moment, whereas Raman is active when there is a change in polarisability. The difference in selection rules means that generally, peaks that are intense in Raman spectra are not necessarily intense in IR vibrations and vice versa. For example, water is very visible in IR because it has a strong dipole moment, whilst the electrons are not easily polarised and thus the Raman scattering is not very intense. Moreover, most APIs contain π -electrons which are easily polarised, and therefore they show intense Raman stretch. On the other hand, excipients are primarily σ -bonded molecules with weak Raman signal. Raman spectroscopy could be useful when detecting low levels of drug crystallinity in multi-component pharmaceutical products (Sinclair et al, 2011).

Raman system is suitable for examining high energy molecular transitions with sufficiently broad range and good resolution (Agrawal & Samal, 2018). Raman technique has been reported to show data of molecular dynamics at time-scales of nanoseconds during glass transition and can also characterize the overall stability of glasses (Sibik & Zeitler, 2016a). Polymorphic screening has been frequently tested using Raman spectroscopy. Raman spectroscopy has been used to investigate the solid-state transformation of crystallized form of indomethacin from amorphous form (Hédoux et al, 2009). Amorphous indomethacin prepared from cryogrinding partially crystallized to a specific polymorphic form well below the glass transition temperature. Polymorphic transformation of a caffeine crystal form to another was observed under moderate pressurization and to a disordered form under further pressurisation, using Raman spectroscopy (Hédoux et al, 2011). Considering that Raman scattering signal comes directly from the material at molecular level, use of Raman technique could potentially be a valuable tool to facilitate investigation of the role of alpha and beta relaxation

events in the physical stability of pharmaceuticals, however to date this has not been reported beyond a small handful of examples.

1.3.6.7 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measures thermodynamic properties associated with thermally induced transitions (Chiu & Prenner, 2011). It is commonly used to determine glass transition temperature but recent studies have shown it is possible to probe beta relaxations in amorphous materials (Vyazovkin & Dranca, 2006). Beta relaxation exists at predominantly lower temperatures compared to alpha relaxation. DSC can measure beta relaxation by annealing an amorphous material at around $\sim 0.8T_g$ followed by rapid cooling, where it slowly relaxes and loses its enthalpy (Vyazovkin & Dranca, 2006). Upon reheating, the lost enthalpy could be recovered in the form of small endothermic peak that appears before the glass transition step. Increasing the annealing time, the associated enthalpy also increases (Chen, 1981). In DSC study, indomethacin has been detected with beta relaxation annealed at as low as -20°C , which is approximately $T_g - 65^{\circ}\text{C}$ (Vyazovkin & Dranca, 2005). Such substantial experimental evidence at temperature far below T_g indicates slow molecular mobility that is much smaller than the alpha relaxation. There has been a study of beta relaxations using DSC measurements, usually involving careful annealing treatments for $\text{Zr}_{65}\text{Cu}_{27.5}\text{Al}_{7.5}$ metallic glass. A pronounced exothermal peak develops below T_g in the annealed sample and was regarded as an indirect sign of the beta relaxation (Yu et al, 2013).

1.4 Literature review

The dynamics of relaxation related to glass transition have been studied almost exclusively on α -relaxation process, which includes the Kohlrausch stretched exponential correlation function, and others (Kob et al, 1997). In some study, beta relaxation showed a strong similarity to alpha relaxation in that their relaxation time are not that different from each other in order of

magnitude (Ngai, 1998). That local mobility in JG beta relaxation could play a significant role in recrystallization (both nucleation and crystal growth) and that it could be regarded as a precursor for structural relaxation, makes it important to investigate secondary relaxation events in dielectric spectra. There have been only few observations that account for the influence of beta relaxation on recrystallization in the amorphous state because the beta relaxation peak is often too much smaller than that of alpha relaxation peak in the dielectric spectra. A weak or no intermolecular local mobility is detected by dielectric spectroscopy. For instance, beta relaxation process of Ortho-terphenyl was barely observable using dielectric spectroscopy (Wagner & Richert, 1999). In the dielectric spectra of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (ROY), no evidence for JG beta relaxation process was found (Sun et al, 2009).

A study used terahertz time-domain spectroscopy to investigate dielectric losses in the supercooled hydrogen-bonded polyalcohols, such as glycerol, threitol, xylitol, and sorbitol, at terahertz frequencies, at temperatures both above and below the glass transition temperature (T_g). The results reveal several universal features among the samples. The glasses were heated from low temperature to above $0.65 T_g$, and the dielectric losses increased steadily with temperature. There could be evidence that the change in dielectric losses originated from the high-frequency tail of the JG beta relaxation. It is best observed in the case of sorbitol, whereas it remains unobservable in the case of glycerol. Temperatures in the proximity of $0.65 T_g$ appeared to be the universal region for the secondary glass transition due to the JG beta relaxation in several systems (Sibik et al, 2014a).

There has been a study that provides terahertz time domain spectroscopy evidence on the crystallization of amorphous paracetamol. The terahertz spectra showed differences in mechanism and level of absorption at terahertz frequencies in different phases when heating supercooled liquid paracetamol from 295 to 470 K in 5 K steps. This information was used to determine the phase transition in amorphous materials. Heating amorphous

paracetamol from 295 K, crystallization to form III has been observed at 330 K, followed by transformation of form III to II at 375 K, form II. The absorption coefficient over the full temperature range of 295 to 470 K at several chosen frequencies was shown as a function of temperature in Figure 1.8 (Sibik et al, 2014b).

In another study, temperature-controlled Terahertz spectroscopy was used to collect terahertz absorption spectra from naproxen, paracetamol, indomethacin, flufenamic acid, and simvastatin, in the frequency range of 0.2 to 2.0 THz and in the temperature range of 80 to 320 K with 10 K steps.

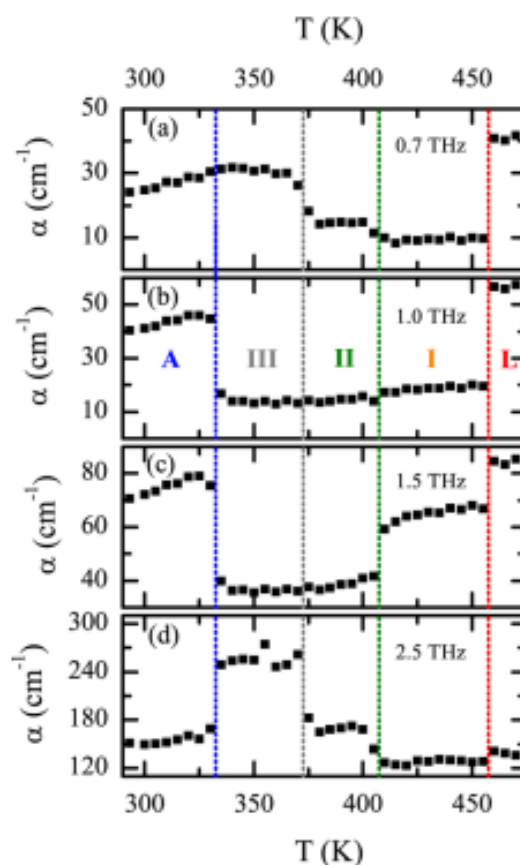


Figure 1.8. Absorption coefficient of paracetamol at various terahertz frequencies in the temperature range of 295 to 470 K with 5 K increments. A = amorphous, III, II, I = crystalline polymorphs, L = liquid (Sibik et al, 2014b)

Figure 1.9 shows terahertz absorption spectra of amorphous paracetamol, flufenamic acid, indomethacin, and simvastatin at 1.0 THz. The temperature was rescaled by T_g and the absorption coefficient at frequency of 1 THz. Increase in terahertz absorption due to the onset of molecular mobility was observed above alpha and beta transition temperatures. It shows that the terahertz molecular dynamics is strongly related to the molecular movements that control the stability of the amorphous drugs (Sibik et al, 2015).

Raman effect can be described as the inelastic scattering that transfers energy from the light to a vibration of the molecule (Smith et al, 2015). The amount of energy is exactly the amount required to excite the molecular vibration of the molecule. Vibrations of functional groups appear in Raman spectrum, which are useful when monitoring reactions that involve the functional groups.

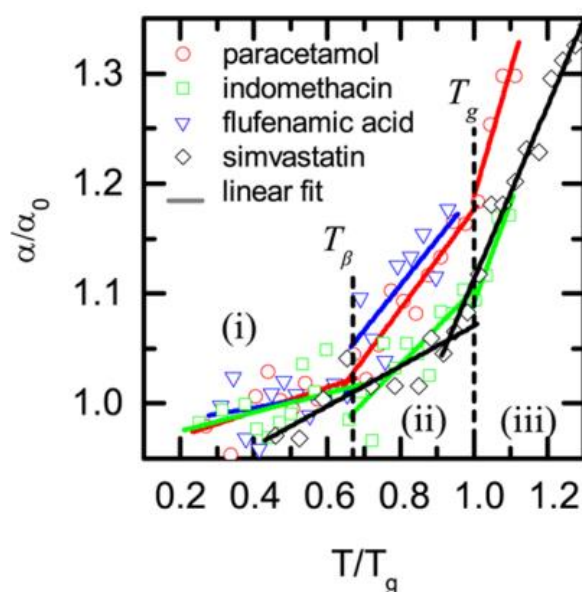


Figure 1.9. Terahertz absorption spectra of amorphous paracetamol, flufenamic acid, indomethacin and simvastatin at 1.0 THz (Sibik et al, 2015)

Raman spectroscopy can measure temperature dependent response of the functional groups of a molecule arising from the glass amorphous transition. It has been used to study dynamical properties of the amorphous salol near the glass transition temperature (Li et al, 1992).

The glass transition of poly(itraconate) was studied by Raman spectroscopy and the value of T_g was consistent with different techniques such as Differential scanning calorimetry and dynamic thermal degradation (Samith & Ramos-Moore, 2015). Raman spectroscopy has been widely used to characterize semiconductors (Ruf, 1997). Temperature dependent Raman scattering has been performed on Si, Ge, diamond, GaAs, and GaN (Liu et al, 1999). The technique is widely used in material science and is considered a powerful tool for the characterization of pharmaceuticals (Šašić, 2008).

A study has shown that with Raman spectroscopy, the alpha, beta and gamma relaxation temperatures of polymer nylon 6,6 (N66) were found and this indicates the possibility of Raman spectroscopy as a tool to determine relaxation temperatures (Bertoldo Menezes et al, 2016). The nylon 6,6 polymer was measured in the spectral range from 100 to 3500 cm^{-1} , using 532 nm laser. The laser power on the sample was 9.0 mW and the 10 $\times$ objective was used. All spectra were collected with fifteen accumulations with an exposure time of 10.0 seconds for each accumulation. The temperature of the sample was heated at rate of 20 $^{\circ}\text{C}$ per minute, in the range from -120 to 120 $^{\circ}\text{C}$, with steps of 10.0 $^{\circ}\text{C}$ each. Temperature-dependent changes of peak parameters of the Raman bands associated with C=O, C-C, and N-H stretching vibrations were observed at known temperature ranges of the alpha, beta and gamma relaxation of N66 (Turi, 2012). Figure 1.10 shows the peak areas as normalised Integrated Raman Intensity (IRI) for N66 as function of temperature. Three characteristic temperature dependent changes were identified with IRI for the three peaks (C=O, C-C, and N-H stretching) which could be attributed to alpha, beta and gamma relaxation.

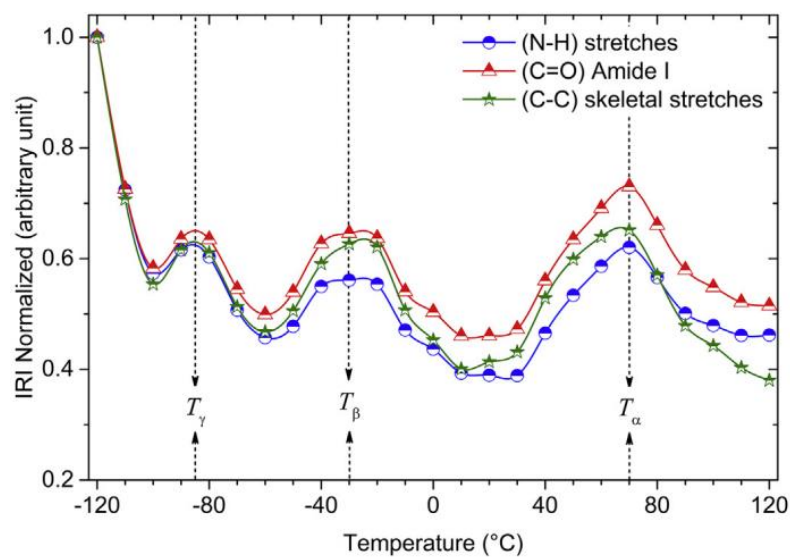


Figure 1.10. Temperature dependence of the normalized Integrated Raman Intensity (IRI) of the three Raman peaks at known temperature ranges of alpha, beta and gamma relaxation and these correspond to C=O, C-C, and N-H stretching vibrations of nylon 6,6 (Bertoldo Menezes et al, 2016)

To study molecular mobility in supercooled liquids and amorphous states, the most useful methods would need to explore the molecular motions with high resolution in the wide range of thermodynamic conditions. Dielectric spectroscopy has been used the most to study beta relaxation processes (Matousek et al, 2005; Matousek & Morris, 2010). Its measurements are made in a range of temperature over a broad frequency band, which facilitate the characterization of alpha and beta relaxation by distinguishing the relaxation motions of different time scales (Grzybowska et al, 2016). Despite the fact that dielectric relaxation is sensitive to alpha and beta relaxation events, dielectric techniques could assign molecular motions to particular structural groups only with simple systems and the differentiation between the crystalline and amorphous systems is not quantitative most of the time (Hancock & Zografi, 1997).

Vibrational technique such as Raman spectroscopy, could be used to study relaxation processes and generate Raman scattering signal by vibrational motions of functional groups which are sensitive to conformational changes and microscopic deformations. Hence, Raman scattering signal can

deliver information directly from the source where the relaxation processes happen, i.e. at molecular level (Bertoldo Menezes et al, 2016). In addition, in dielectric system, careful sample preparation is expected due to strong absorption line from water. On the other hand, Raman spectroscopy is not affected by water and requires almost no sample preparation. The potential advantage of studying relaxation with Raman spectroscopy is that the light scattering signal is from the vibrational motions of the functional groups within the molecule that are sensitive to conformational changes (Bertoldo Menezes et al, 2016). Raman scattering signal comes directly from the material at molecular level, hence will facilitate investigation of the role of molecular changes in the physical stability of pharmaceuticals. The improvement in stability and wavelength specificity of Raman spectrometers enables access to broad frequency region unlike Terahertz spectroscopy (Parrott & Zeitler, 2015). Raman spectroscopy has high spectral band width, which gives access to the vibrational response from terahertz to mid-Infrared (IR) frequencies in a single measurement. Simultaneous use of Raman spectroscopy and another technique, such as dielectric spectroscopy could provide complementary information and help investigate the local mobility in pharmaceutical drugs (Bertoldo Menezes et al, 2016).

1.5 The study aims

The study aim is to investigate any correlations between Raman spectra collected as a function of temperature and the alpha and the beta relaxations. Our hypothesis is that we will see spectral changes around the alpha and beta relaxations which we can interpret chemically and allow molecular understanding of the basis of the alpha and beta relaxations. To achieve this, we will undertake following experiments:

- We intend to do Raman experiments with well-studied drug that has reported alpha (23.85°C) and beta (-79.15°C) transition temperatures, such as paracetamol at various temperatures, and observe if Raman spectral peaks change at around the reported temperature of alpha and

beta relaxation. If so, we want to see which of the peaks change at the alpha and beta transition temperature and link with specific functional groups or molecular interactions. We want to see if the results are reproducible. This is addressed in Chapter 3.

- Based on the result from the paracetamol experiment, we will look at nifedipine and felodipine. These are the model drugs and they both have similar alpha transition temperatures. Nifedipine has glass transition temperature at around 41.9°C (Ishida et al, 2007), and felodipine at around 44.7°C (Saboo et al, 2021), but very different reported physical stabilities. We want to observe if any changes happen below the alpha transition, and possibly around the beta transition temperature that may account for the different physical stabilities. This is addressed in Chapter 4 for nifedipine and Chapter 5 for felodipine.

Chapter 2 Materials and methods

2.1 Materials

2.1.1 Paracetamol

Paracetamol (Figure 2.1) is a common analgesic and antipyretic. It is classified as a BCS class I drug - a drug of high solubility and intestinal permeability (Somani et al, 2016). It has been used in the present study due to abundance of existing data on its solid-state behaviour. It is known to have three crystalline forms and additional forms IV and V at high pressure (Smith et al, 2014). Paracetamol has been used as a model in the study of stability of amorphous drugs (Martino et al, 2000; Nanubolu & Burley, 2012). Its amorphous form and polymorphism associated with crystallization could show interesting properties for investigation of the physical stability of drug at various temperatures. In present study, paracetamol was used as a reference BCS class I drug.

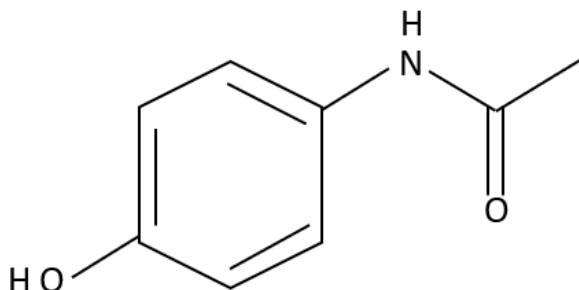


Figure 2.1. Chemical structure of paracetamol

2.1.2 Nifedipine

Nifedipine (Figure 2.2) is a calcium channel blocker used for the treatment of hypertension (Guazzi et al, 1977). It has low water solubility and high intestinal permeability. It is classified as a BCS class II drug (Nader et al, 2016). Nifedipine is known to have three polymorphic forms, alpha, beta and gamma (Gunn et al, 2012). Amorphization of nifedipine could possibly improve respective insolubility issue to enhance bioavailability and was used in present

study as BCS II reference drug to investigate molecular mobility of the amorphous form at various temperatures.

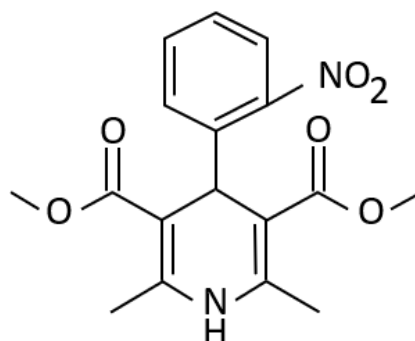


Figure 2.2. Chemical structure of nifedipine

2.1.3 Felodipine

Felodipine (Figure 2.3) is a poorly water-soluble calcium channel blocker (Staessen et al, 2003). It is classified as a BCS class II drug (Tapas et al, 2009). It has four polymorphic forms (Surov et al, 2012). Amorphous felodipine was used as BCS II reference drug to investigate molecular mobility at various temperatures.

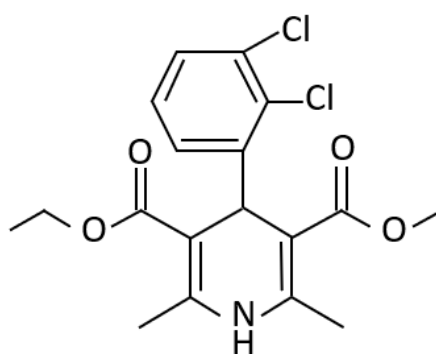


Figure 2.3. Chemical structure of felodipine

2.2 Methods

2.2.1 Differential scanning calorimetry (DSC)

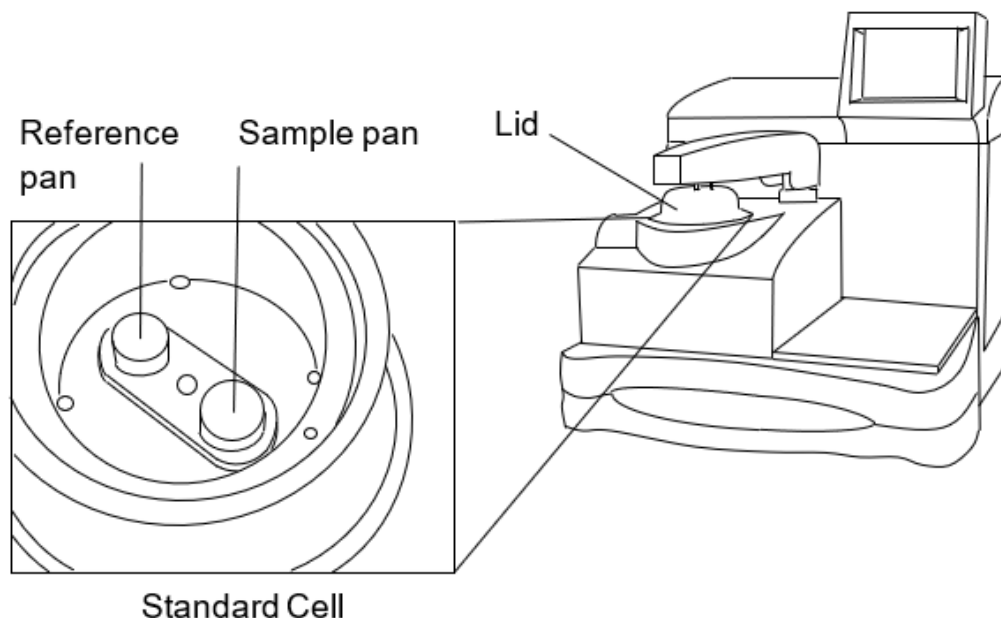


Figure 2.4. A diagram of differential scanning calorimetry (DSC).

Differential scanning calorimetry (DSC) is a thermal analysis technique which measures the difference in the heat flow rates between a sample and reference as a function of time and temperature, while the sample is under controlled temperature program (Lombardi, 1980). DSC consists of two pans, a pan filled with sample and an empty reference pan, resting on top of heaters (Figure 2.4). The principle of this technique entails that the sample and reference are both maintained at the same temperature, and when the sample goes through a phase transition, more or less heat will need to flow to the sample to compensate for the temperature difference to the reference. By measuring the difference in heat flow between the sample and the reference, DSC could be used to determine important parameters of a material such as, heat capacity, glass transition temperature, crystallization temperature, and melting point (Basu, 2018). Figure 2.5 shows a DSC trace for zidovudine with the position of the melting peak (Araújo et al, 2010). Crystallisation and melting events, generally considered to be first-order phase transitions are shown as

large exotherms and endotherms. By comparison, the second-order thermodynamic event of glass transition is displayed as a change in heat capacity (Debenedetti & Stillinger, 2001).

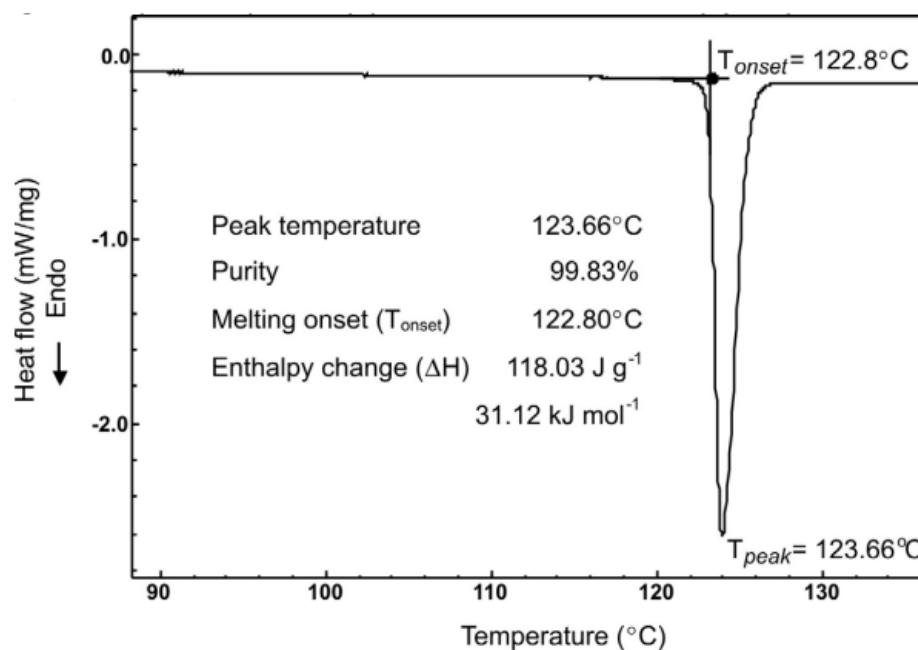


Figure 2.5. DSC trace for zidovudine (Araújo et al, 2010)

DSC technique has been selected and used in this study to obtain characteristic temperatures – glass transition temperatures, melting temperatures, and other phase transition temperatures. TRIOS software was used for viewing and analysing of the data.

2.2.2 X-Ray diffraction (XRD)

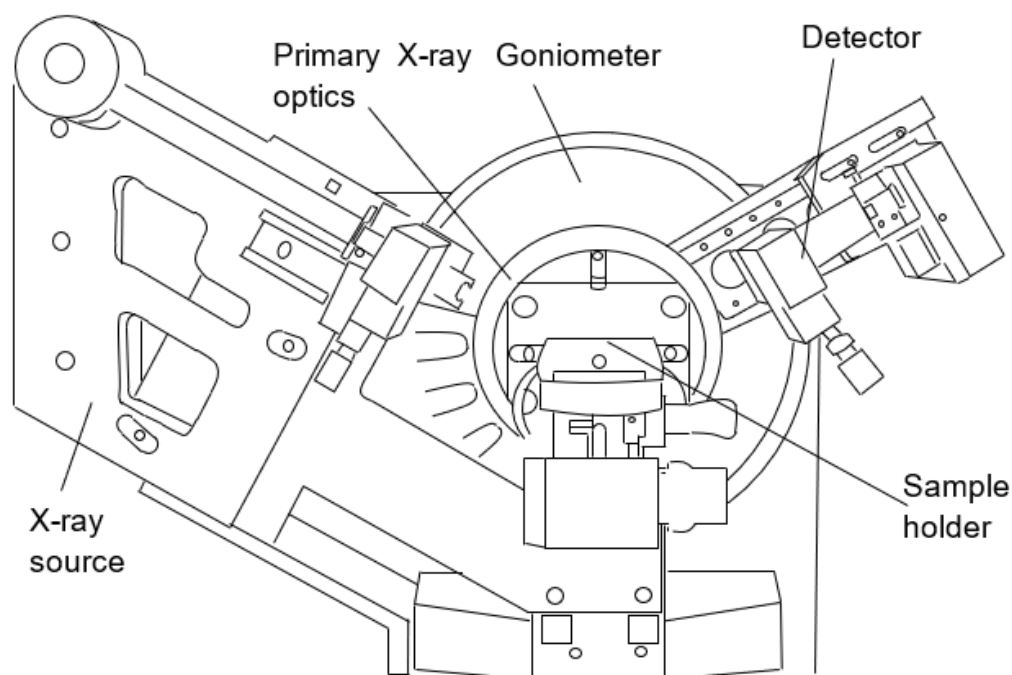


Figure 2.6. A diagram of x-ray diffraction (XRD).

X-ray diffraction (XRD) (Figure 2.6) is a technique that provides information about the crystalline composition and the phase purity, nature of the materials as crystalline or amorphous (Khan et al, 2020). When x-ray beam reaches the crystalline sample, diffraction patterns form. Each diffraction pattern is unique for each sample like a fingerprint, which could be distinguished by comparison with reference diffraction pattern. In XRD crystalline samples are observed as sharp peaks of high intensity, whereas amorphous are broad peaks (Yadav, 2021). XRD of amorphous and various polymorphic crystalline patterns of indomethacin are shown in Figure 2.7 (Okumura et al, 2006). The broader features correspond to amorphous nature of the indomethacin particles. The small sharp peaks imply the crystalline nature of the sample.

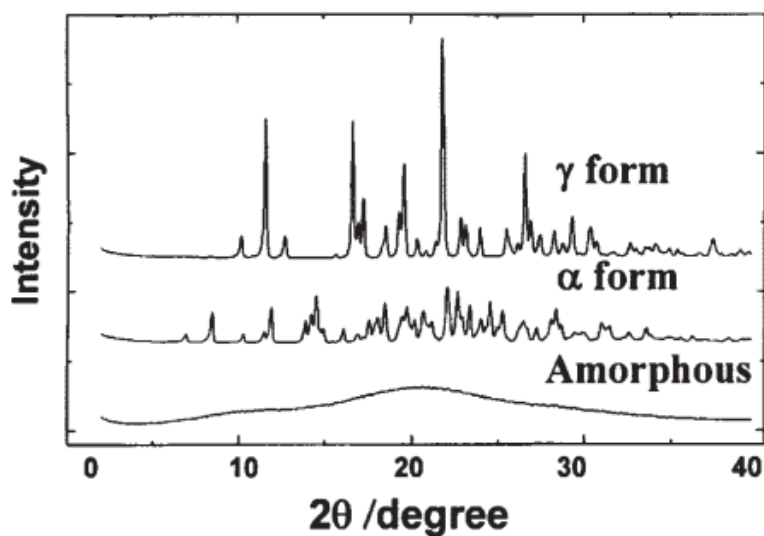


Figure 2.7. X-ray diffraction patterns of amorphous, alpha and gamma forms of indomethacin. Used with permission of Elsevier Science & Technology Journals, from (Okumura et al, 2006); permission conveyed through Copyright Clearance Center, Inc.

2.2.3 Fourier transform infrared (FTIR)

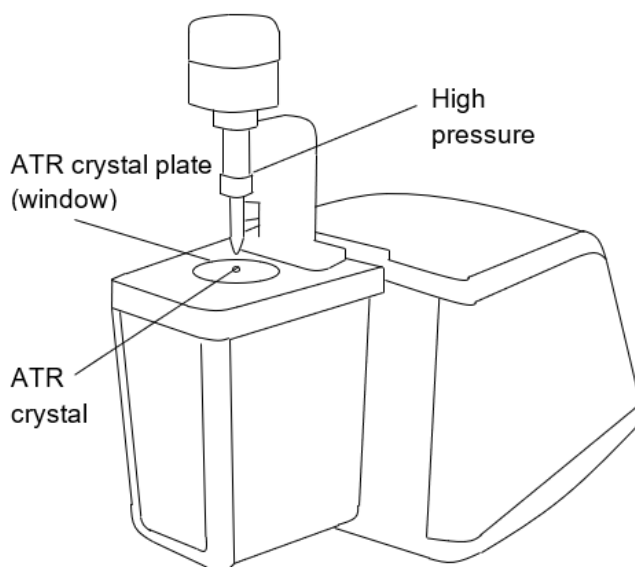


Figure 2.8. A diagram of fourier transform infrared (FTIR).

Fourier transform infrared (FTIR) spectroscopy (Figure 2.8) is a form of vibrational spectroscopy based upon the identification of chemical bonds within molecules when irradiated with mid-infrared light, in the wavelengths of 4000 to 400 cm^{-1} (Hermann et al, 2017; Sauer et al, 2021). Infrared (IR)

analysis using transmission technique is commonly encountered with difficulty where IR passes through a sample of controlled pathlength (Grdadolnik, 2002). Attenuated total reflection (ATR)-fourier transform infrared (FTIR) is a technique that could use samples to be analysed directly in either solid or liquid state. Figure 2.9 shows principle of ATR experiments. When IR light enters ATR crystal, the light can reflect and create an evanescent wave, which can interact with samples. The sample may absorb the evanescent wave, resulting in a spectrum (Barbeş et al, 2014).

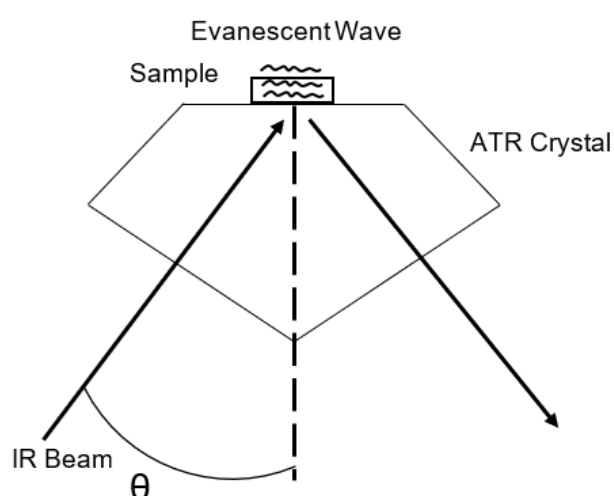


Figure 2.9. Schematic of an ATR experiment – Evanescent waves on top of ATR crystal

The ATR-FTIR technique measures distinct vibration and rotation of molecules at specific wavelength. It gives spectral fingerprints to different functional groups within the molecule, and each spectral peak could be analysed individually. Figure 2.10 shows FTIR spectrum of sparfloxacin. Characteristic peaks of sparfloxacin were demonstrated at 3460 cm^{-1} and 3340 cm^{-1} (primary amine in NH_2), 1714 cm^{-1} (carbonyl in COOH), 1639 cm^{-1} (carbonyl in 4-quinolone ring) (Kowalczyk & Pitucha, 2019). FTIR and Raman spectroscopy are complementary analytical techniques (Mehta et al, 2020) and both were used in this study. IR and Raman spectroscopy have different vibrational activity, in some modes both are active, but in some others only IR or Raman is active.

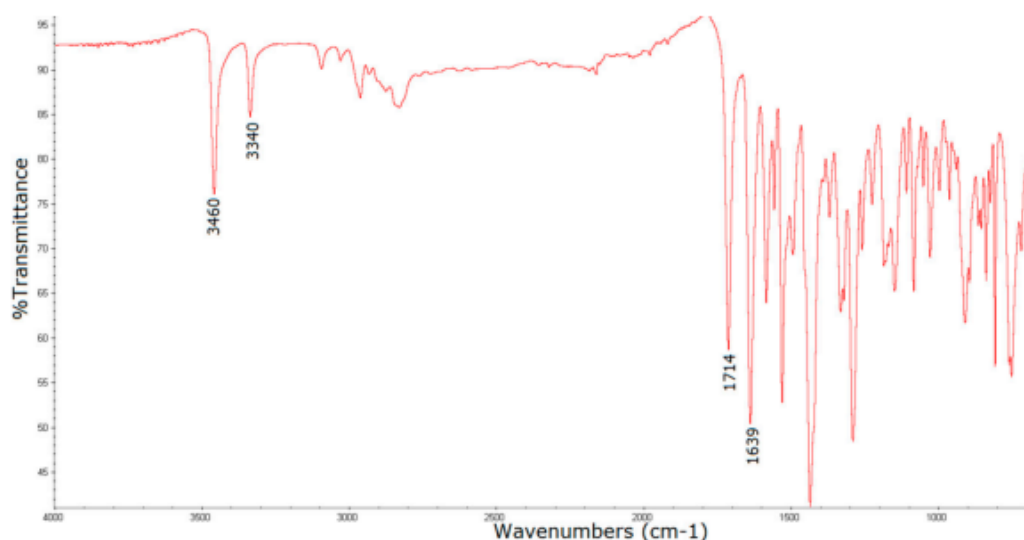


Figure 2.10. FTIR spectrum of sparfloxacin (Kowalczyk & Pitucha, 2019)

2.2.4 Raman spectroscopy

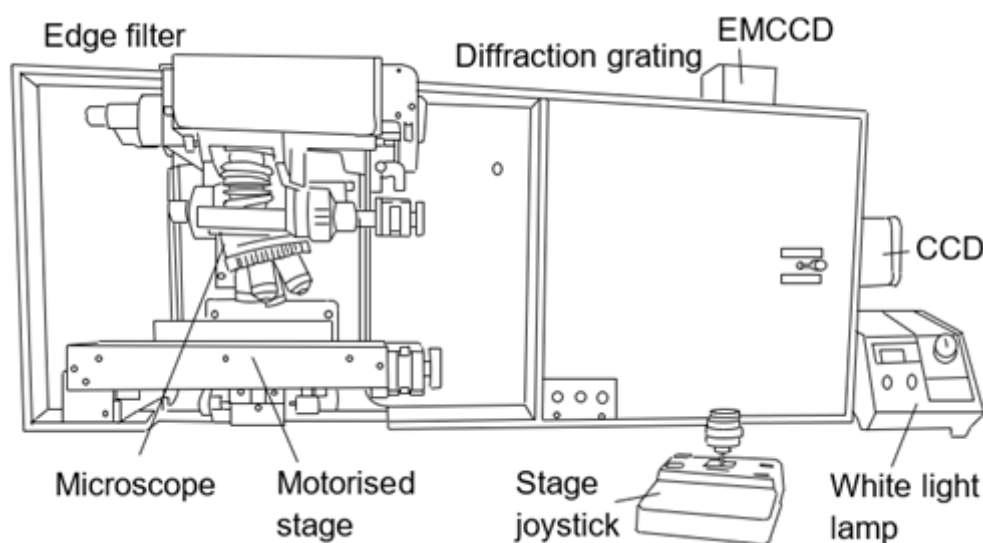


Figure 2.11. A diagram of Raman microscope – Horiba LabRAM HR confocal microscope/spectrometer.

Raman spectroscopy (Figure 2.11) is a vibrational spectroscopic technique based on inelastic scattering phenomenon of light for investigating the unique fingerprint of samples (Le Pevelen, 2017). When light of specific wavelength is shined to the sample, the light gets either absorbed to the

sample molecule or scattered. Rayleigh scattering describes most of the lights that will be scattered elastically with no change to the wavelength. On the other hand, Raman scattering describes light that will be scattered inelastically, resulting in a wavelength change. The Raman scattering occurs only 1 photon in 10^5 - 10^7 photons (quantum of light) (Ferraro, 2003). Such change to the wavelength of the photons is called “Raman shift” and it is unique to sample molecules. The Raman technique could be used to probe chemical bonds in molecules. Figure 2.12 is the Raman spectrum of amorphous solid, alpha, and gamma crystalline forms of indomethacin (Hédoux et al, 2011). The Raman shift is within 1550 - 1750 cm^{-1} region that is mostly represented by C=O chemical bonding.

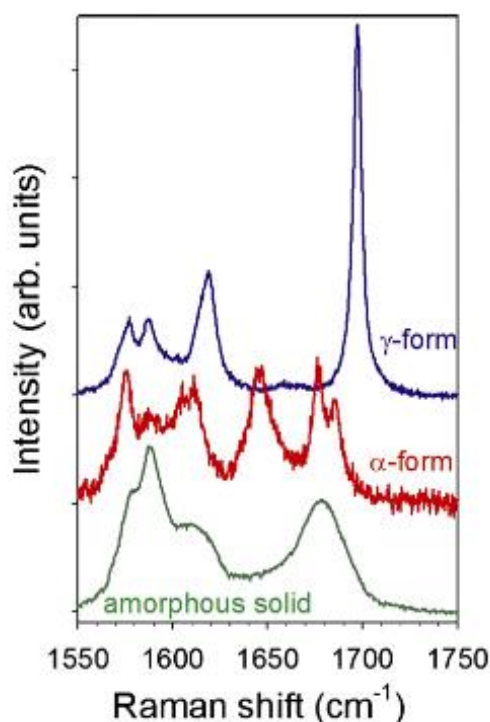


Figure 2.12. Raman spectra of the amorphous state, alpha, and gamma crystalline forms of Indomethacin. Used with permission of Elsevier Science & Technology Journals, from (Hédoux et al, 2011); permission conveyed through Copyright Clearance Center, Inc.

The Raman spectrum contains a number of peaks. Each of those peaks correspond to a particular vibrational mode. It is the characteristics of those peaks that provide valuable information on the origin of that particular

vibrational mode. There are three specific parameters that need to be considered when diagnosing peaks within the Raman spectrum (Figure 2.13).

The first is that of the absolute height (or intensity) of the peak, which is typically used to diagnose concentration, in the knowledge that there should be a linear relationship between concentration and the height of the peak. So, if the concentration of material is doubled, the height or intensity of the peak is expected to become twice as big. This does come with a slight caveat with Raman spectroscopy that the absolute height is not directly proportional to the absorption cross-section in the same way that would be expected regarding the intensity of peaks in the infrared spectrum. This is simply because the height of the peak is a manifestation of the experimental setup, as much as the fundamental properties of the material itself. So, the Raman peak height should not be overinterpreted and treated relatively, rather than absolutely, in any case.

The width of the peak could give useful information on structural disorder and crystallinity. A general rule of thumb is that an increase in peak broadness is associated with a transition from a crystalline to an amorphous state. Hence, a particularly broad peak might be indicative of a range of different environments around a particular chemical bond. Therefore, this might be associated with a more amorphous phase, rather than a crystalline phase, which would have a much narrower full width.

The last parameter is that at the absolute peak position. This often is the most diagnostic because it gives specific information on the nature of the chemical species involved in the vibration. It could identify molecular structure from Raman spectra. It could also give further information on crystal phases and alloy compositions.

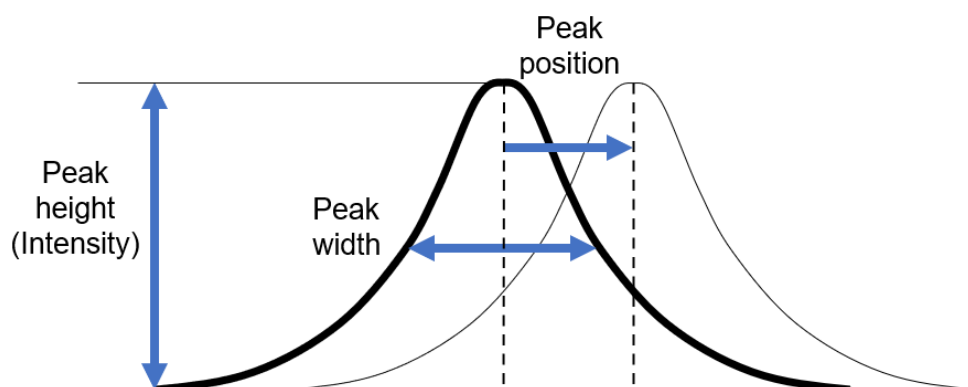


Figure 2.13. The properties of Raman peaks -peak height (intensity), peak width and peak position

Analytical techniques such as DSC or XRD cannot provide enough information about the structures of the molecules and the chemical bonds within amorphous sample (Bo et al, 2021). Vibrational spectroscopy such as FTIR, Raman spectroscopy can give information about the structure of the molecules and molecular interactions within the sample (Ayala et al, 2012; Zhang et al, 2012). Terahertz (THz) spectroscopy could assign molecular motions to particular structural groups only with simple systems (Hancock & Zografi, 1997). THz could be expensive and require large sample sizes and preparation. The samples used in these techniques are sometimes restricted to be in a specific physical form or experimental settings in regard to moisture or air. Raman spectroscopy has been used in this study because it is non-destructive or non-invasive, hardly requires any sample preparation. Raman scattering signals from the vibrational motions of the functional groups within the sample molecule are sensitive to conformational changes and therefore more suited for the purpose of the experiment (Bertoldo Menezes et al, 2016).

Raman spectroscopy has been used to determine temperature of a material from ratio of the Stokes and anti-Stokes signal strengths of the same Raman peak (Kauffmann et al, 2019). The Stokes/anti-Stokes intensity ratio could calculate in real time and locally the temperature of the sample based on a Boltzmann distribution law. The equation describing the Boltzmann

distribution of the ground and first excited state populations is shown in Equation 2 (Tuschel, 2016):

Equation 2

$$\frac{I_{AS}}{I_S} = \frac{(V_1 + V_V)^3}{(V_1 - V_V)^3} e\left(-\frac{hV_V}{kT}\right) \quad (2)$$

where I_S Stokes Raman scattering strength, I_{AS} is anti-Stokes Raman scattering strength, T is temperature, k is Boltzmann's constant, h is Planck's constant, V_1 is the frequency of the laser, V_V is the frequency of the vibrational mode.

For the variable-temperature analysis, the Horiba Raman microscope system could be used in combination with a Linkam thermal stage LTS350 (Figure 2.14). Temperatures could be controlled with TMS94 temperature controlling unit and LNP94 cooling system. The temperature can be operated over the range of -196 to 420°C.

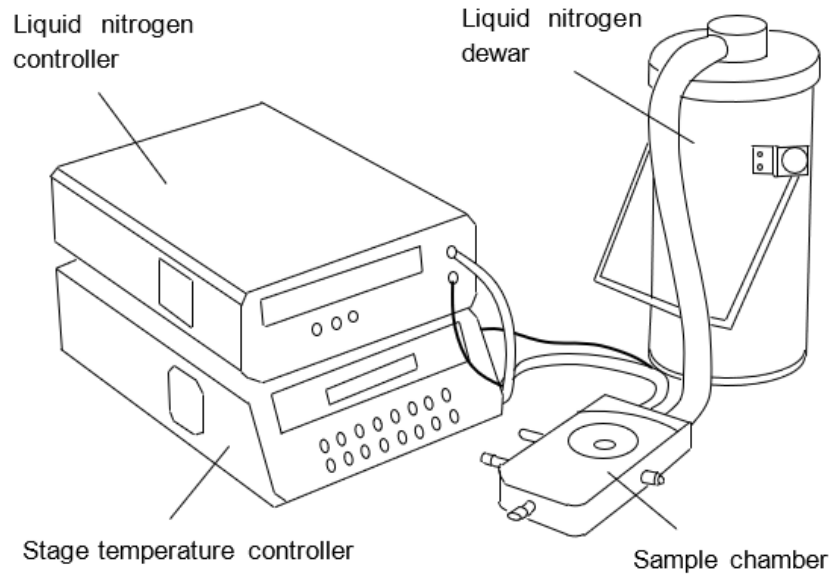


Figure 2.14. A diagram of Linkam thermal stage LTS350 and temperature controlling units that used with Raman systems for variable temperature set up experiments.

2.2.5 Data analysis

LabSpec6 software generates spectra collected in Raman microscope LabRAM HR experiment. The generated files were plotted in the open source statistical software R (R Core Team, 2021). The spectral data were normalized so that each spectrum is mean-centered and then divided by its root mean-square prior to data analysis. R program was written to fit peaks of selected vibrational modes to the Gaussian model. Gaussian peak shape was chosen so that a parameter of interest, such as peak position, height, and width, could be calculated from graphically measurable parameters. In order to fit peaks a model comprising several Gaussians was coded. The code performs peak fitting for multiple data sets. Variables employed are peak position, height, and width. The script generates image files containing changes in the peak position, height and width of the entire data set. The script for the peak fitting and generated peak fitting images is provided in the appendix A. Background, gradient and intercept on either side of the peak model were linear. Gaussian fitting was optimised using `nls()` function in R, which helps fit nonlinear regression models.

Chapter 3 Investigation of the applicability of Raman spectroscopy to probe the alpha and beta relaxation events in paracetamol

3.1 Introduction

As noted in the introduction no work has been done in investigating molecules involved in alpha and beta relaxation events. We selected paracetamol as simple model molecule that has been characterised by Raman spectroscopy before but not at around the beta relaxation temperature. The aim of the present study is to collect the spectra across the alpha and beta transition temperature range in detail, to see if anything happens at around beta relaxation temperature, and to see if this is reproducible. Finally, our study aims to understand which (if any) part of the molecules is important for the alpha and beta relaxation events. In addition to variable temperature set up Raman experiments where we investigate changes in paracetamol molecules around alpha and beta transition temperatures from low to high temperature, we are going to do cycling experiments to check the reproducibility and whether we could observe molecular changes in heating and cooling, or just on cooling, or just on heating the paracetamol sample.

3.2 Materials

Paracetamol was purchased by Sigma-Aldrich ($\geq 99.0\%$). It is in the form of white crystalline powder and was used as a model drug in the study. The melting point, alpha transition temperature (T_α) and beta transition temperature (T_β) of paracetamol are 169°C , 23.85°C and -79.15°C , respectively (Sibik et al, 2015).

3.3 Methods

3.3.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to measure the characteristic melting point of the compound to get an indication of the purity. Thermal analysis was performed using DSC Q2000. Nitrogen flow was set at 20 mL/min and samples were heated from 20 to 180°C at a rate of 5°C/min. Modulated DSC has been performed to investigate beta transition temperature of paracetamol. The DSC was set up to modulate ± 1.00 °C at every 60 seconds with ramp of 0.50 °C/min from -100 to 180.00 °C. TRIOS software was used for viewing and analysing of the data.

3.3.2 X-ray Diffraction (XRD)

X-ray Diffraction (XRD) data was collected on a Panalytical X'pert diffractometer to determine the purity of the compound. Samples were spun at 30 rpm and exposed to a Cu-K α radiation at a voltage of 45 kV and a current of 40 mA. After being smeared onto the holder, samples were scanned from 5° to 70° 2 θ , with a 25 s exposure per 0.02° 2 θ increment. Peak analysis was performed using R software.

3.3.3 Fourier transform infrared (FTIR)

Fourier transform-infrared spectroscopy (FTIR) was used to assess purity of the compound. FTIR spectra were obtained using Cary 630 FTIR spectrometer equipped with attenuated total-reflectance (ATR) accessory. The spectra were recorded in the range of 4000-650 cm⁻¹ at a resolution of 8 cm⁻¹. Peak analysis was performed using Agilent MicroLab software.

3.3.4 Raman spectroscopy

In order to investigate the potential structural changes in amorphous drugs at around alpha and beta transition temperature, both amorphous and crystalline paracetamols were prepared for the variable temperature

experiments. For the amorphous paracetamol experiment, sample slide was placed in Linkam thermal stage LTS350 (Figure 2.14) to be melted up to high temperature of 180°C, which is higher than the melting point of paracetamol, i.e. 169°C, to ensure that the sample was completely melted (Frank, 2002). The sample was then cooled rapidly to low temperature (-100°C) in cooling rate of 30°C min⁻¹. For the crystalline paracetamol experiment, the sample was cooled straight from the room temperature to -100°C, without the melting and quench cooling procedures.

The samples were placed under the 50x objective of the Horiba LabRAM confocal Raman microscope/spectrometer to collect spectral data as a function of temperature. Numerical aperture and working distance were 0.55 and 8.0 mm, respectively. Green laser (532 nm) of 20 mW power were employed in the experiments allowing a choice of wavelength depending on the sample. Using different lasers for different pharmaceutical samples helped enhance stability of Raman scattering intensity and fluorescence suppression. The Raman instrument was calibrated with the Rayleigh line at 0 cm⁻¹ and on a standard silicon peak position at 520.7 cm⁻¹. The spectra were collected using a 300 µm confocal hole. The groove density of 300 gr/mm grating was used. Raman spectra were acquired using a SYNAPSE CCD (charged couple device) detector. Laser spot is defined by the Duoscan - shaped square, 5 × 5 µm to avoid any direct burn on samples. The spectra acquired included the typical range of both phonon-mode region (40-400 cm⁻¹) and fingerprint (500-1800 cm⁻¹) regions. Each spectrum was collected for 5 seconds, repeated once to remove spikes from cosmic rays.

3.4 Results

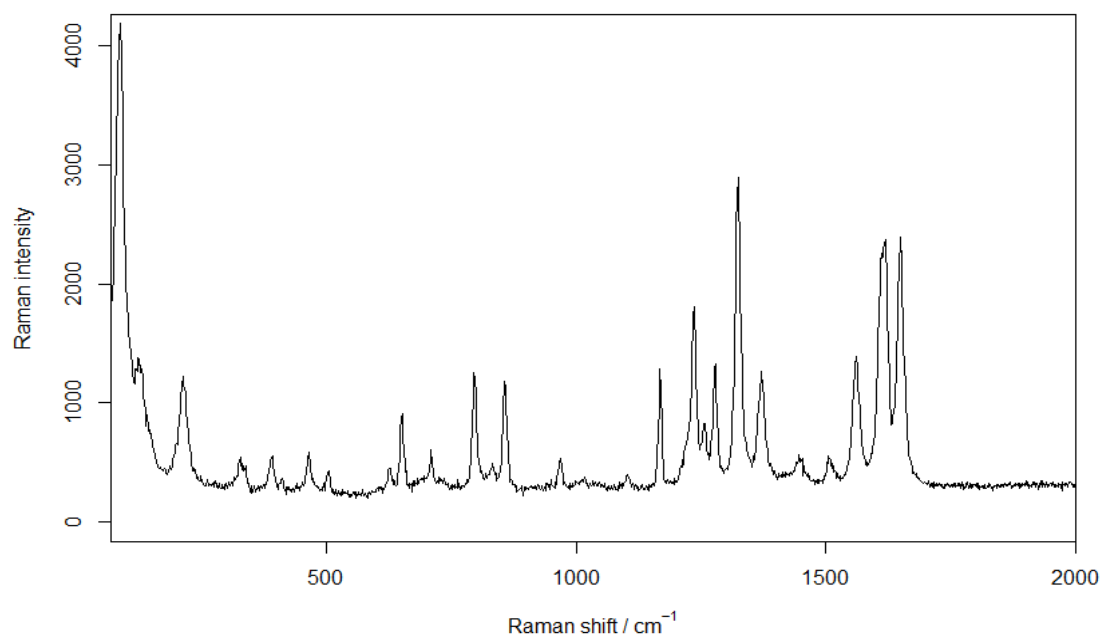
3.4.1 Characterisation of the paracetamol sample

The DSC data in the appendix (Figure A 3 of Appendix A) showed evidence of endothermal event (melting point) at around 169°C confirming the purity of the sample when compared to the reported melting point (Figure A 4 of Appendix A) in the literature (de Oliveira et al, 2017), which showed good

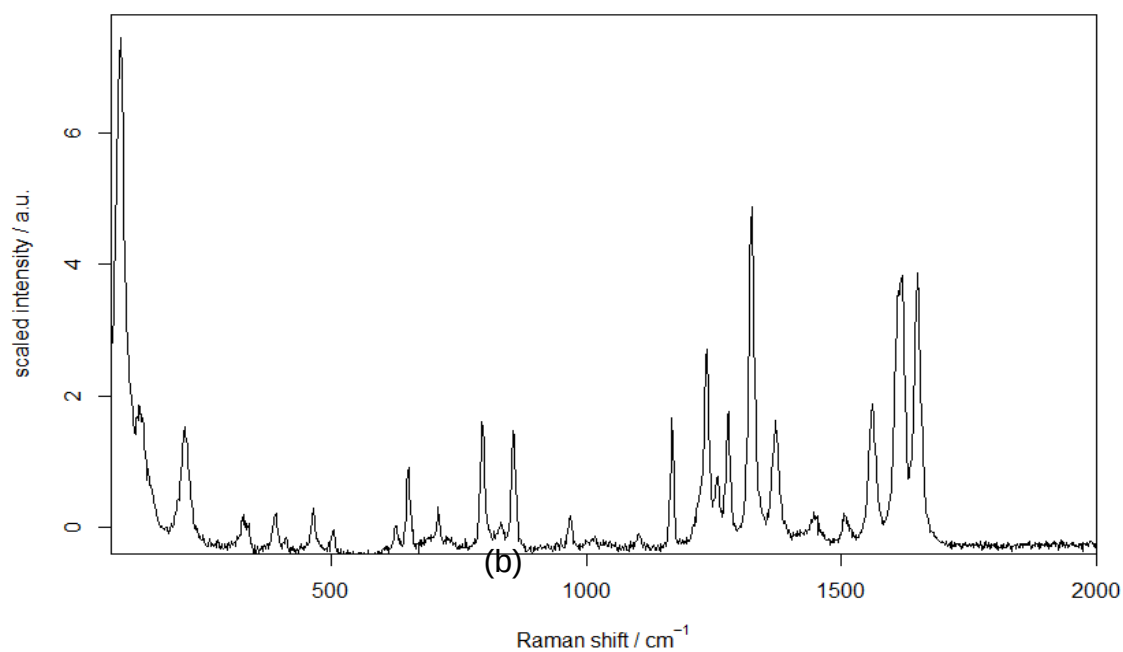
correspondence to the experimental data. The plot of the modulated DSC thermogram could be found in the appendix (Figure A 5 of Appendix A) and does not show any obvious evidence of beta relaxation. The PXRD data (Figure A 6 of Appendix A) closely resembled pattern of COTZAN07 crystal structure from the Cambridge structural database (Groom et al, 2016) and showed no extra peaks in the crystal pattern, hence confirmed the purity of the material as crystalline form and the structure of crystalline form to be polymorph I. Fourier transform-infrared spectroscopy (FTIR) was used to assess purity of the compound. The infrared spectral data (Figure A 7 of Appendix A) confirmed the characteristic peak positions of pure paracetamol (Form I) at around 1561 cm^{-1} (C=O group) and 3233 cm^{-1} (N-H group) as shown in the reference diagram (Khaled et al, 2018).

3.4.2 Scaling of Raman spectra

Figure 3.1 (a) and (b) show unscaled and scaled Raman spectra of paracetamol collected at 25°C , respectively. The spectral data of Figure 3.1 (a) were normalized with a built-in “scale” function from statistical software, R. The scale function in R normalises the spectral data set using mean value and standard deviation. The scale function subtracts the value of every column of the Raman intensity dataset by the corresponding mean value and divides the values of every column by the corresponding standard deviation value (R Core Team, 2021). Scaling enables comparison of the data that is not measured in the same approach and therefore used in this study to normalise the spectral data set from different repeat experiments and obtain meaningful results for further analysis.



(a)



(b)

Figure 3.1. Raman spectra of powdered paracetamol collected at 25°C: Not scaled (top), scaled (bottom)

3.4.3 Measurement of height of paracetamol at variable temperature prior to the variable temperature set up Raman experiments to determine minimum and maximum values for automatic focus

One concern prior to commencing these experiments was the length of time taken to do the experiment, which seem to have impact on the granularity of the temperature steps that we can use. The Raman spectra were collected from quench-cooled paracetamol sample prepared as outlined in Section 3.2 and the sample was heated from -100 to 180°C with step sizes of 50°C, in the wavenumber range of 0 to 2000 cm^{-1} . Figure 3.2 shows height differences of the sample from the initial z stage (0 μm at -100°C) plotted versus corresponding temperature into a graph. The value of the percentage error bar is 5%. The measurement of temperature dependent height changes of amorphous paracetamol gave a good estimate of the expansion and contraction behaviour of the sample. The changes in sample volume were used as z limits minimum and maximum values for automatic focus, to ensure that an optimal focus is reached before acquisition of spectra in Raman spectrometer during the variable temperature experiment. For example, the value of 10 μm at -50 °C refers to the distance z stage had to move 10 μm upward in height to stay in focus and compensate for the loss in working distance from the decrease in volume of the sample. What was achieved is the understanding of the range of search needed when setting up auto focus of the sample so that the stage doesn't have to go up and down unnecessarily prior to collection of each spectrum.

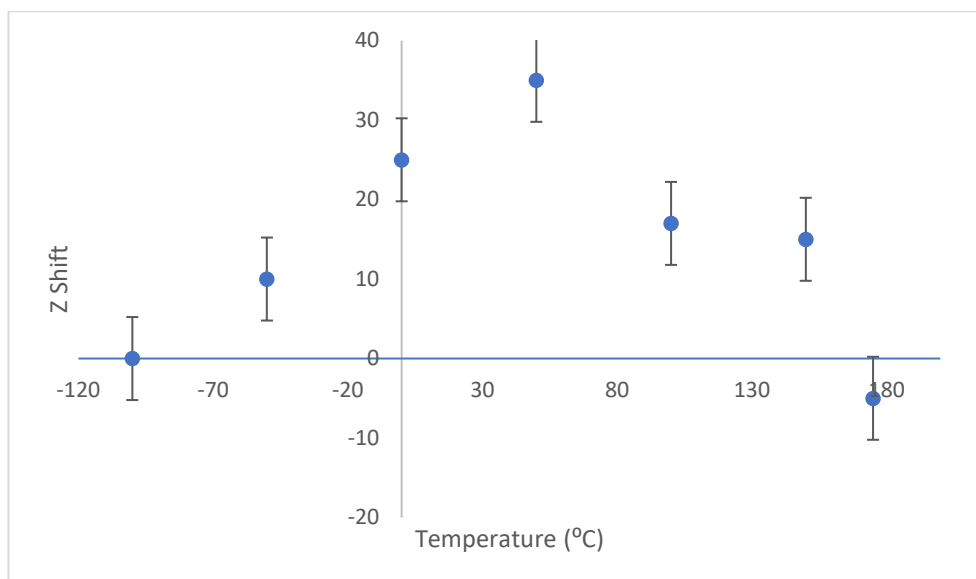


Figure 3.2. Graph illustrating the height (Z stage) change of paracetamol sample at various temperature from -100 to 175°C. The value of the percentage error bar is 5%.

3.4.4 Heat map - Variable temperature in Raman spectra

In order to investigate potential Raman spectral changes which can be linked with to alpha (23.85°C) and beta (-79.15°C) relaxation processes, contour maps were plotted (Sibik et al, 2015). Raman imaging via Raman intensity contour map could provide a picture that shows potential structural changes at the temperature of our interest. Figure 3.3 is the contour map of the normalised Raman spectra plotted as a function of temperature. The Raman spectra were collected from quench-cooled paracetamol sample prepared as outlined in Section 3.2 and the sample was heated from -100 to 180°C with step sizes of 1°C, in the wavenumber range of 0 to 2000 cm^{-1} . The Raman shift is on the Y axis and temperature on the X axis. The scaled intensities are plotted on the colour scale, where white and red represent high intensities, and blue and black represent low intensities as shown in the key to the right-hand side of the Figure 3.3. The intensities range from -1.5 to 8. Peaks typically appear as horizontal bands on the plot with colours that are light, often red, white or yellow compared to area where there are no peaks, which are blue or black.

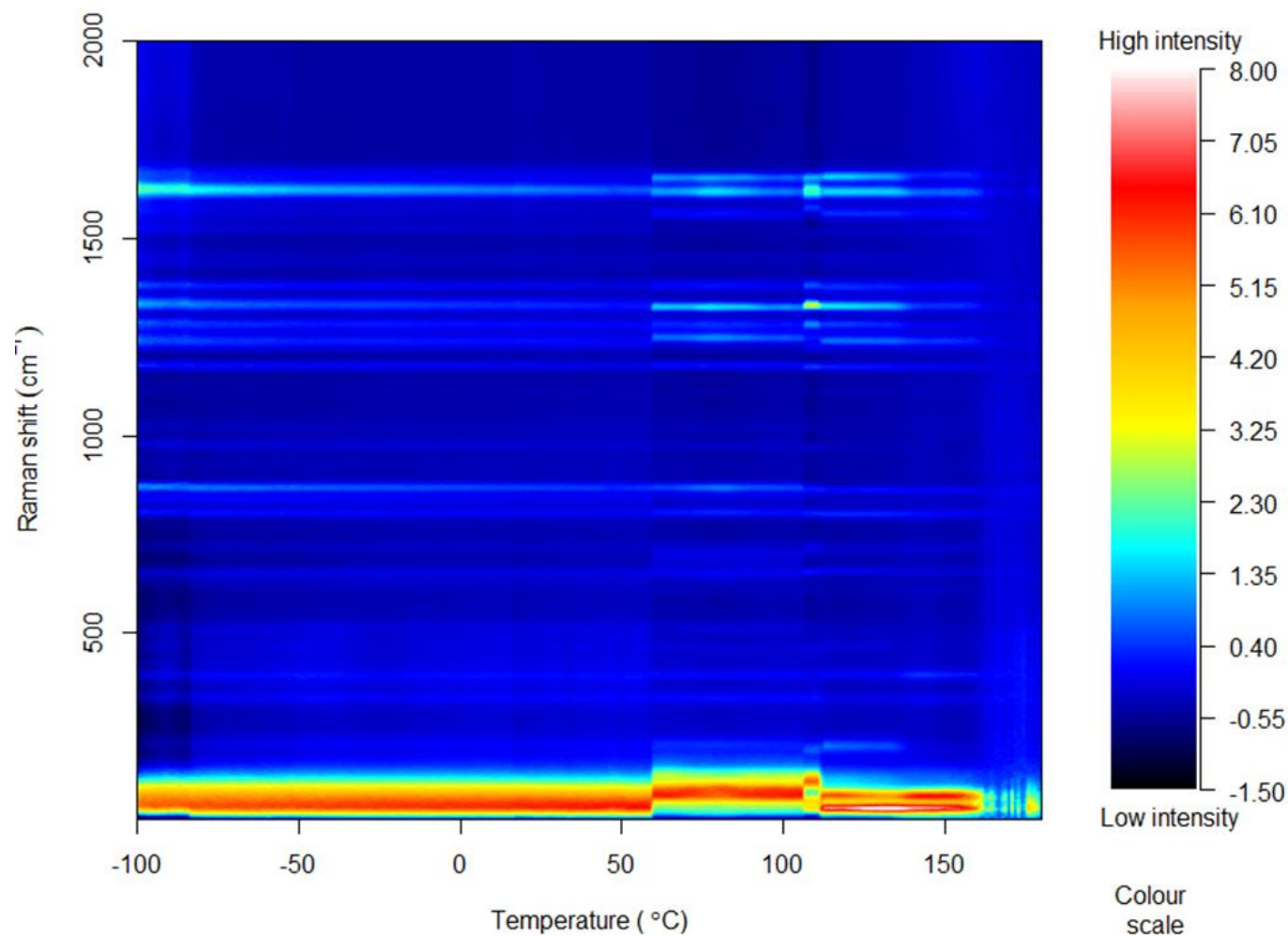


Figure 3.3. Paracetamol experiment 1 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to 180°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

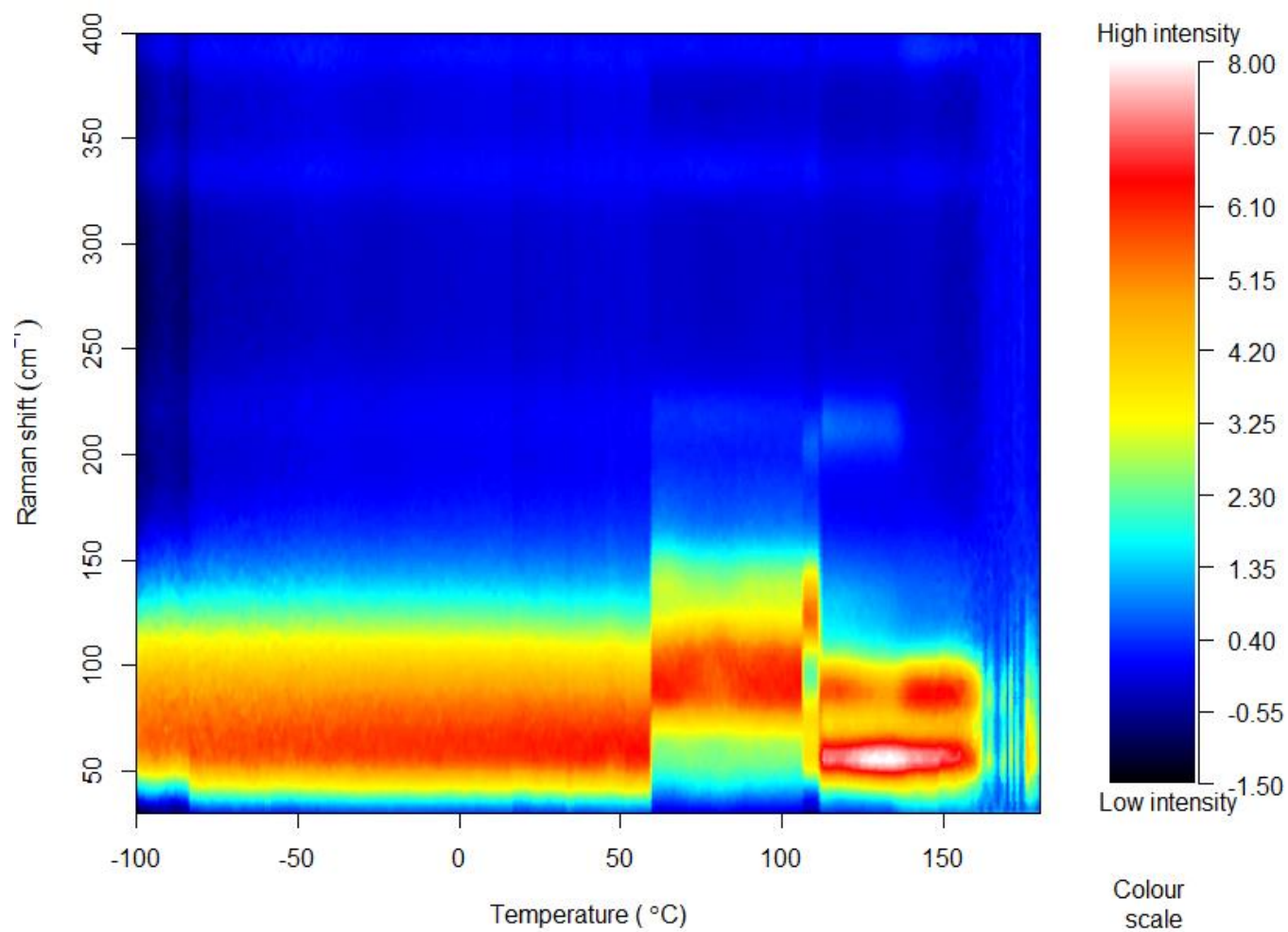


Figure 3.4. Paracetamol experiment 1 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 180°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

In Figure 3.3, the peak intensity is high at low wavenumber peaks at around 100 cm^{-1} which are red and yellow at the bottom of the screen. In this region there is also a noticeable change to the background at around -80°C , which is not too far from the reported beta relaxation temperature of paracetamol, -79.15°C . For clarity this region is presented zoomed-in in the next figure. No obvious change is observed at temperature around the reported alpha transition temperature of paracetamol, 23.85°C . At around 60°C there is a clear abrupt change to the position and intensity of several peaks. This is apparent in low wavenumber where there is discontinuity at around 200 cm^{-1} which shifts to a slightly higher number. At 110°C the peak shifts up and splits. At 120°C it shifts and splits down again.

Figure 3.4 is the zoomed in version of the previous contour map. At around -80°C , near beta transition temperature of paracetamol (-79.15°C), there is a vertical band that can be observed. Other than what was observed at -80°C , there is no obvious visual change to the spectra from -100°C up to approximately 60°C . At the left side of low wavenumber, there is a broad high intensity area mostly in red and yellow. It is essentially broad up to 60°C and then it narrows as it moves up in wavenumber. There are two split peaks at around 110°C . Above 110°C , there are high peaks at about 60 to 90 cm^{-1} but the intensity of these peaks drops very rapidly after 150°C and the peaks seem to disappear. Two more repeat experiments were carried out to test the reproducibility of the data. Figure 3.5, Figure 3.6, Figure 3.7 and Figure 3.8 are the key figures.

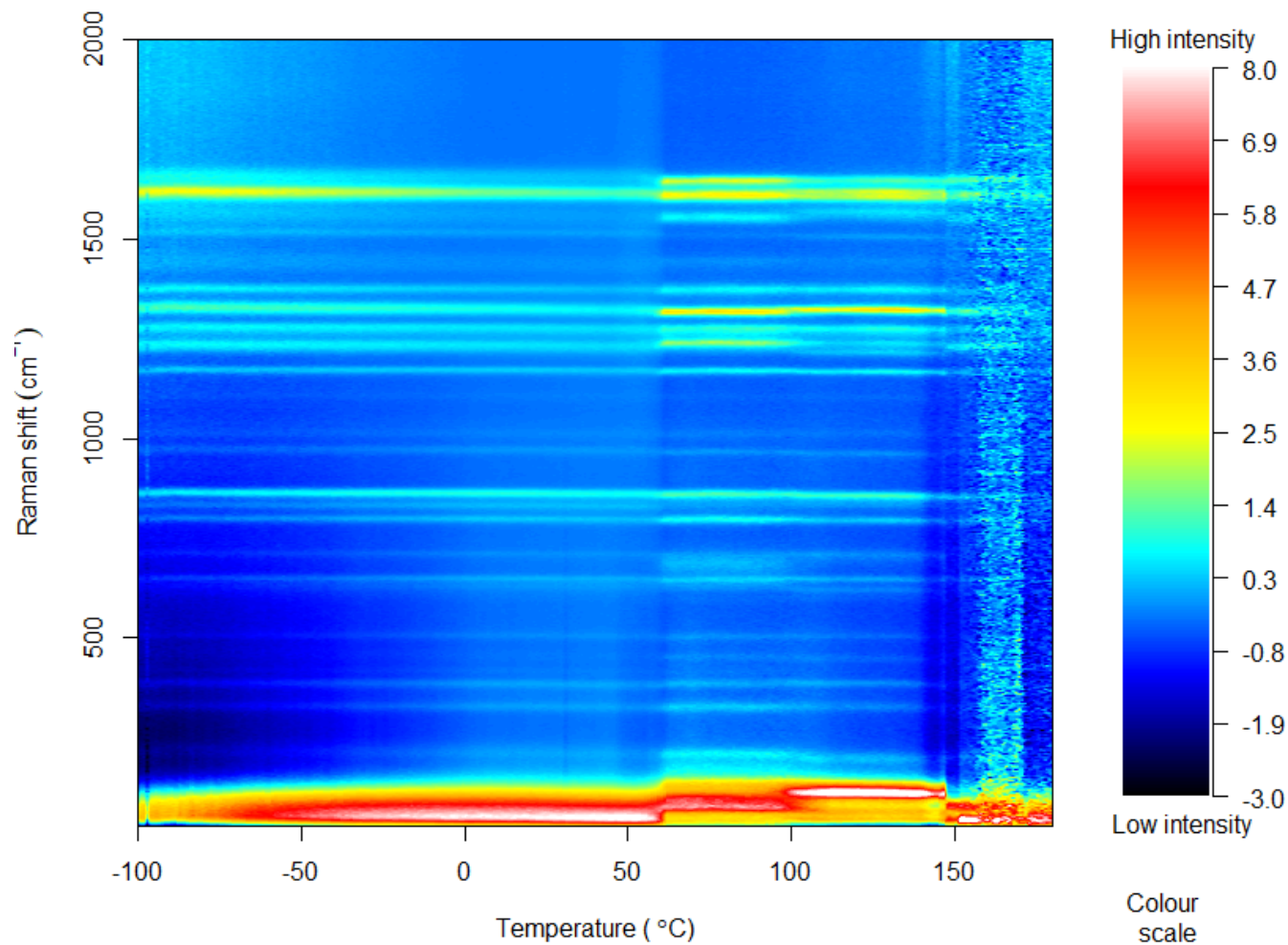


Figure 3.5. Paracetamol experiment 2 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to 180°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

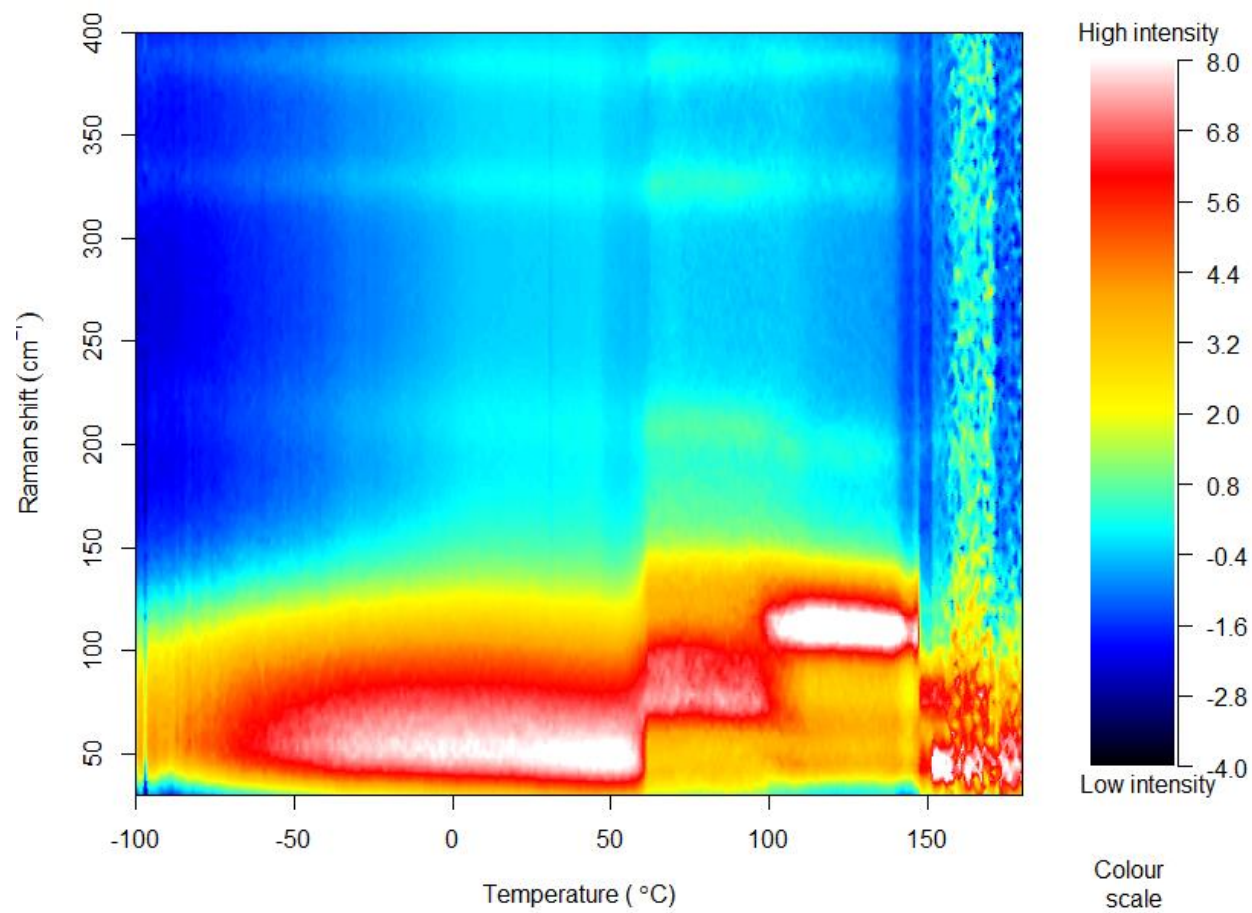


Figure 3.6. Paracetamol experiment 2 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 180°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

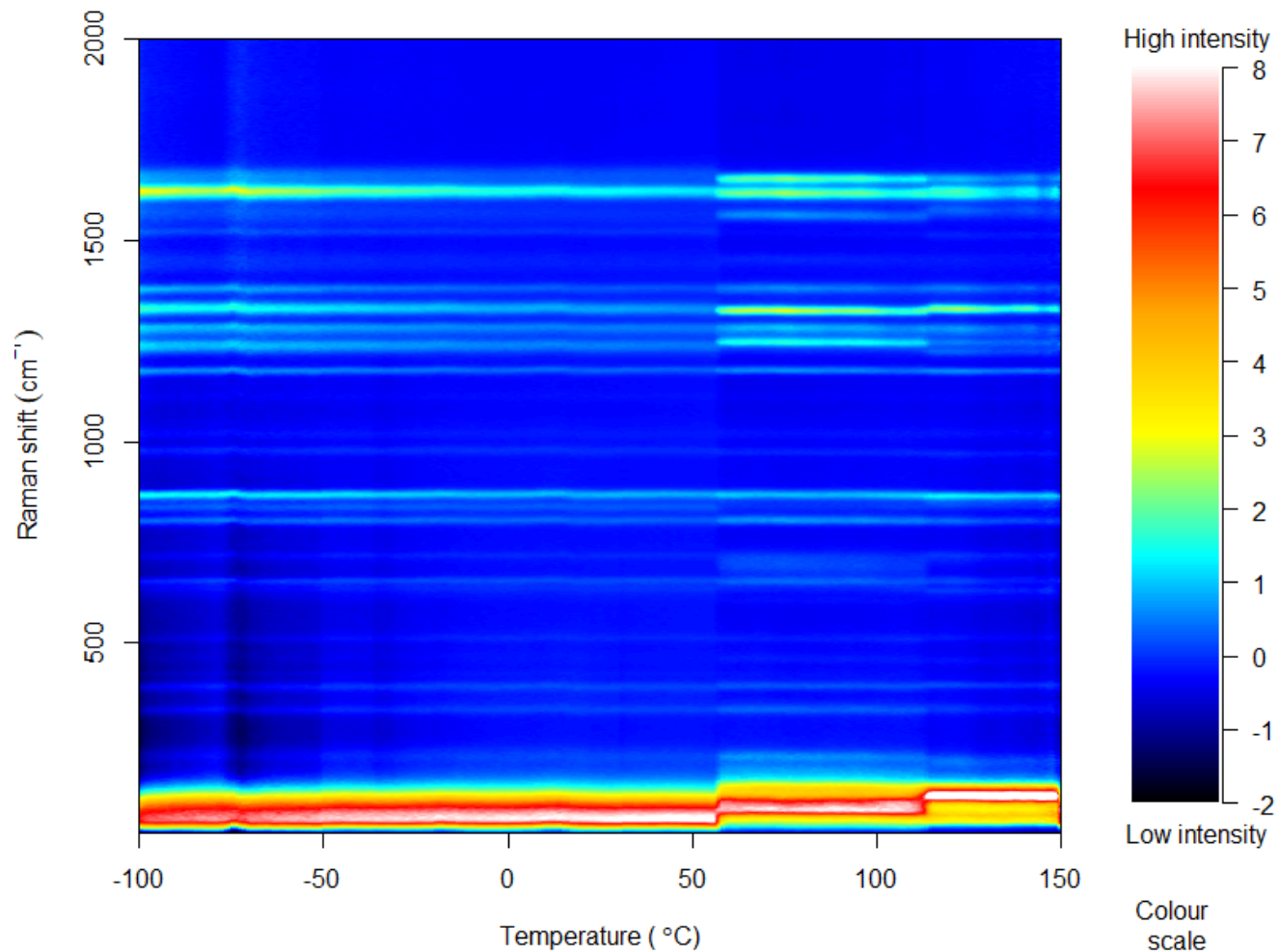


Figure 3.7. Paracetamol experiment 3 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to 180°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

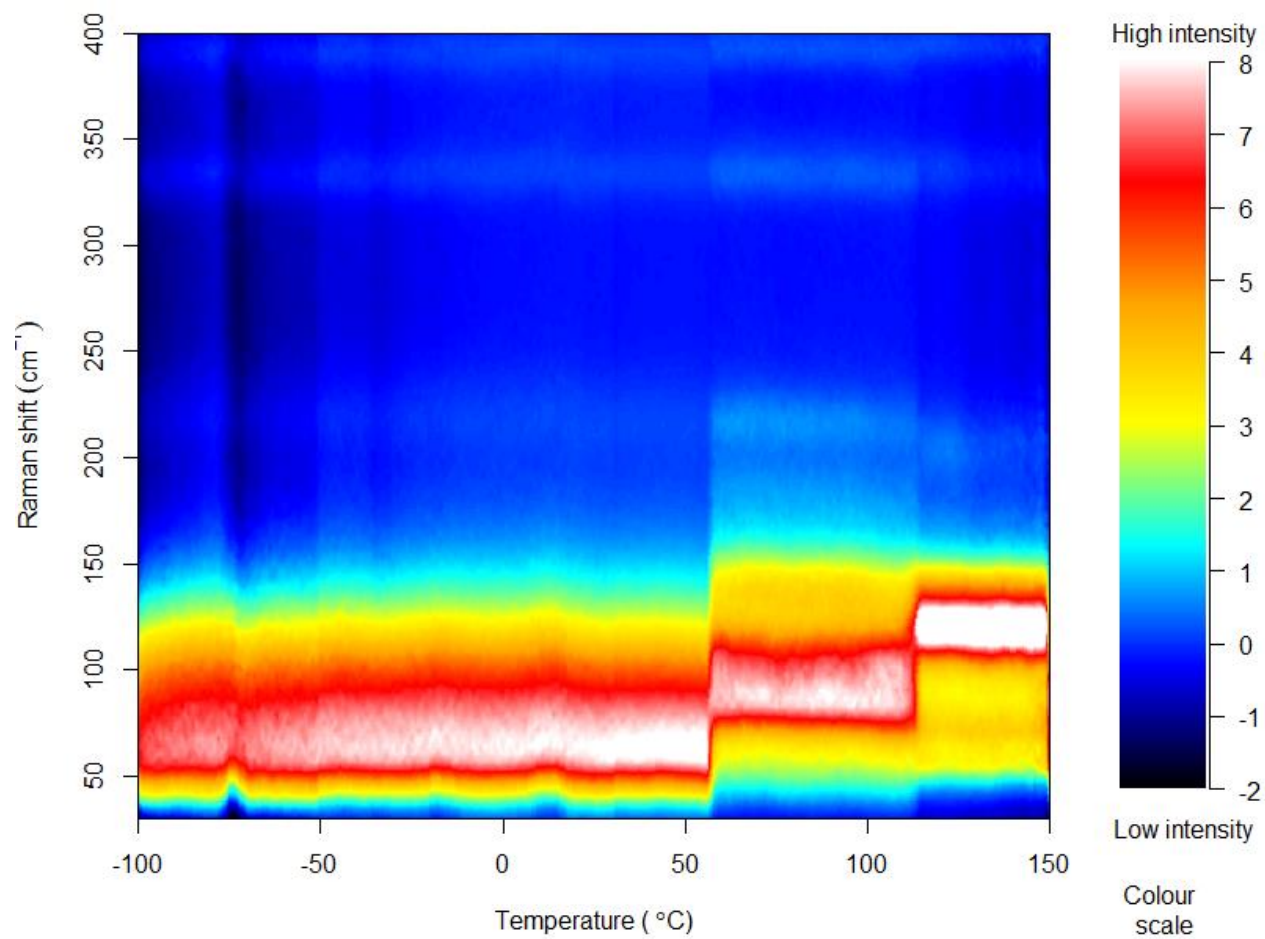


Figure 3.8. Paracetamol experiment 3 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 180°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

Experiment 1, 2 and 3 are all repeats. In all repeat experiments, there are regions where there are not many changes, and then peaks step up so each plot is essentially divided into several regions – amorphous form, form III, form II, form I and the melt. In the second experiment (Figure 3.5), there are amorphous form, form III, form II, and probably form I, which is hardly visible because it does not have much intensity. In the third experiment (Figure 3.7), there are amorphous form, form III, form II, and presumably form I again, and then the melt. The repeat experiments were reasonably reproducible in that we observe amorphous form, form III, form II, form I, and then the melt. For all of the experiments, the quench cooled paracetamol goes from liquid form to crystalline form at around 55 to 60°C. That seems to be consistent across all three experiments. These correspond the temperature at which three phase transformations were observed during heating: crystallization of amorphous material to form III in the temperature range 50 to 56°C, the transformation of form III to II at 112 to 118°C, another transformation from II to I at 148°C and the melting of form I (Nanubolu & Burley, 2012).

From inspection of the contour maps, visual changes were observed in of Raman spectra in experiment 1 (Figure 3.4) and experiment 3 (Figure 3.8) at around the beta transition temperature (-79.15°C), but not around the alpha transition temperature (23.85°C). We found that on repeat experiments, there was no obvious change to peak positions in experiment 3 around the beta and alpha transition temperatures. The visual changes occurring at around the beta transition temperature in the contour maps are not reproducible. Hence, we cannot fully conclude that we see changes to Raman intensity around the beta transition temperature.

3.4.5 Illustration of Raman spectra

3.4.5.1 Form III to II transition of paracetamol

Figure 3.9 shows the 20 to 2000 cm^{-1} wavenumber region of spectra from paracetamol to highlight differences in Raman features caused by change from crystal to crystal (solid) forms. They are spectra at different

temperatures, 106 and 107°C. We selected these two spectra that are most evident changes as a function of temperature among the collected spectra, therefore chosen reference spectra to illustrate the change to peak shape. The spectra at chosen temperatures were vertically superimposed and listed by increasing temperature from the bottom. From 106 to 107°C there is an obvious change to the shape of the spectra and the most striking difference between the spectra can be observed in the region around 1200 to 1700 cm^{-1} . Spectra of paracetamol at around 106°C exhibit lower signal to background ratio, compared to the spectra at around 107°C. At higher temperature of 107°C, the signal to background ratios increases, and the bands grow sharper and taller. The tall peak at around 100 cm^{-1} divides into two peaks after an increase of 1°C.

Comparison with literature (Nanubolu & Burley, 2012), what is being observed here is transformation from crystalline paracetamol form III into crystalline paracetamol form II. While this has been observed before and it is not the subject of this thesis, it is illustrative of the changes that might be expected in a Raman spectrum if we have change between solid forms. Hence, more obvious changes to molecular structure are expected when paracetamol goes from solid to liquid form. We conclude from Figure 3.9 that significant changes in Raman spectra are expected where we have a big change to molecular structure and packing as it is known to occur at temperature in form III to form II transition. Hence, we expect potential spectral changes to occur when the amorphous form of paracetamol goes through beta relaxation or turns into liquid form at around the alpha transition temperature.

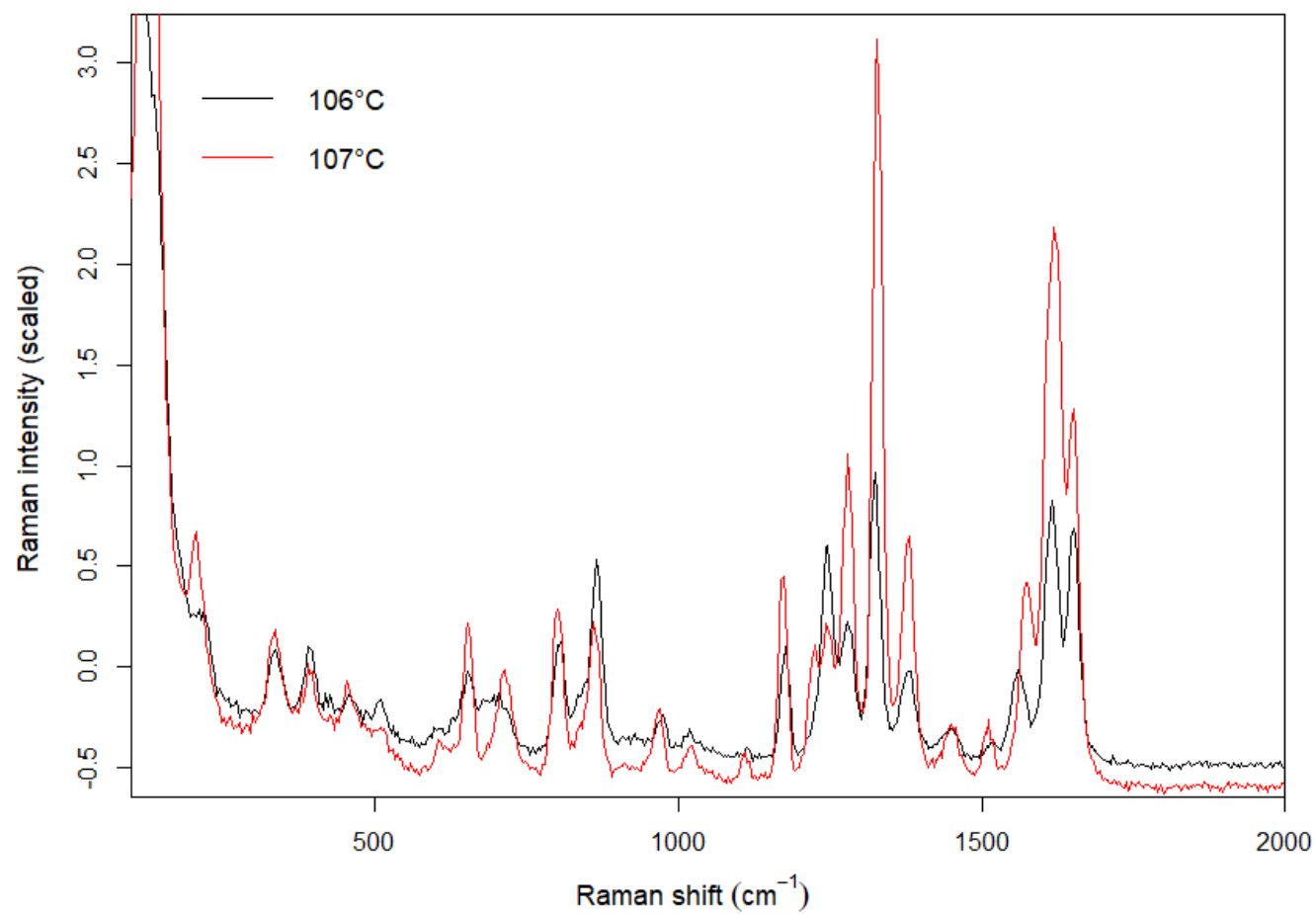


Figure 3.9. The Raman spectra from paracetamol at 106°C (black line) and 107°C (red line)

3.4.5.2 Alpha and beta transition of paracetamol

Having considered crystal to crystal transition, we are now going to look more in depth at the topics of direct interest in this thesis which would be the area where we might expect to see an amorphous to liquid (T_α) or amorphous to amorphous transition (T_β). In Figure 3.10, we show the spectra 5°C below and above the reported alpha transition temperature of paracetamol and the same for beta transition temperature. It is immediately apparent from visual inspection of these figures that any differences, either side of these transitions are very subtle compared to the crystal-to-crystal data shown in Figure 3.9. Given this, we undertook more detailed study in the area we don't see crystals employing peak fitting and we will be reporting peak intensities following in the study of nylon 6,6 by Raman spectroscopy (Bertoldo Menezes et al, 2016), peak width which is an indication of molecular disorder and peak position which is an indication of molecular and conformational studies. All of these changes will be discussed together with alpha and beta transition in the next section.

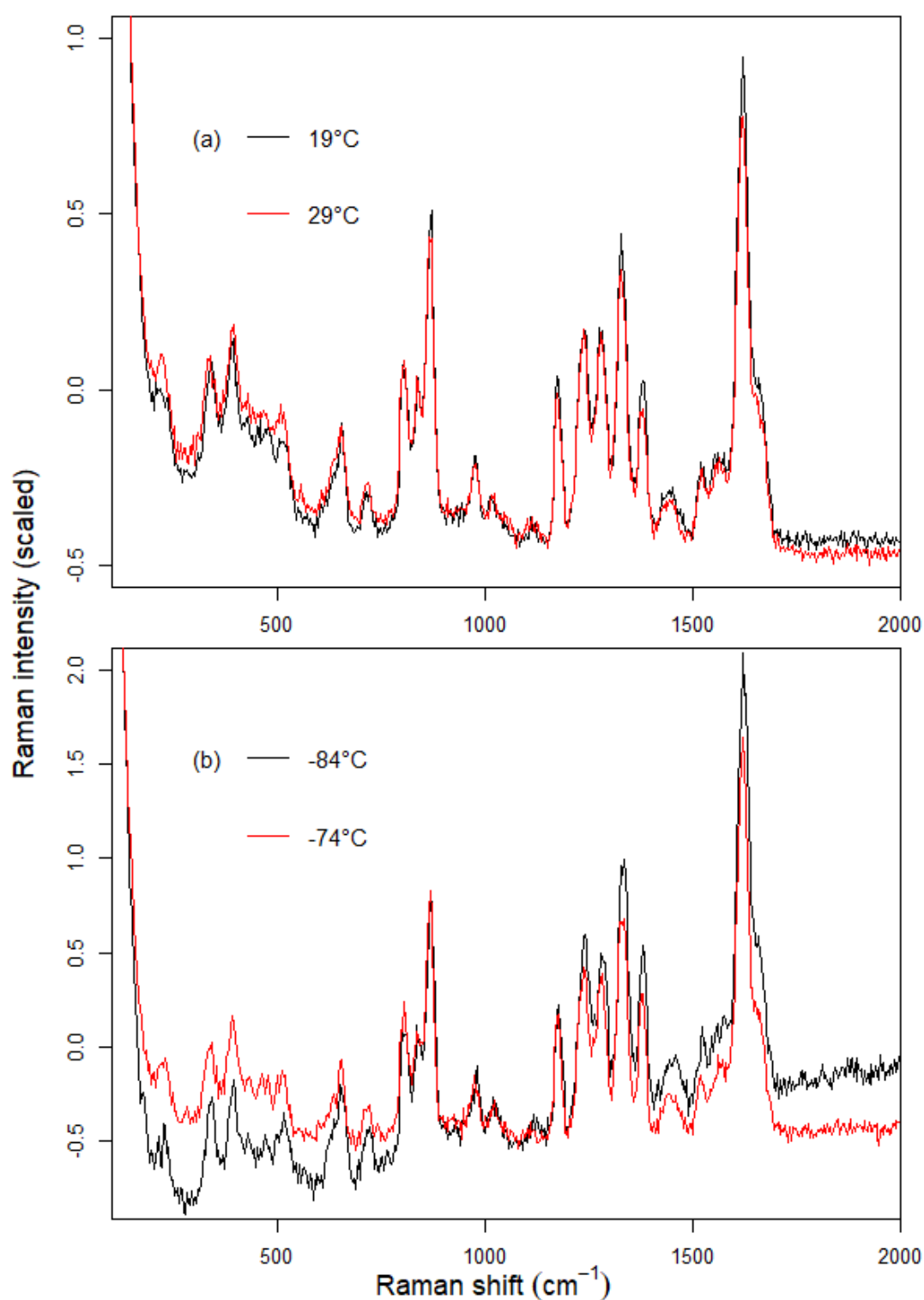


Figure 3.10. The Raman spectra of paracetamol: (a) 5°C below and above alpha transition temperature, 19°C (black line) and 29°C (red line); (b) 5°C below and above beta transition temperature, -84°C (black line) and -74°C (red line)

3.4.6 Gaussian peak fitting

Visual inspection of the Raman bands possibly indicates phase transformation between different forms of paracetamol at various temperatures, but in order to better characterise differences in Raman features caused by temperature changes, quantitative peak shape analysis has been taken for Raman peak characterisation of 10 selected intramolecular vibrational modes (Table 3.1). They correspond to the vibrational modes of paracetamol reported in previous study (Nanubolu & Burley, 2012). Gaussian peak fit model was used to fit peaks of selected vibrational modes as discussed in the previous 2.2.5. data analysis section. We intend to diagnose these Raman peaks with three specific parameters – peak intensity, peak width and peak position - that were mentioned in Section 2.2.4.

Table 3.1. Wavenumbers (cm^{-1}) and suggested assignments derived from Raman spectra of paracetamol polymorphs

Wavenumbers (cm^{-1})	Assignment
388	Whole molecule
862	Symmetric phenyl breathing
970	Deformation of phenyl ring + stretching of C–N amide bond
1168	Bending of phenyl ring and C–OH groups
1230	Deformation of phenyl ring + amide + C–OH groups
1324	Deformation of phenyl ring + C–OH group
1513	Bending of phenyl ring + stretching of CNH group
1560	Bending of amide N–H group
1616	C=C stretching
1660	C=O stretching

3.4.6.1 Analysis of Gaussian fit curves of peak intensity

We will proceed to review Raman data of paracetamol sample captured across all temperatures. Figure 3.11 show Gaussian fit curves of peak intensities plotted as a function of temperature at around 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate, but an exact number is used to allow easy linking with Table 3.1. The spectral peaks were fitted to see different Raman features caused by alpha (23.85°C), beta (-79.15°C) and other phase transition temperatures in the 10 selected intramolecular vibrational modes of paracetamol. Unscaled raw peak intensities were plotted in accordance with the study by Bertoldo Menezes et al who have shown that with Raman spectroscopy, the α -, β - and γ -relaxation temperatures of polymer Nylon 6,6 (N66) were found (Bertoldo Menezes et al, 2016). Each dot on the scatterplot represents peak intensity of selected peaks in the range of -100 to 150°C . The black, blue and purple dots represent experiment 1, 2 and 3, respectively. These plots could help investigate the relationship between peak intensity and phase transformation caused by temperature change at around alpha, beta and other phase transition temperatures.

In experiments 1, 2 and 3 in Figure 3.11, the peak intensities tend to increase as the temperature increases from the origin to around -30°C . The major changes in peak intensity or discontinuity are observed at around 60 and 110°C , which correspond to the temperatures at which there are clear abrupt change to the position and intensity of several peaks in Figure 3.3.

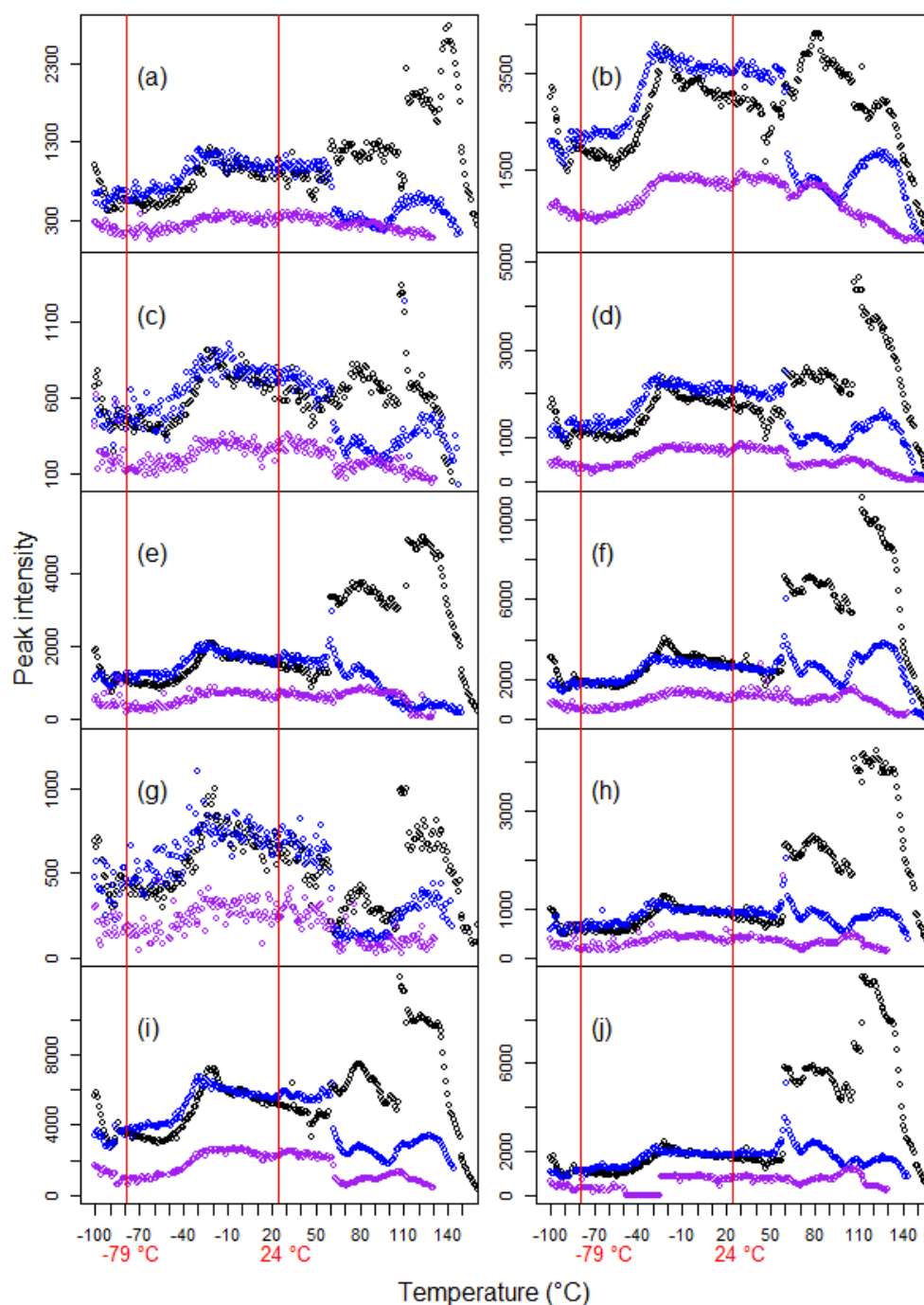


Figure 3.11. The peak fitting of intensity against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

In experiment 1 (Figure 3.11, black dots), there appears to be an obvious discontinuity in many of the peak intensities around the beta (-79.15°C) transition temperature, but not around the alpha (23.85°C) transition temperatures. In experiment 2 (Figure 3.11, blue dots), no obvious change to peak intensity is observed at around alpha or beta transition temperatures. On the other hand, in experiment 3 (Figure 3.11, purple dots), there are changes in peak intensities around the beta and the alpha transition temperature. The peak intensity drops at around the beta transition temperature and shifts to higher peak intensity around the alpha transition temperature. The change in alpha transition temperature is most obvious in peaks at around 865 cm^{-1} , 975 cm^{-1} and 1175 cm^{-1} which correspond to symmetric phenyl breathings, Deformation of phenyl ring and stretching of C–N amide bond, and bending of phenyl ring and C–OH groups, respectively (Figure 3.11 (b), Figure 3.11 (c), and Figure 3.11 (d)).

In experiment 3, there seemed to be an obvious change to peak intensity at around the reported beta transition temperature (-79.15°C). In order to check that the result from the peak fitting is reasonable, we plotted spectra either side of both the beta (-79.15°C) and alpha (23.85°C) transition temperatures, in Figure 3.12 and Figure 3.13, respectively. The spectra at 5°C below and above the temperature of interest were plotted as a function of temperature. The subplots from the experiment 1, 2 and 3 were enumerated with (a), (b), and (c), respectively.

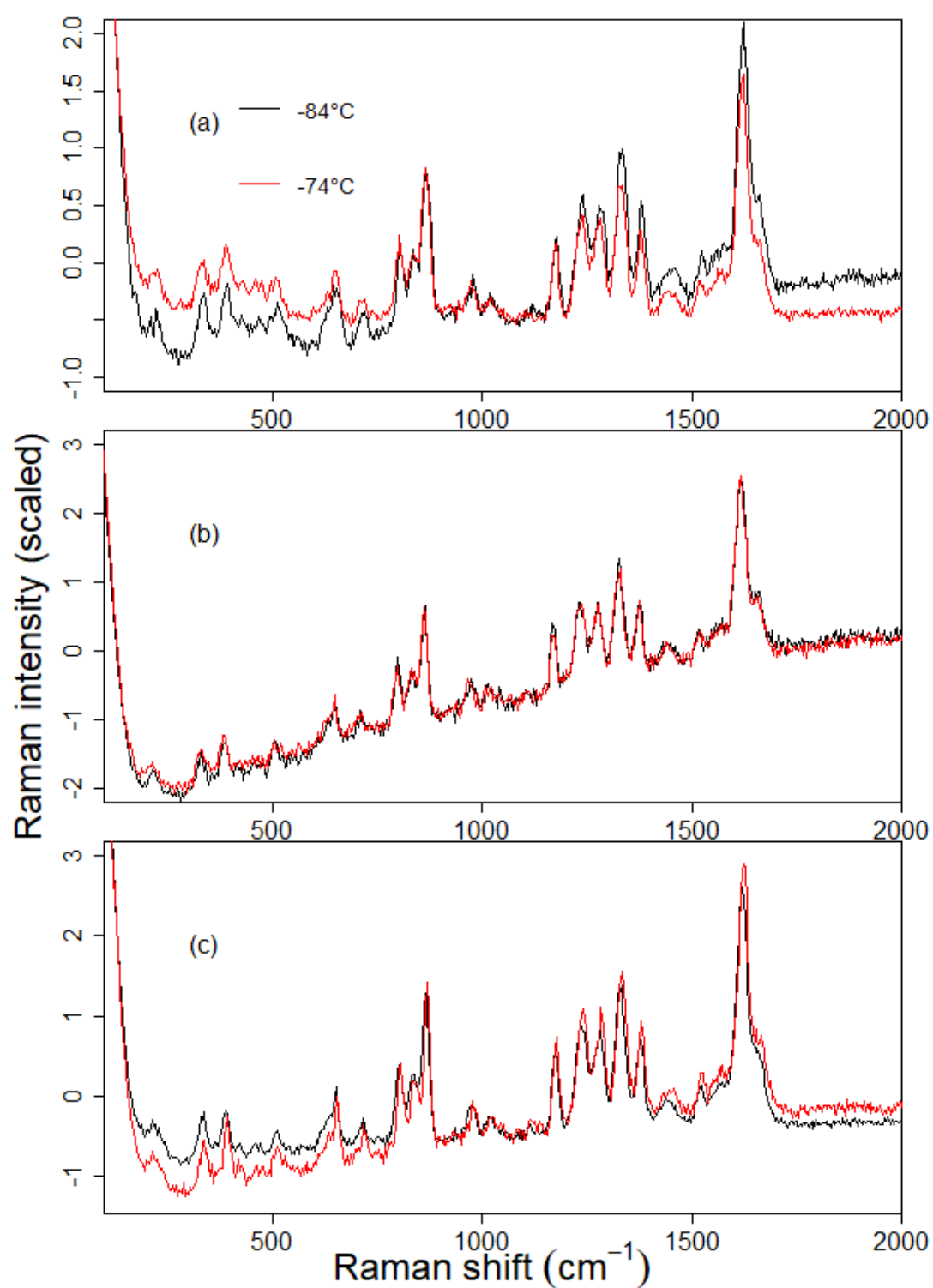


Figure 3.12. The Raman spectra of paracetamol 5°C below and above beta transition temperature (-79.15°C): -84°C (black line) and -74°C (red line), (a) experiment 1 (b) experiment 2 (c) experiment 3

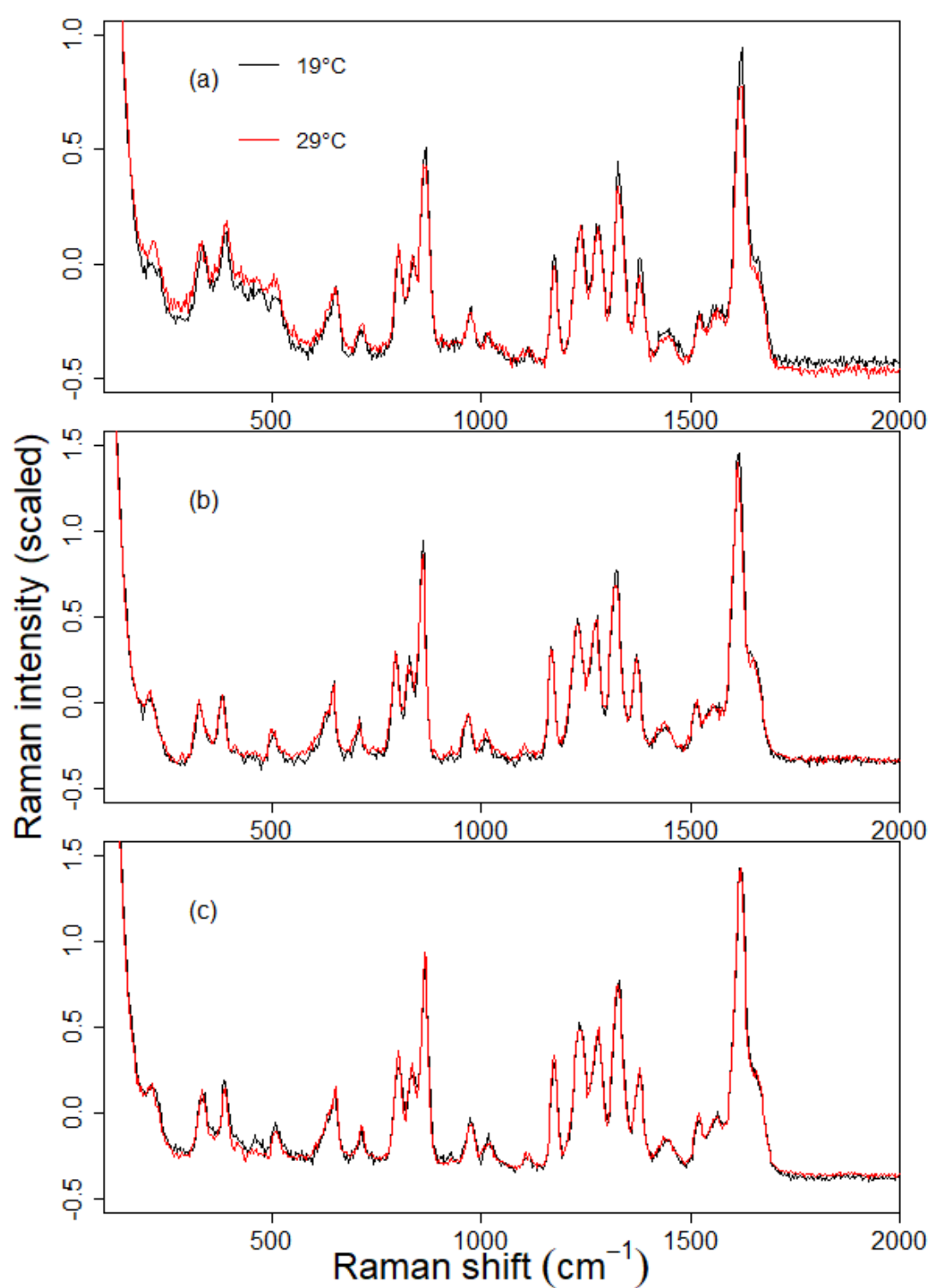


Figure 3.13. The Raman spectra of paracetamol 5°C below and above alpha transition temperature (23.85°C): 19°C (black line) and 29°C (red line), (a) experiment 1 (b) experiment 2 (c) experiment 3

Experiment 1 (Figure 3.12 (a)) and experiment 3 (Figure 3.12 (c)) show obvious changes to peak intensity below and above beta transition temperature (-79.15°C). Experiment 2 (Figure 3.12 (b)) hardly shows any obvious changes to peak intensity below and above beta transition temperature. On the other hand, no significant change to intensity 5°C below and above alpha transition temperature (23.85°C) is observed in the experiments 1,2 and 3 (Figure 3.13). Hence, for paracetamol based upon these studies, we cannot fully conclude that temperature could lead to detectable changes in Raman scattering of functional groups that are associated with alpha or beta relaxation as reported in the study of nylon 6,6 by Raman spectroscopy (Bertoldo Menezes et al, 2016).

3.4.6.2 Analysis of Gaussian fit curves of peak width

Figure 3.14 are Gaussian fit curves of peak widths of paracetamol plotted as a function of temperature at 10 selected vibrational modes (Table 3.1). We intend to diagnose width of the peak as a function of temperature, which could give useful information on structural disorder and crystallinity. It has been reported that Raman bandwidth and band shapes of some glass samples exhibit changes during glass transition (Ganguly et al, 1984). Amorphous forms of samples tend to have Raman bandwidths that are broader than for crystalline forms and hence bandwidths could represent disorder of the system. These plots could help investigate the relationship between structural disorder and phase transformation caused by temperature change at around alpha (23.85°C), beta (-79.15°C) and other phase transition temperatures.

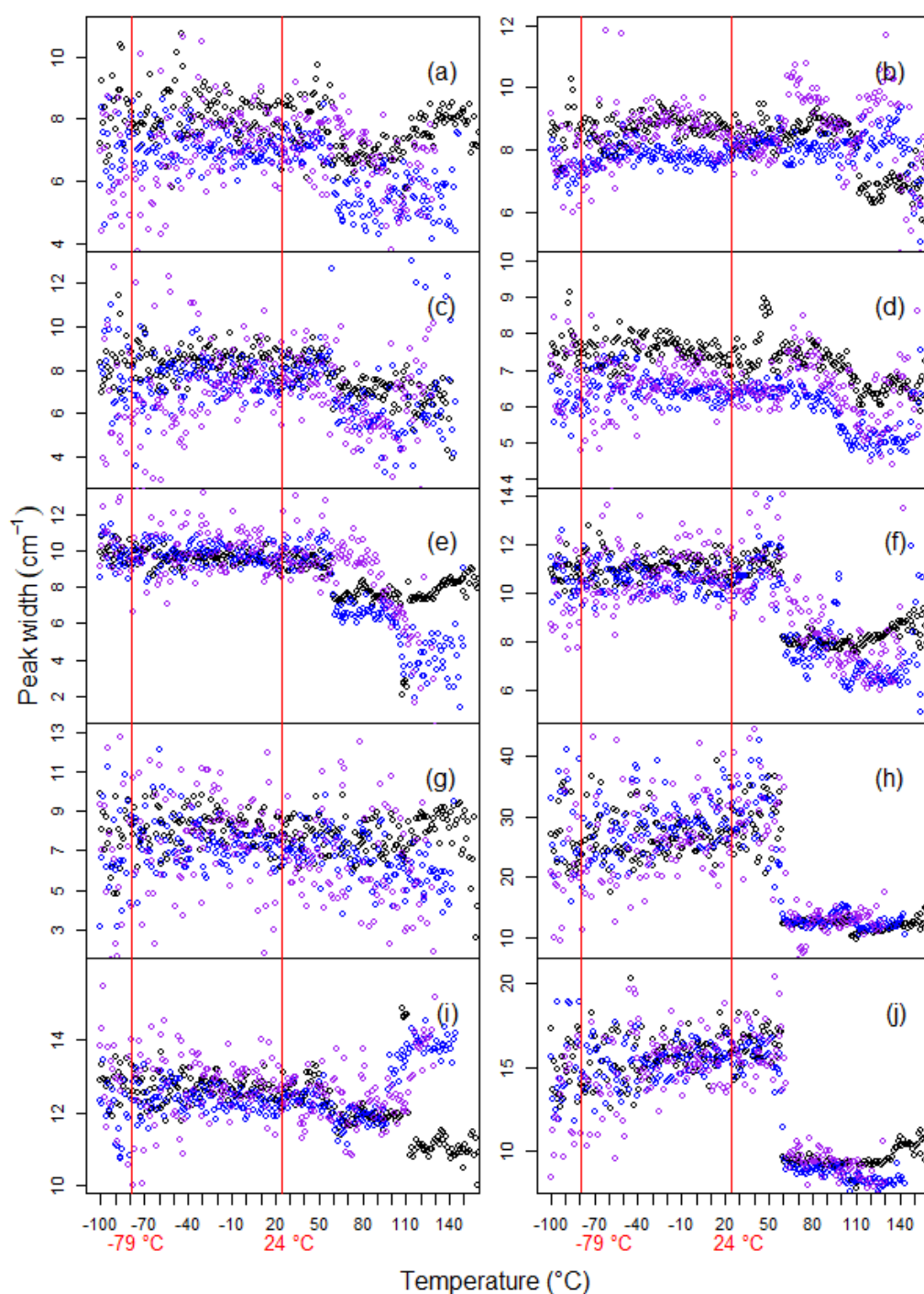


Figure 3.14. The peak fitting of width against temperature for the 10 selected peaks of paracetamol: 390 cm⁻¹, 865 cm⁻¹, 975 cm⁻¹, 1175 cm⁻¹, 1230 cm⁻¹, 1330 cm⁻¹, 1520 cm⁻¹, 1560 cm⁻¹, 1620 cm⁻¹, and 1660 cm⁻¹. The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

In experiments 1, 2 and 3 in Figure 3.14, peak width decreases as the temperature approaches 60°C. The peaks at around 1520 cm⁻¹ present very unevenly distributed broad peaks and fluctuate in the area from the origin to 180°C (Figure 3.14(g)). Peak at 1520 cm⁻¹ correspond to bending of phenyl ring and stretching of CNH group. Most peaks show change to peak width at 106°C. Hence, the peak width shows most obvious changes at around 60 and 106°C, which correspond to the reported phase transformation temperatures during crystallization of amorphous paracetamol to form III at around 70 to 94°C and phase transition from form III to form II at around 95 to 149°C (Nanubolu & Burley, 2012). These changes are most obvious in peaks at around 1330 cm⁻¹, 1560 cm⁻¹ and 1660 cm⁻¹ (Figure 3.14 (f), Figure 3.14 (h) and Figure 3.14 (j)). The peak at around 1330 cm⁻¹ corresponds to deformation of phenyl ring and C-OH group. The peak at around 1560 cm⁻¹ corresponds to bending of amide N-H group and at 1660 cm⁻¹ to C=O stretching. These changes in peak width are reasonable as we expect paracetamol to go from liquid to crystalline at around 60°C, and from other crystal to crystal transition at around 110°C.

We have looked at a selection of peak widths from experiment 1,2, and 3 and no obvious change to peak width is seen in the non-crystalline region. No obvious change is observed at around alpha (23.85°C) or beta (-79.15°C) transition temperatures. This contrasts with major changes in peak width that are observed at around 60 and 106°C, when the paracetamol sample crystallizes or when there is crystal to crystal transitions. Hence, for paracetamol based upon these studies described above, Raman band width as a function of temperature shows no evidence of detectable changes in order/disorder of paracetamol structure that are associated with alpha or beta transition temperatures.

3.4.6.3 Analysis of Gaussian fit curves of peak shift

Figure 3.15 presents Gaussian fit curves of peak positions of paracetamol plotted as a function of temperature at around 10 selected Raman

spectra (Table 3.1). The Gaussian fit curves of peak positions as a function of temperature could help us in identifying change to molecular structure at various temperatures. We intend to investigate potential structural changes caused by temperature change at around alpha, beta and other phase transition temperatures.

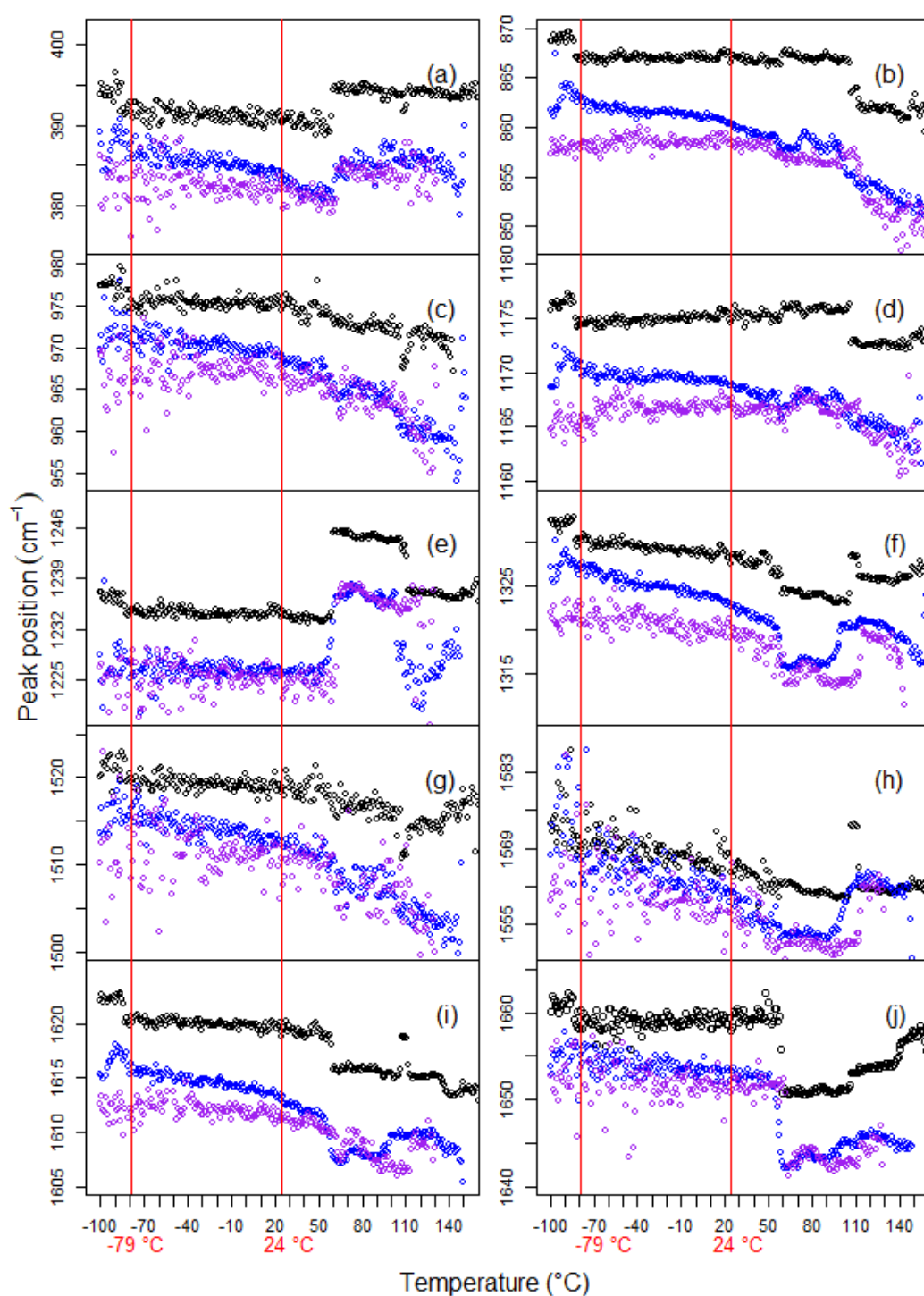


Figure 3.15. The peak fitting of position against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

In experiment 1, 2 and 3 in Figure 3.15, the peak positions generally show similar shift patterns from -100 to -80°C. The peaks shift to lower wavenumber as the temperature approaches -80°C, which is not too far from the reported beta relaxation temperature of paracetamol (-79.15°C). The peak shift is most noticeable in experiment 1 (Figure 3.15, black dots) and experiment 3 (Figure 3.15, purple dots). These changes are not as obvious in peaks at around 1520 and 1560 cm^{-1} (Figure 3.15 (g) and Figure 3.15 (h)). The peak at 1520 cm^{-1} corresponds to Bending of phenyl ring and stretching of CNH group. The peak at 1560 cm^{-1} corresponds to ending of amide N-H group. No obvious change to peak position is observed at alpha transition temperature around 23.85°C. In all three experiments, the most obvious changes in peak position are observed at around 60 and 110°C. The peaks either show discontinuity or shift to different wavenumber at 60°C and 110°C. These findings on peak shifts were promising for the temperature at around the beta transition temperature but not the alpha transition temperature. We conclude from our findings that some structural changes may occur at around the beta transition temperature, but not around the alpha transition temperature.

From inspection of the peak fit plots, we could immediately conclude that for paracetamol based upon the studies described above, only the peak position (and not the peak intensity or peak width) of the Raman spectra exhibits reproducible changes around the beta transition (-79.15°C) temperature, but not the alpha transition temperature (23.85°C). There were detectable changes in peak position when the paracetamol went through phase transition from liquid to crystal, which we see at around 55 to 60°C data and from crystal to crystal as exemplified in the 106 and 107°C. Peak shifts around these temperatures are detectable. Hence, in order to investigate these peak shifts, we are going to do further analysis by plotting Raman spectra either side temperature of interest and investigate the potential peak shift cause by change to temperature.

3.4.6.4 Further analysis of Gaussian fit curves of peak shifts at around the alpha and beta transition temperatures

Peak shifts of Raman spectra of 10 selected vibrational modes of paracetamol will be compared at two temperature points around the beta relaxation temperature (-79.15°C), alpha transition temperature (23.85°C), temperature at which amorphous material crystallizes to form III (60°C), and form III transforms to form II (106°C).

Two temperature points, lower and higher than the temperature of interest, were selected to create two peak plots that represent peak shift below and above the temperature of interest to identify direction (given the Raman shift is on the X axis, peak shift to the right would indicate decrease in wavenumber, whereas shift to the left indicates increase in wavenumber) to which peak shifts in respect to temperature increment and to test reproducibility of these changes. Two temperature points, -95 and -65°C were selected to plot peaks that represent peaks before and after temperature increase around the beta transition temperature of -79.15°C . Similarly, two temperature points 17 and 35°C were selected to show peak shift before and after alpha transition temperature of 23.85°C ; 59 and 61°C for 60°C (temperature at which amorphous material crystallizes to form III); 106 and 107°C for 106°C (form III to II transition temperature).

The data from experiment 1 and 3 seems to be more promising in terms of reproducibility and are shown from Figure 3.16 to Figure 3.23. The plots from experiment 2 are found in the Appendix B (Figure B 1 to Figure B 4 of Appendix B).

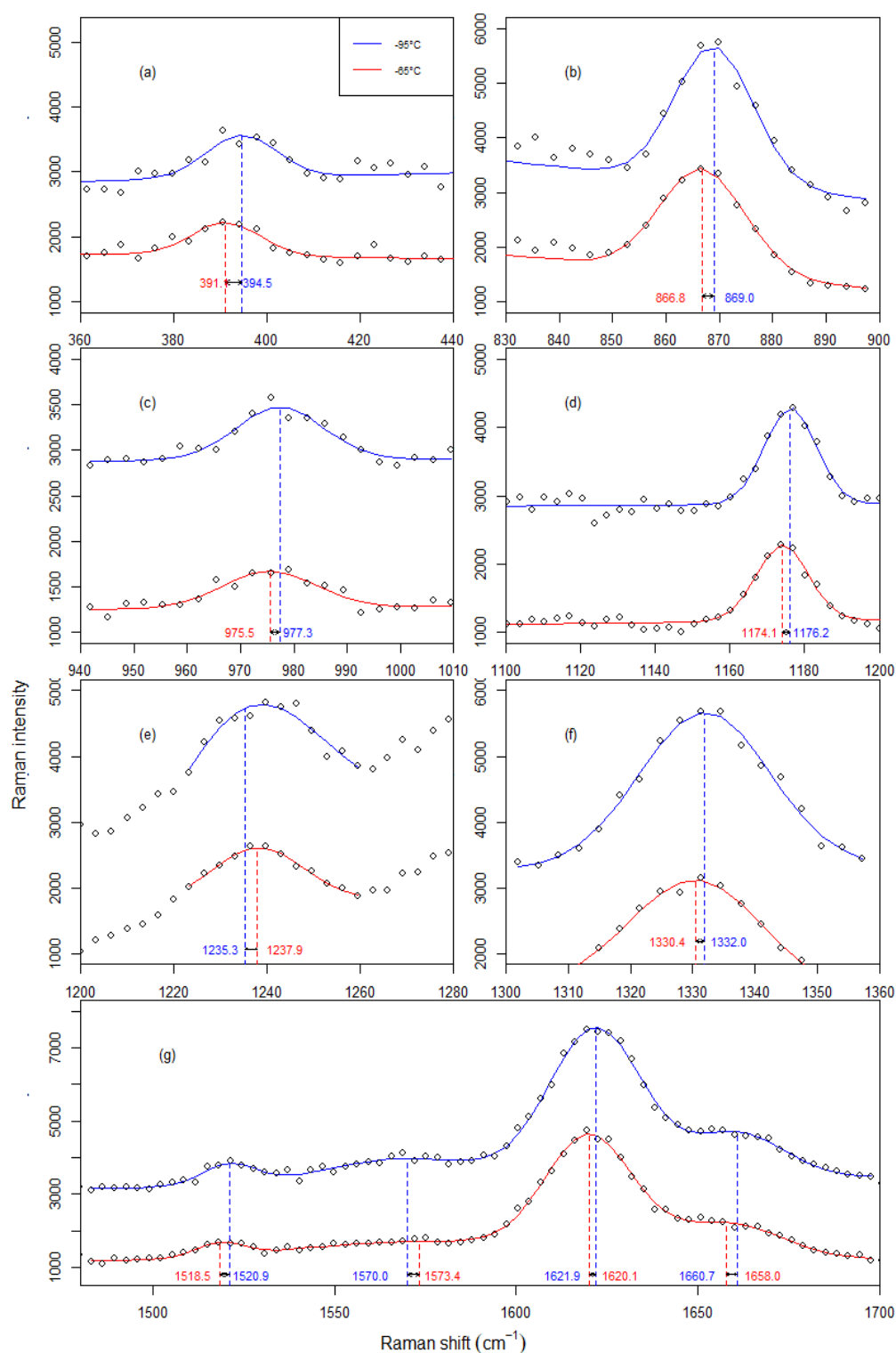


Figure 3.16. Experiment 1 Raman peaks at -95 and -65°C to illustrate peak shift around beta transition temperature (-79.15°C): (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

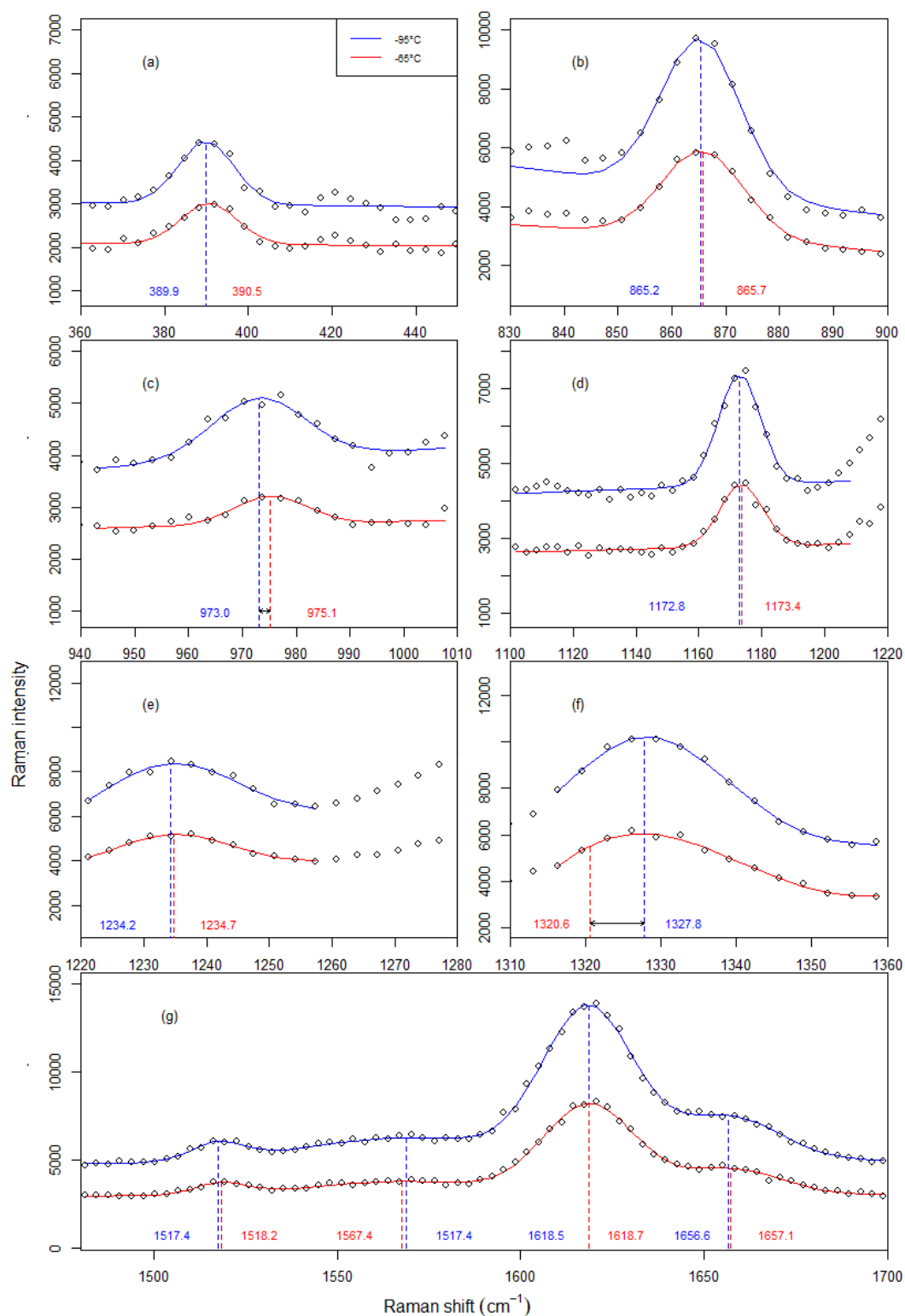


Figure 3.17. Experiment 3 Raman peaks at -95 and -65°C to illustrate peak shift around beta transition temperature (-79.15°C): (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate

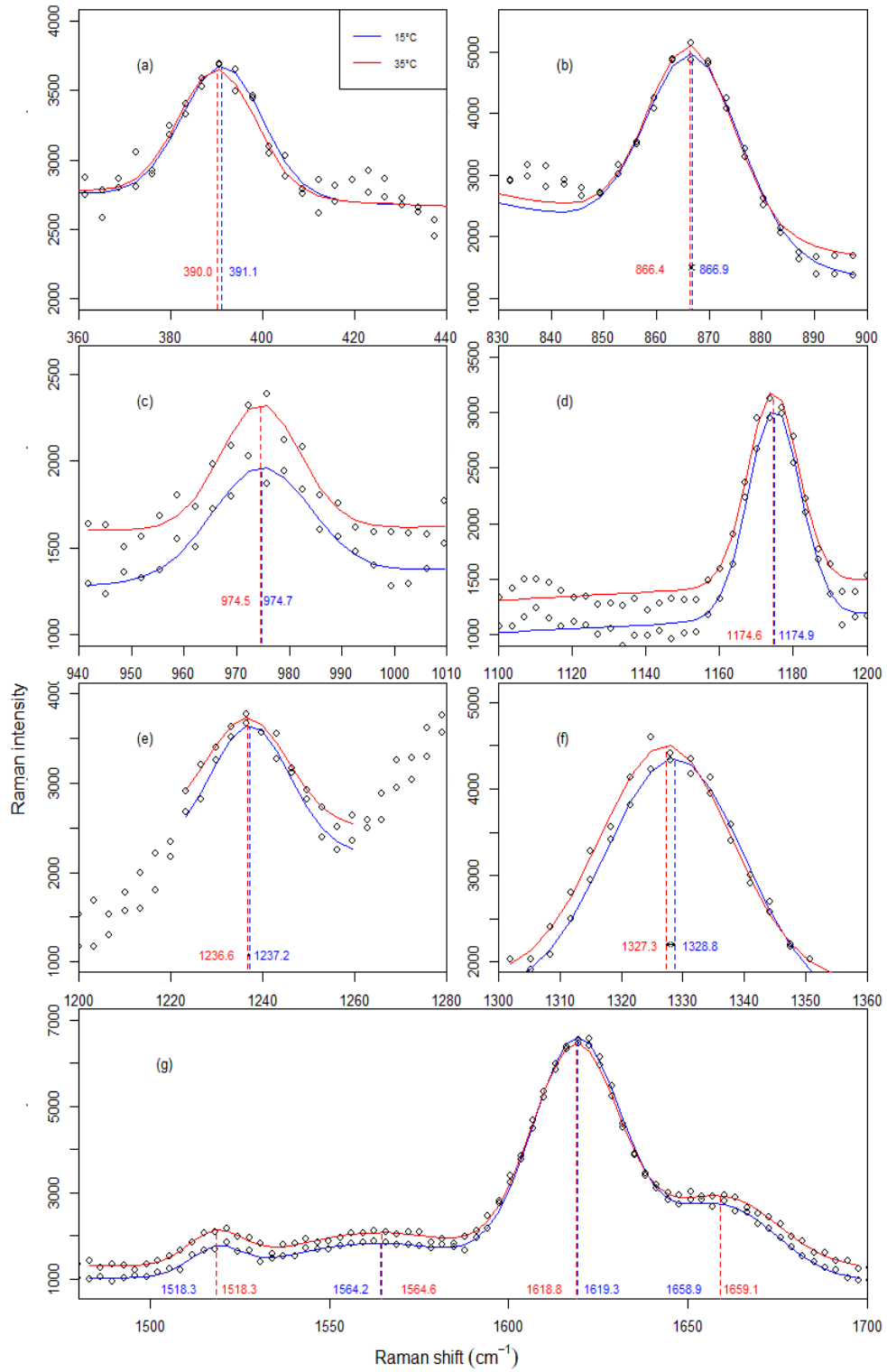


Figure 3.18. Experiment 1 Raman peaks at 15 and 35°C to illustrate peak shift around alpha transition temperature (23.85°C): (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

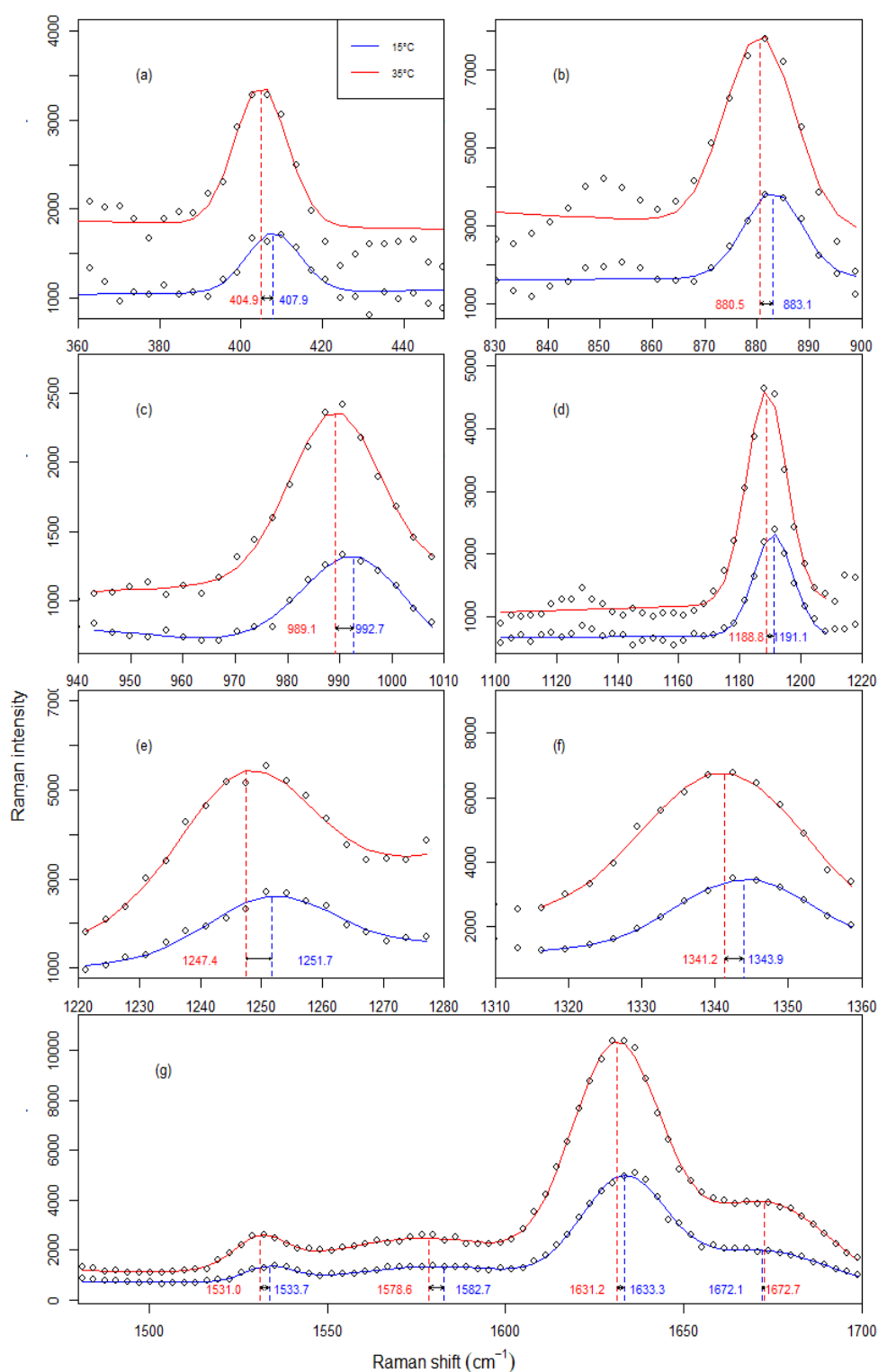


Figure 3.19. Experiment 3 Raman peaks at 15 and 35°C to illustrate peak shift around alpha transition temperature (23.85°C): (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

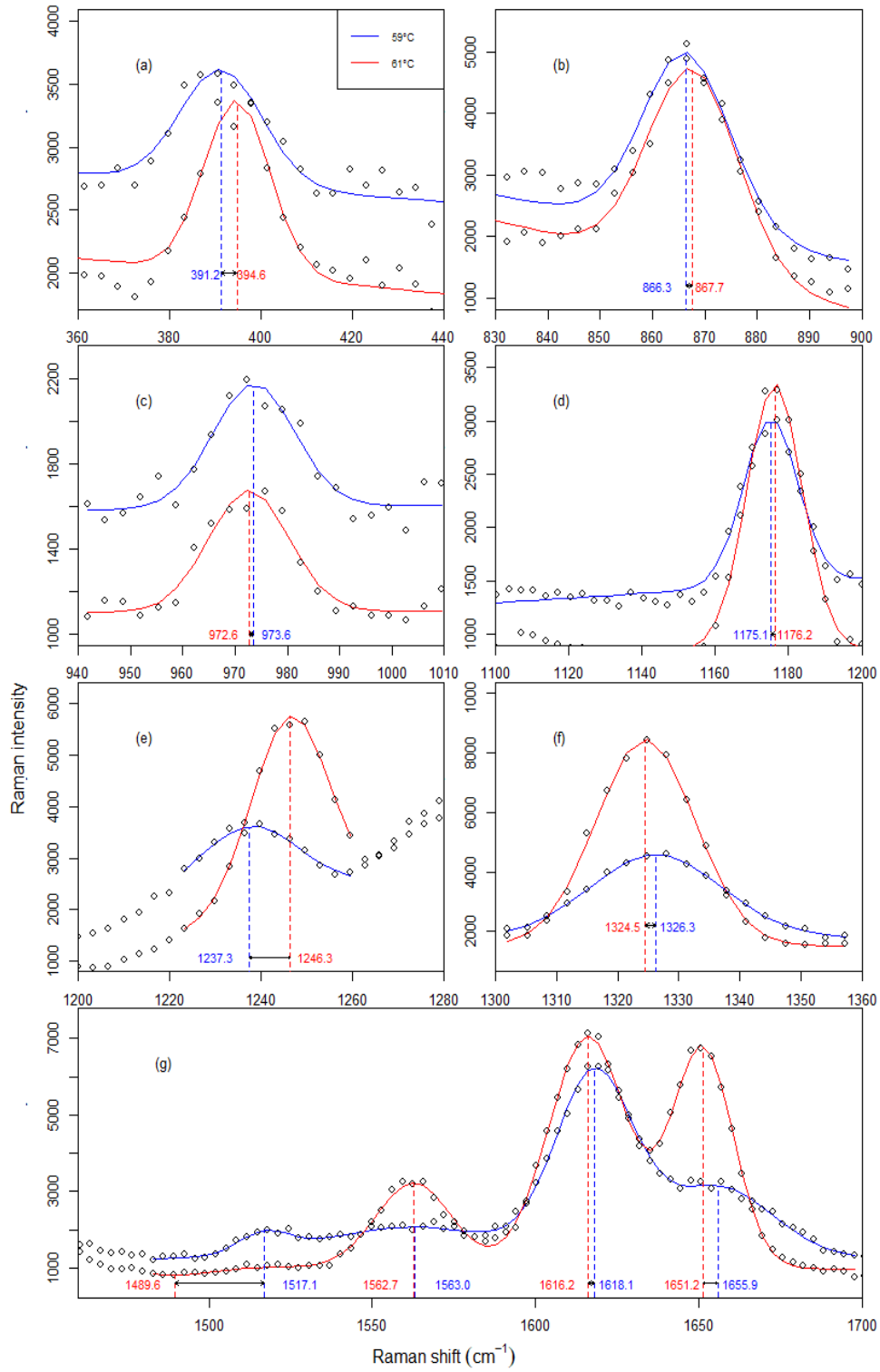


Figure 3.20. Experiment 1 Raman peaks at 59 and 61°C to illustrate peak shift around form III phase transition temperature: (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

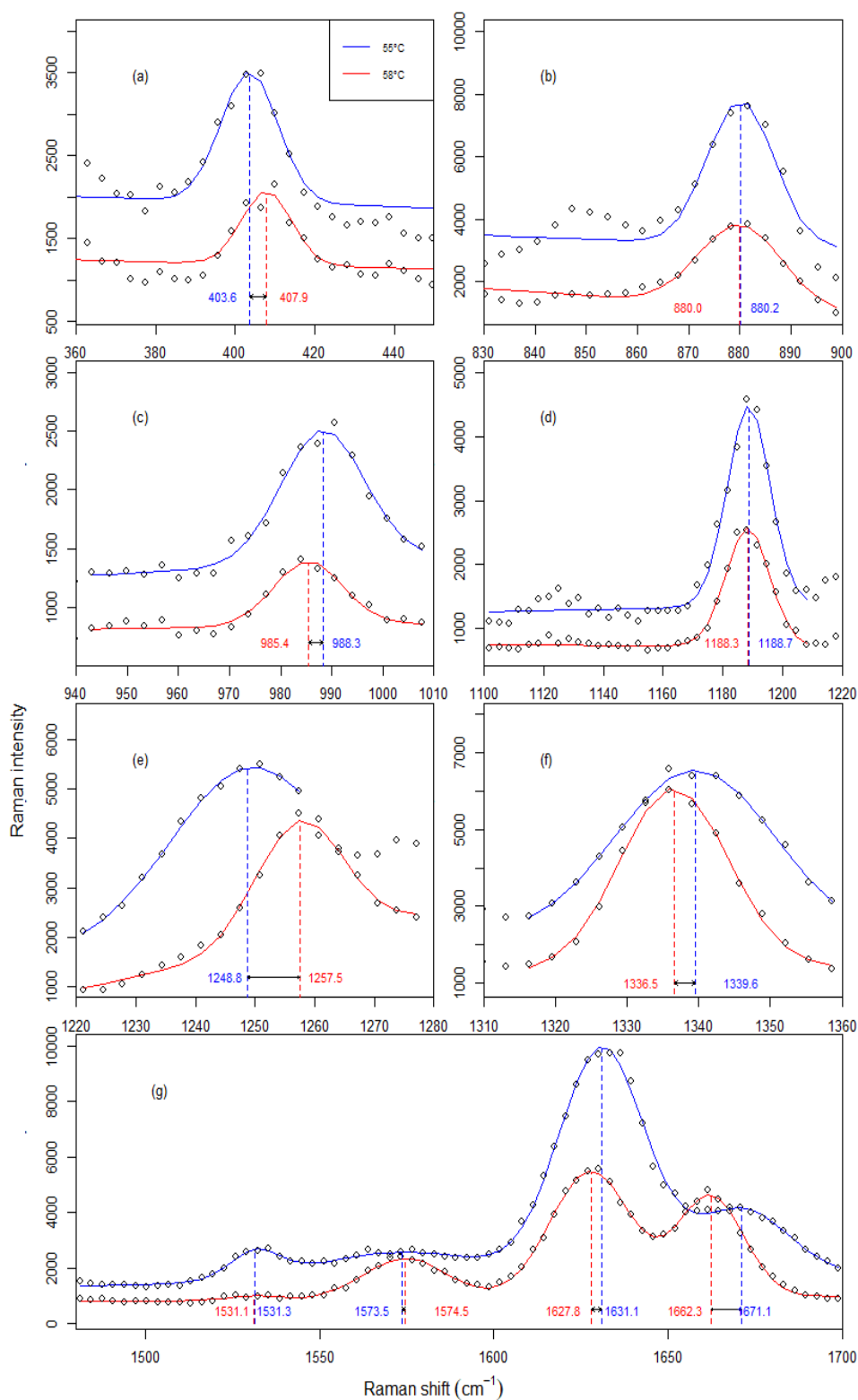


Figure 3.21. Experiment 3 Raman peaks at 55 and 58°C to illustrate peak shift around form III phase transition temperature: (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

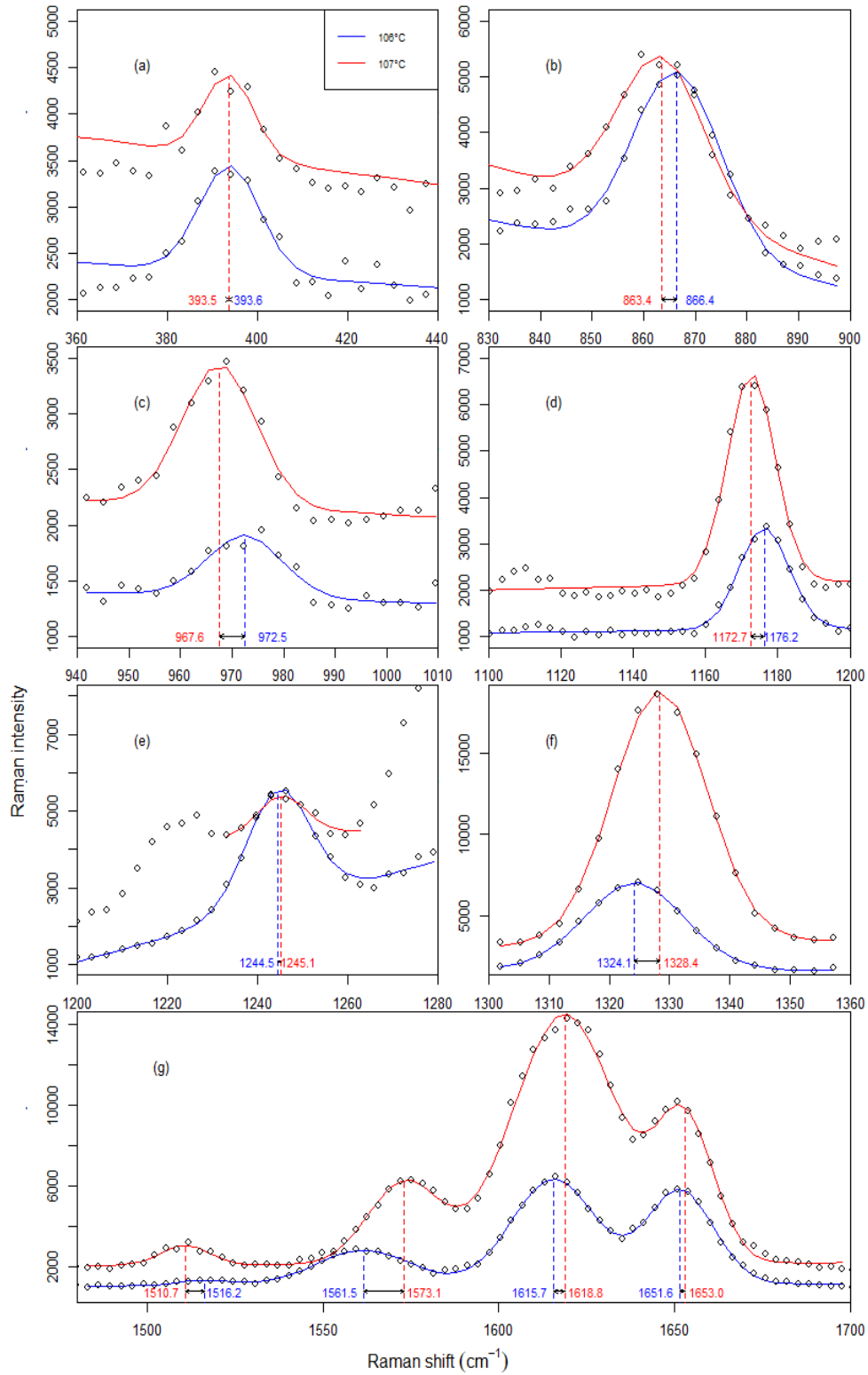


Figure 3.22. Experiment 1 Raman peaks at 106 and 107°C to illustrate peak position shift around form II phase transition temperature: (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

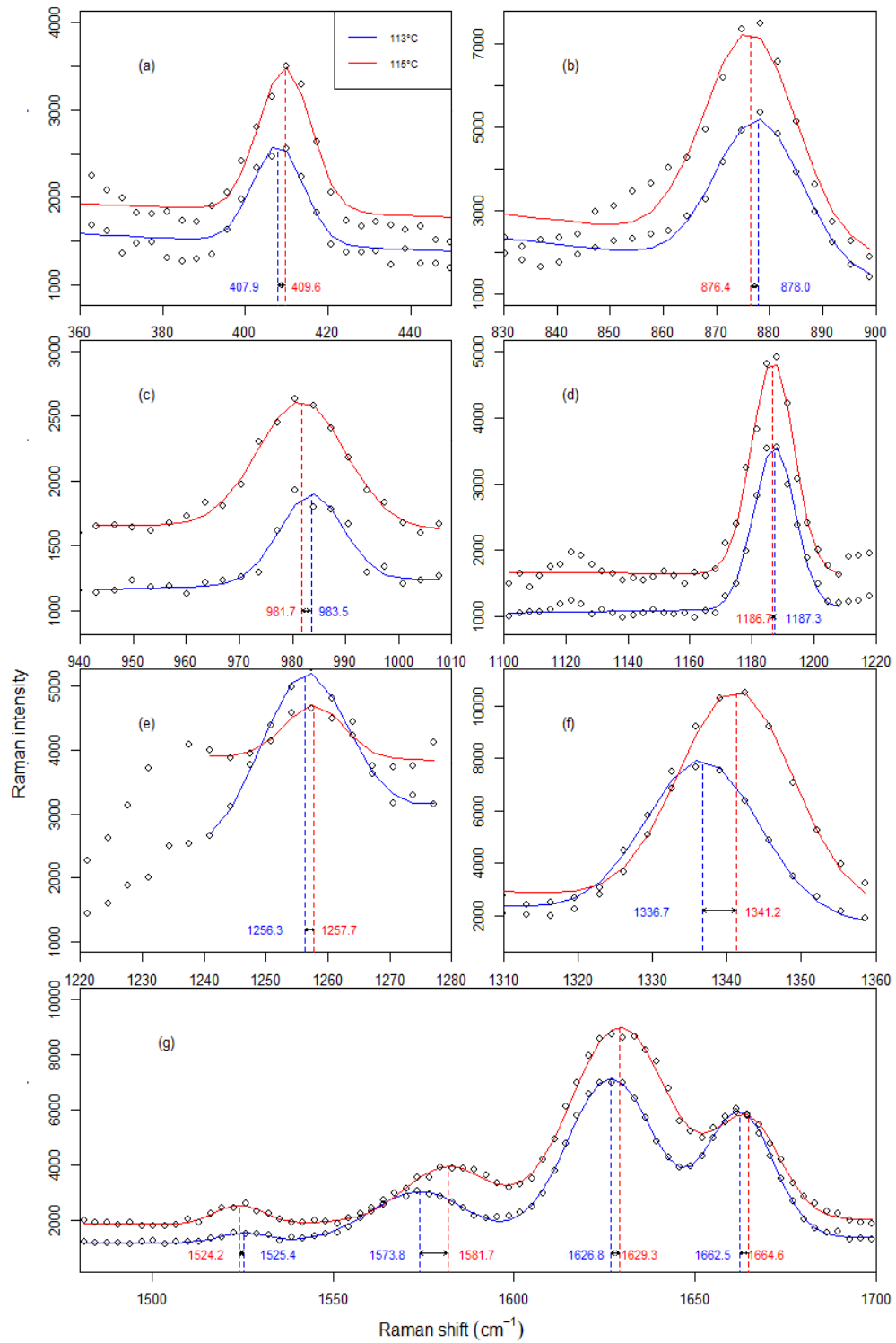


Figure 3.23. Experiment 3 Raman peaks at 113 and 115°C to illustrate peak shift around form II phase transition temperature: (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

Experiment 1 (Figure 3.16) shows peak shift to lower wavenumber as temperature increases from -95 and -65°C which is near the beta transition temperature (-79.15°C), but peaks at around 1230 cm⁻¹ (deformation of phenyl ring, amide and C–OH groups) and 1560 cm⁻¹ (bending of amide N–H group) show peak shift to higher wavenumber. In experiment 3 (Figure 3.17), peaks show no significant change to peak position at around -95 and -65°C. The peak shifts between the two temperature points are very subtle and show no pattern in terms of shift in wavenumber. On the other hand, as temperature increases from 17 to 35°C near the alpha transition temperature (23.85°C) in the experiment 1 (Figure 3.18) and experiment 3 (Figure 3.19), peaks generally shift to lower wavenumber, except for peak at around 1660 cm⁻¹ (C=O stretching), which shifts to higher wavenumber. The changes to peak shifts during form III phase transition are similar in experiment 1 (Figure 3.20) and experiment 3 (Figure 3.21). Most peaks show position shift to higher wavenumber, except for peaks at around 970 cm⁻¹ (deformation of phenyl ring and stretching of C–N amide bond) and 1230 cm⁻¹ (deformation of phenyl ring, amide and C–OH groups) which shift to lower number. In experiment 1 (Figure 3.22) and experiment 3 (Figure 3.23), peaks at 865 cm⁻¹ (symmetric phenyl breathings), 975 cm⁻¹ (deformation of phenyl ring and stretching of C–N amide bond), 1175 cm⁻¹ (bending of phenyl ring and C–OH groups), 1230 cm⁻¹ (deformation of phenyl ring, amide and C–OH groups) and 1520 cm⁻¹ (bending of phenyl ring and stretching of CNH group) shift to lower wavenumber when others at 1330 cm⁻¹ (deformation of phenyl ring and C–OH group), 1560 cm⁻¹ (bending of amide N–H group), 1620 cm⁻¹ (C=C stretching) and 1660 cm⁻¹ (C=O stretching) shift to higher wavenumber during form II phase transition. Peak at 390 cm⁻¹ (whole molecule) shows no observable change to peak shift in experiment 1 and a slight shift in experiment 3 despite change. Hence, the peak shift in experiment 1 and 3 are in agreement during form III and II phase transition.

As a guidance, the peak shifts of 10 Raman spectral peaks from the experiments 1, 2 and 3 are summarised in Table 3.2. We could conclude that for paracetamol based upon the studies described above, peak shifts around

beta transition temperature fluctuate and show no consistent pattern. On the other hand, peak shift at around alpha, form III and form II phase transition temperature are more reproducible and consistent, compared to those at around beta transition temperature. Peak shift around the alpha transition temperature is the prominent in peak at around 1520 cm^{-1} (bending of phenyl ring and stretching of CNH group) 1560 cm^{-1} (bending of amide N-H group) and 1660 cm^{-1} (C=O stretching). In reference to form III transition, we observe consistent peak shift to higher wave number in peaks at around 388 cm^{-1} (whole molecule) and 1230 cm^{-1} (deformation of phenyl ring, amide and C-OH groups). During form II transition, we also see a pattern of consistent peak shift to higher wavenumber at around 1230 cm^{-1} (deformation of phenyl ring, amide and C-OH groups), 1330 cm^{-1} (deformation of phenyl ring and C-OH group), 1560 cm^{-1} (bending of amide N-H group), 1620 cm^{-1} (C=C stretching) and 1660 cm^{-1} (C=O stretching).

Table 3.2. Summary of 10 Raman spectral peaks at two temperature points to illustrate peak position shift versus temperature

Temperature range	Experiment No.	Peak positions (cm ⁻¹)									
		388	862	970	1168	1230	1324	1520	1560	1620	1650
		Whole molecule	Symmetric phenyl breathing	Deformation of phenyl ring + stretching of C-N amide bond	Bending of phenyl ring and C-OH groups	Deformation of phenyl ring + amide + C-OH groups	Deformation of phenyl ring + C-OH group	Bending of phenyl ring + stretching of CNH group	Bending of amide N-H group	C=C stretching	Bending of amide N-H group
Beta transition (-95 to -65°C)	Experiment 1										
	Experiment 2										
	Experiment 3										
Alpha transition (15 to 35°C)	Experiment 1										
	Experiment 2										
	Experiment 3										
Form III phase transformation* (55 to 61°C)	Experiment 1										
	Experiment 2										
	Experiment 3										
Form II phase transformation* (95 to 115°C)	Experiment 1										
	Experiment 2										
	Experiment 3										
Peak shift to higher wavenumber Peak shift to lower wavenumber Peak shift to slightly higher wavenumber (Peak shift < 0.5 cm⁻¹) Peak shift to slightly lower wavenumber (Peak shift < 0.5 cm⁻¹)											

3.4.7 Low temperature repeat tests

From inspection of the contour maps and Gaussian peak fit curves of paracetamol, some visual changes were observed for Raman spectra at around the beta transition temperature (-79.15°C), but not around the alpha transition temperature (23.85°C). These results were not obviously reproducible in terms of the beta transition temperature. There seemed to be sample to sample variation in each of the experiments, which was not expected given the samples were prepared in the same way, so the temperature cycling experiments of a single sample (three repeats for each sample) were repeated at low temperature (-100 to -59°C) to test the reproducibility of the data around the beta transition temperature. Three sets of experiments were repeated, and each experiment consisted of three heated cycling experiments at low temperature.

3.4.7.1 Heat map – low temperature repeat tests

Figure 3.24 to Figure 3.29 are the key figures of the contour maps of the normalised Raman spectra collected for a quench-cooled paracetamol from the low temperature repeat tests. The figures were plotted as a function of temperature similar to those in Section 3.4.4, but during heating of the sample from -100 to -59°C . The scaled intensities are plotted on the colour scale, similar to those in Section 3.4.2.

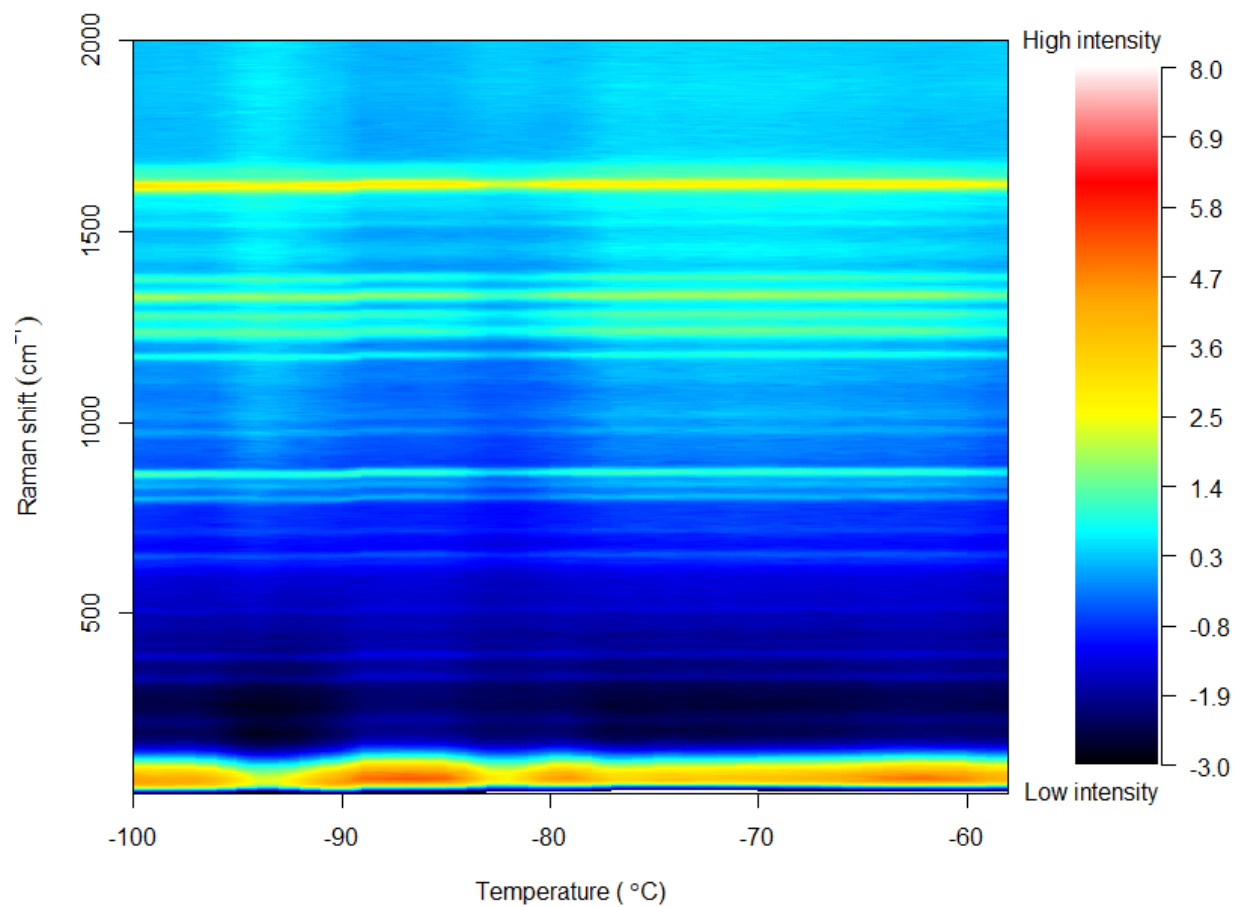


Figure 3.24. Low temperature paracetamol repeat experiment 1 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

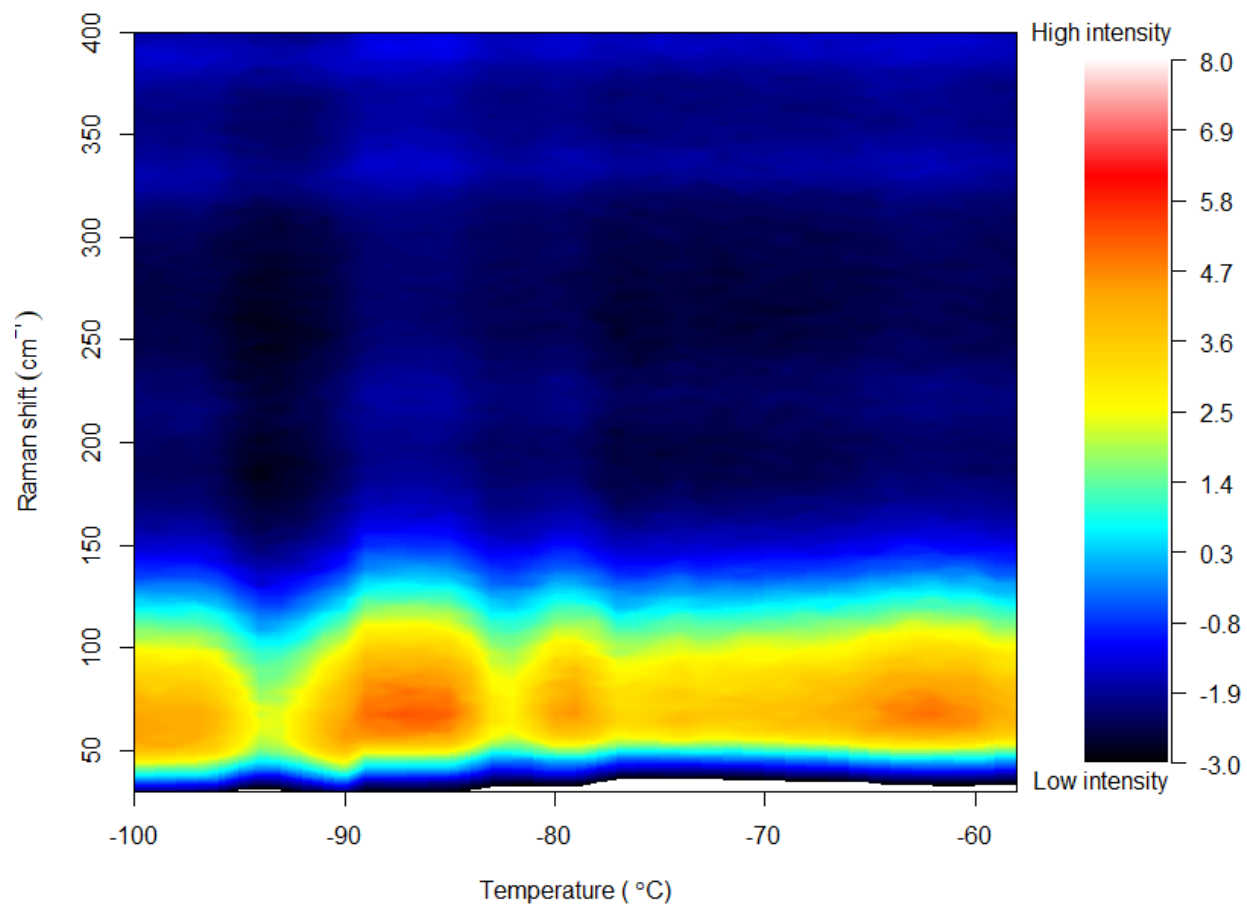


Figure 3.25. Low temperature paracetamol repeat experiment 1 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

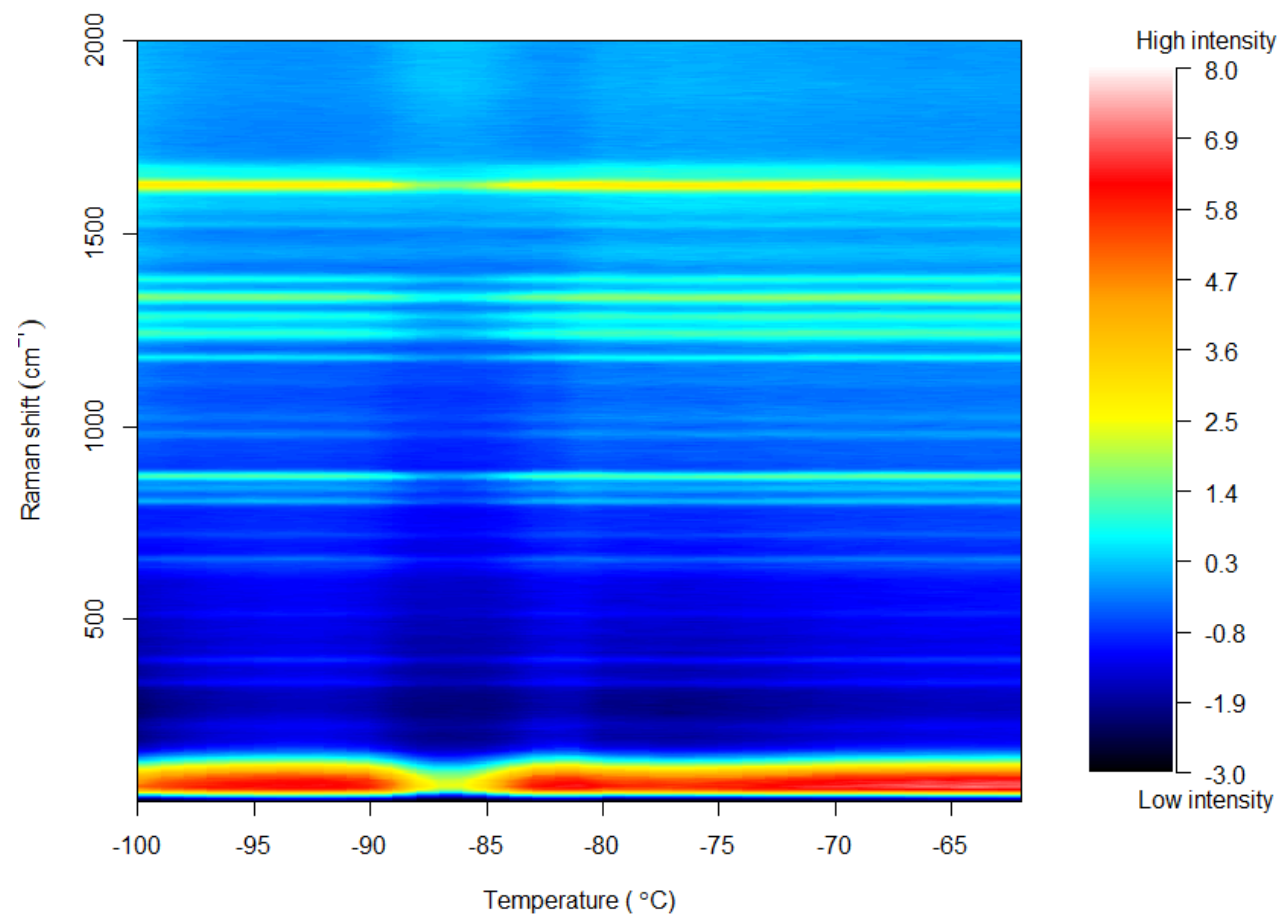


Figure 3.26. Low temperature paracetamol repeat experiment 1 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

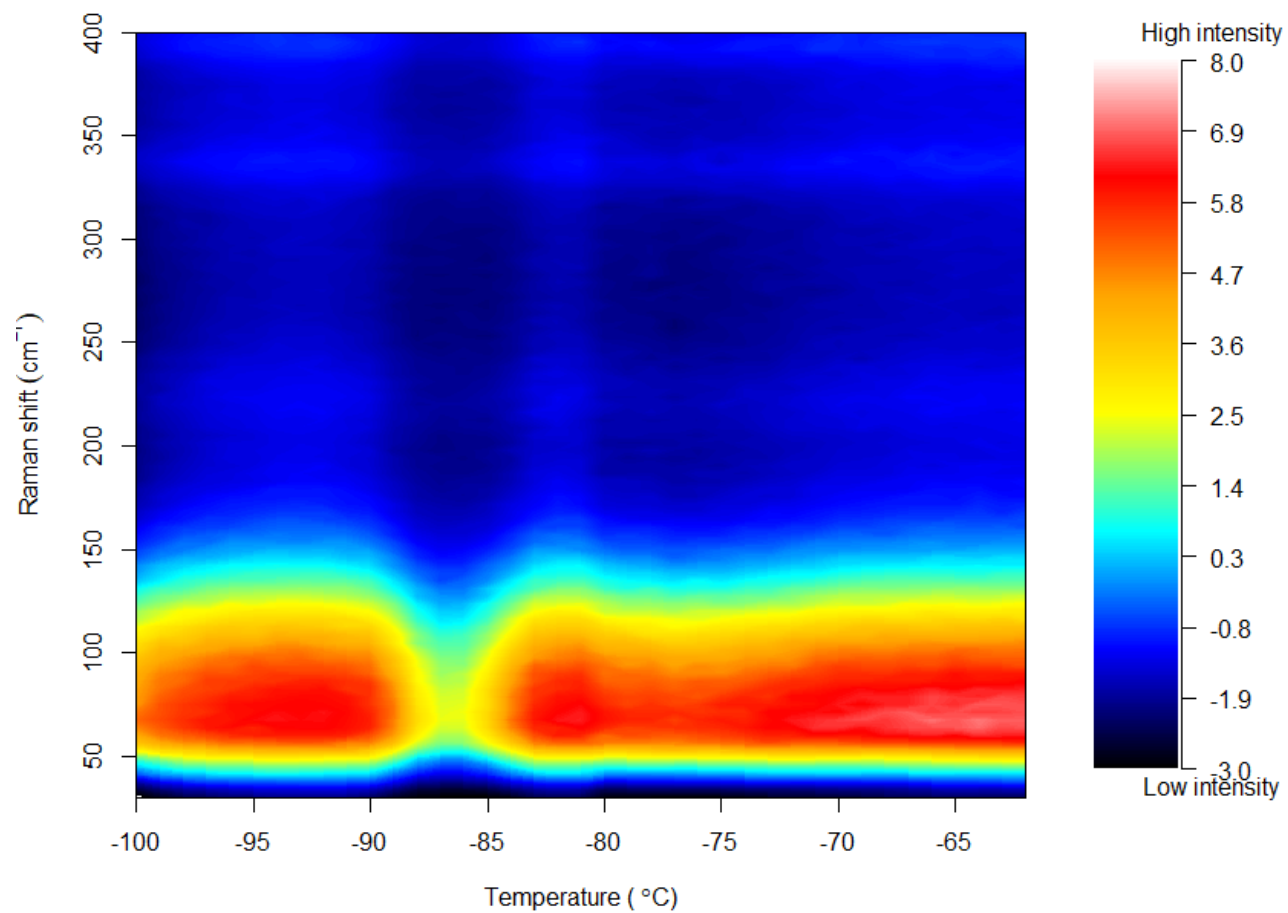


Figure 3.27. Low temperature paracetamol repeat experiment 1 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

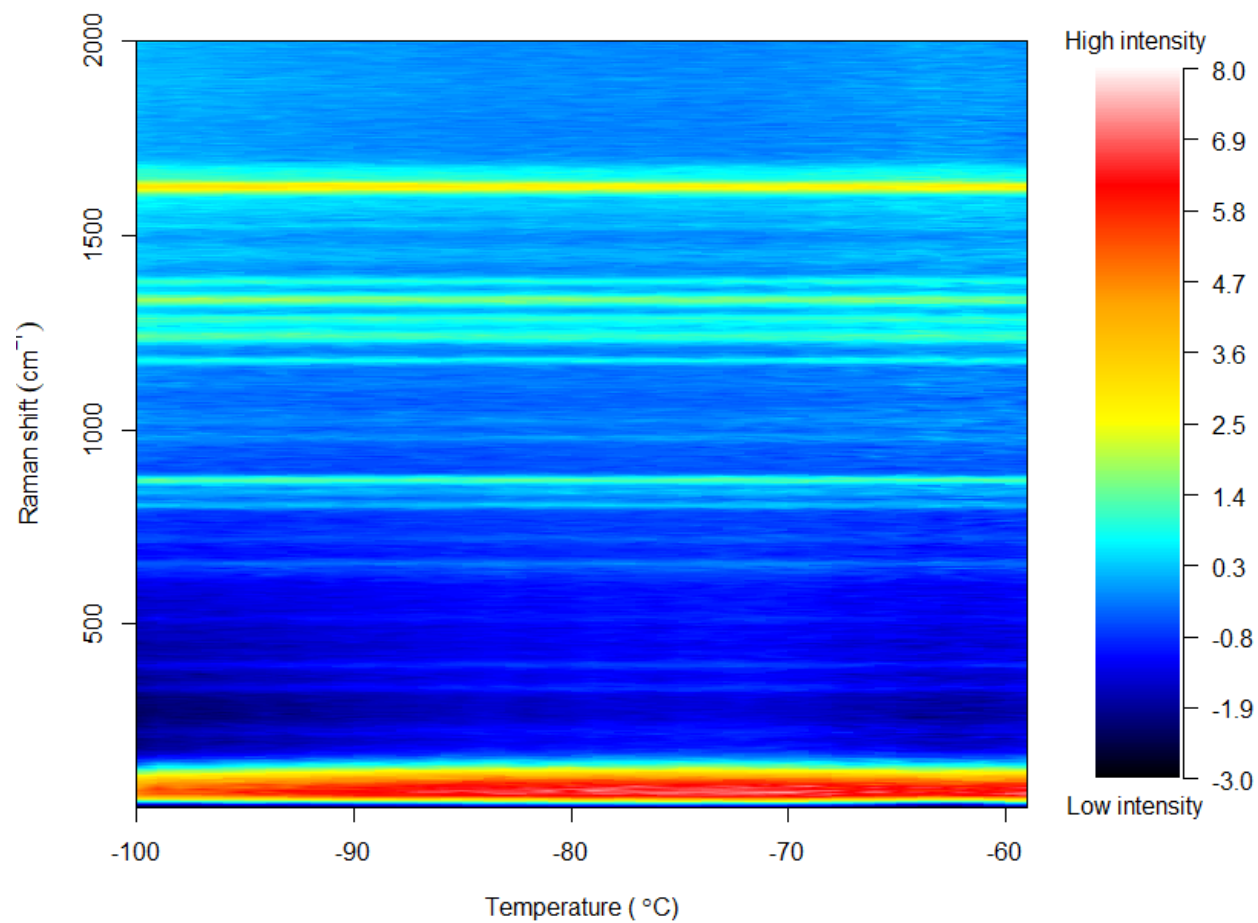


Figure 3.28. Low temperature paracetamol repeat experiment 1 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

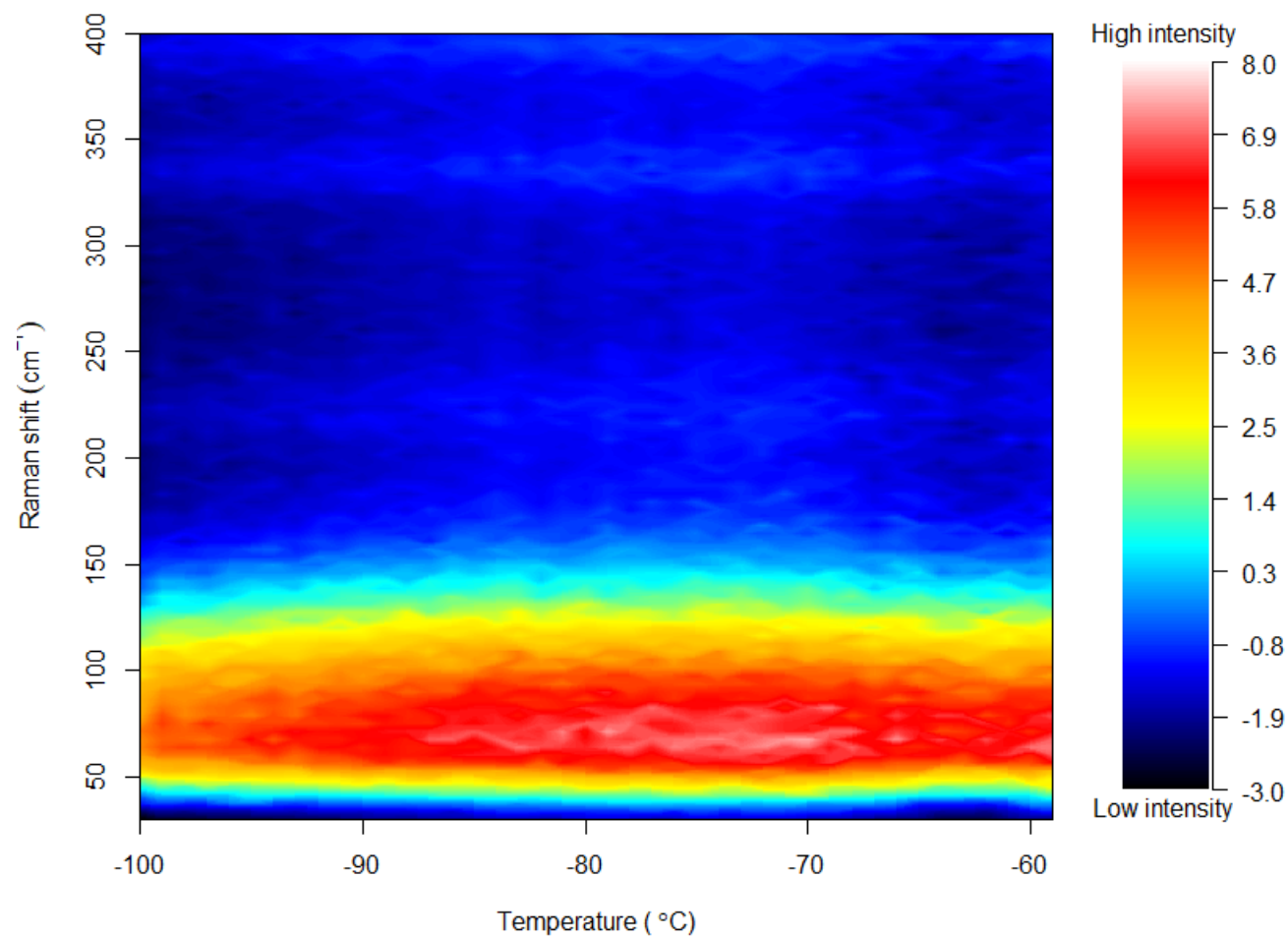


Figure 3.29. Low temperature paracetamol repeat experiment 1 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

In the first low temperature cycling experiment, there were changes to peak intensity in cycle1 (Figure 3.24), therefore repeated the experiment of the same quench-cooled paracetamol sample by re-freezing and reheating, for cycle 2 (Figure 3.26) and cycle 3 (Figure 3.28). Changes around the beta transition temperature of paracetamol (-79.15°C) were observed in the second cycle but there were no obvious changes observed in the third cycle. We did not see anything at around -80°C , which is near the reported beta transition temperature of paracetamol. We repeated two more sets of the experiments but did not see obvious changes around the beta transition temperature. The data from the second (Figure B 5 to Figure B 10 of Appendix B) and third (Figure B 11 to Figure B 16 of Appendix B) sets of experiments are in the appendix. Hence, for paracetamol based upon these studies, we cannot fully conclude that temperature could lead to detectable changes in Raman scattering of functional groups that are associated with beta relaxation.

3.4.7.2 Analysis of Gaussian fit curves of peak intensity in low temperature repeat tests

We have seen the contour maps of the low temperature repeat experiments and we proceed to test the reproducibility of the data around the beta transition temperature of paracetamol (-79.15°C). Figure 3.30, Figure 3.31 and Figure 3.32 are Gaussian fit curves of peak intensities at around 10 selected vibrational modes (Table 3.1) plotted as a function of temperature. Figure 3.30, Figure 3.31 and Figure 3.32 correspond to the peak fit curves of cycle 1, 2, and 3 of the low temperature experiments, respectively. Each dot on the scatterplot represents peak intensity of selected peaks in the range of -100 to -59°C . The black, blue and purple dots represent low temperature experiment 1, 2 and 3, respectively.

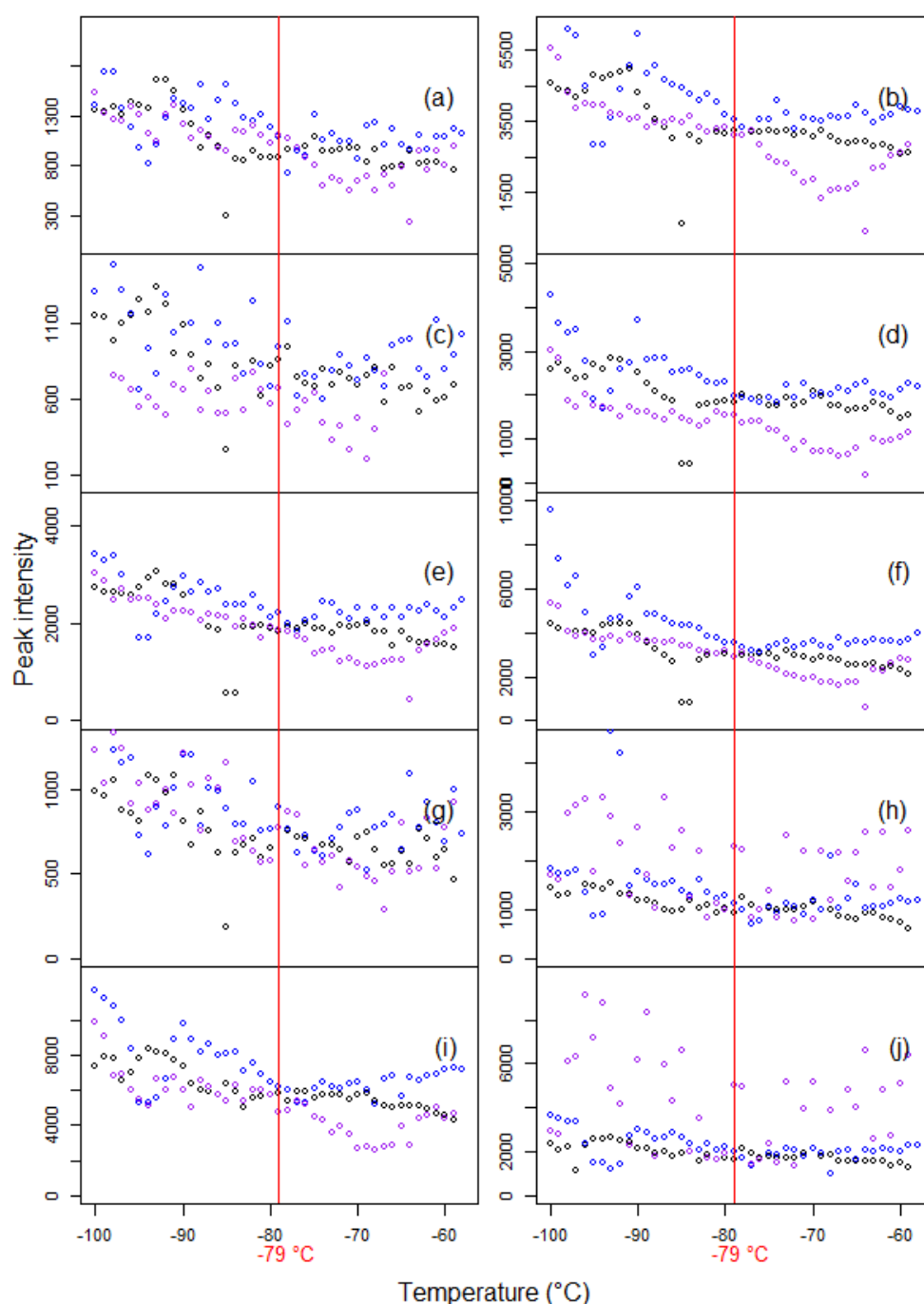


Figure 3.30. Low temperature experiment (cycle 1) - The peak fitting of intensity against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

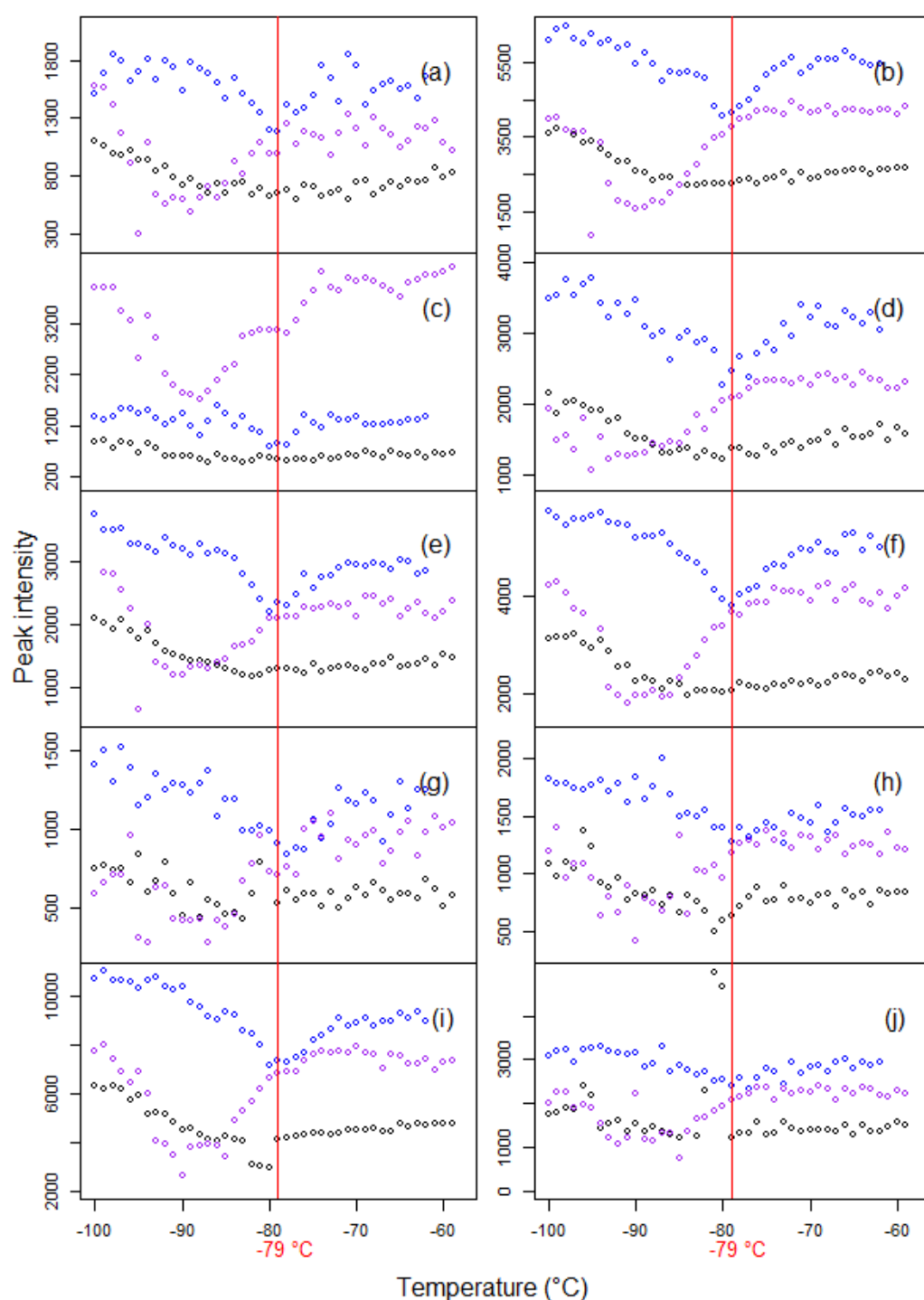


Figure 3.31. Low temperature experiment (cycle 2) - The peak fitting of intensity against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

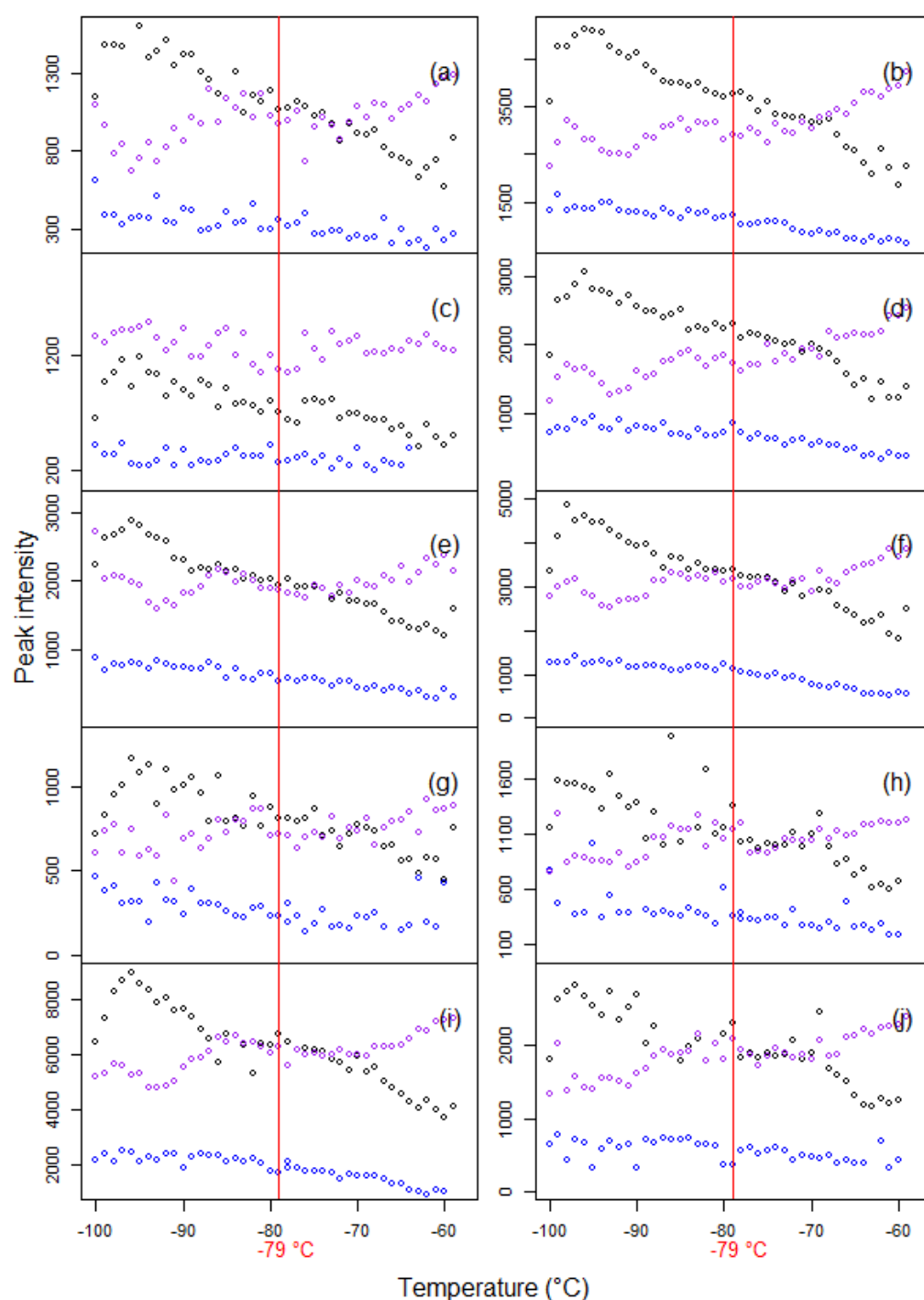


Figure 3.32. Low temperature experiment (cycle 3) - The peak fitting of intensity against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

Cycle 1 (Figure 3.30) shows drop in intensities as the temperature goes from the origin to -59°C, which is not far from the beta transition temperature of paracetamol (-79.15°C). The change to peak intensity is not observed in and cycle 3 (Figure 3.32). An obvious change around beta transition temperature is only observed in cycle 2 of the experiment 2 (Figure 3.31, blue dots). The changes in peak intensities are not as obvious in peaks at around 1660 cm⁻¹ which correspond to C=O stretching (Figure 3.31 (j)). Overall, we observe some changes in peak intensities around the beta transition temperatures in the low temperature experiments, but question the reproducibility of these changes. Given the figures are not reproducible, for paracetamol based upon these studies described above, we conclude that the beta temperature does not appear to cause changes in Raman scattering of functional groups.

3.4.7.3 Analysis of Gaussian fit curves of peak width in low temperature repeat tests

The Gaussian fit curves of peak width were plotted to investigate different Raman features caused by the beta transition temperatures in the 10 selected intramolecular vibrational modes of paracetamol (Table 3.1). The Gaussian fit curves of peak width in Figure 3.33, Figure 3.34 and Figure 3.35 correspond to the peak fit curves of cycle 1, 2, and 3 of the low temperature experiments, respectively.

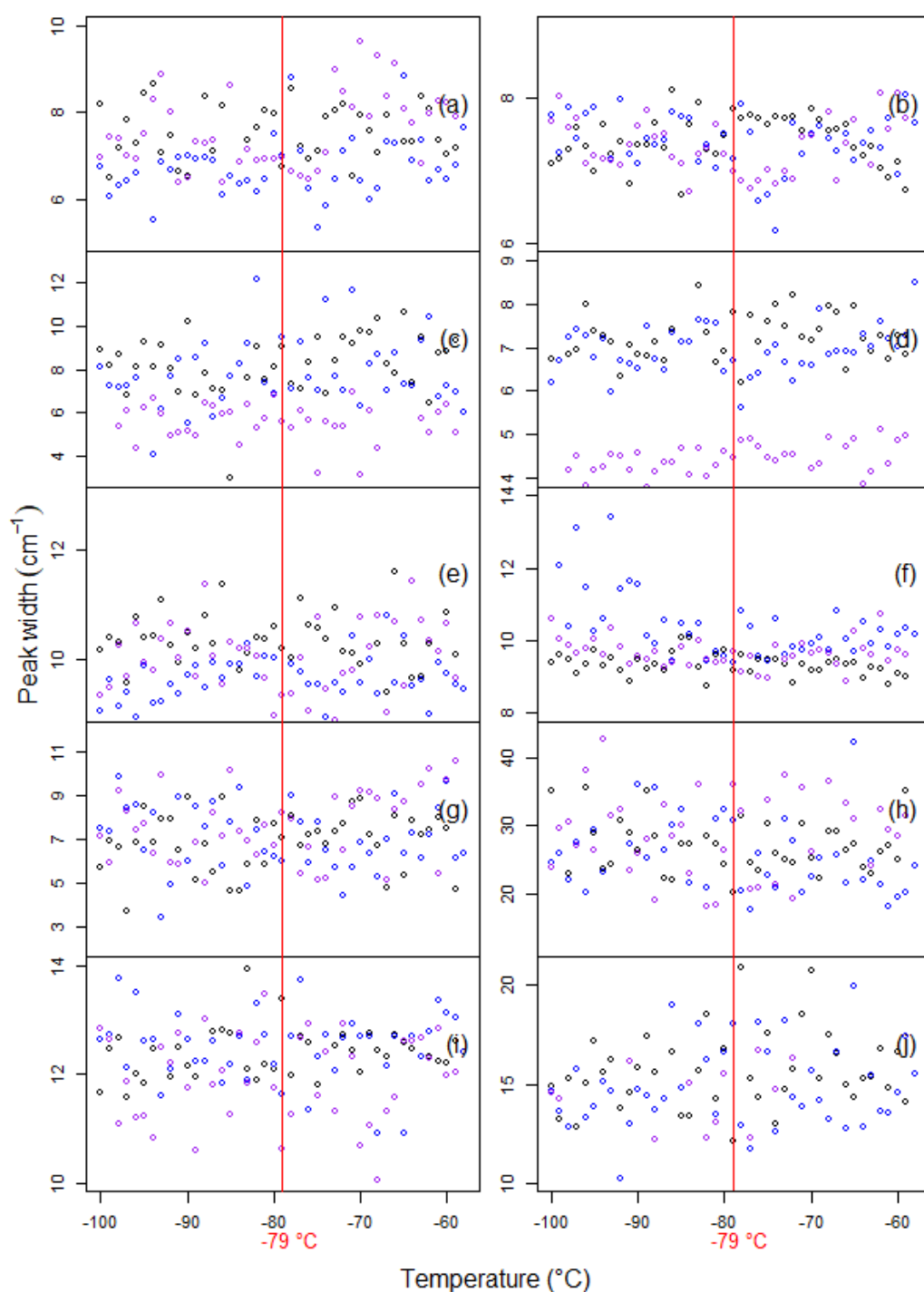


Figure 3.33. Low temperature experiment (cycle 1) - The peak fitting of width against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

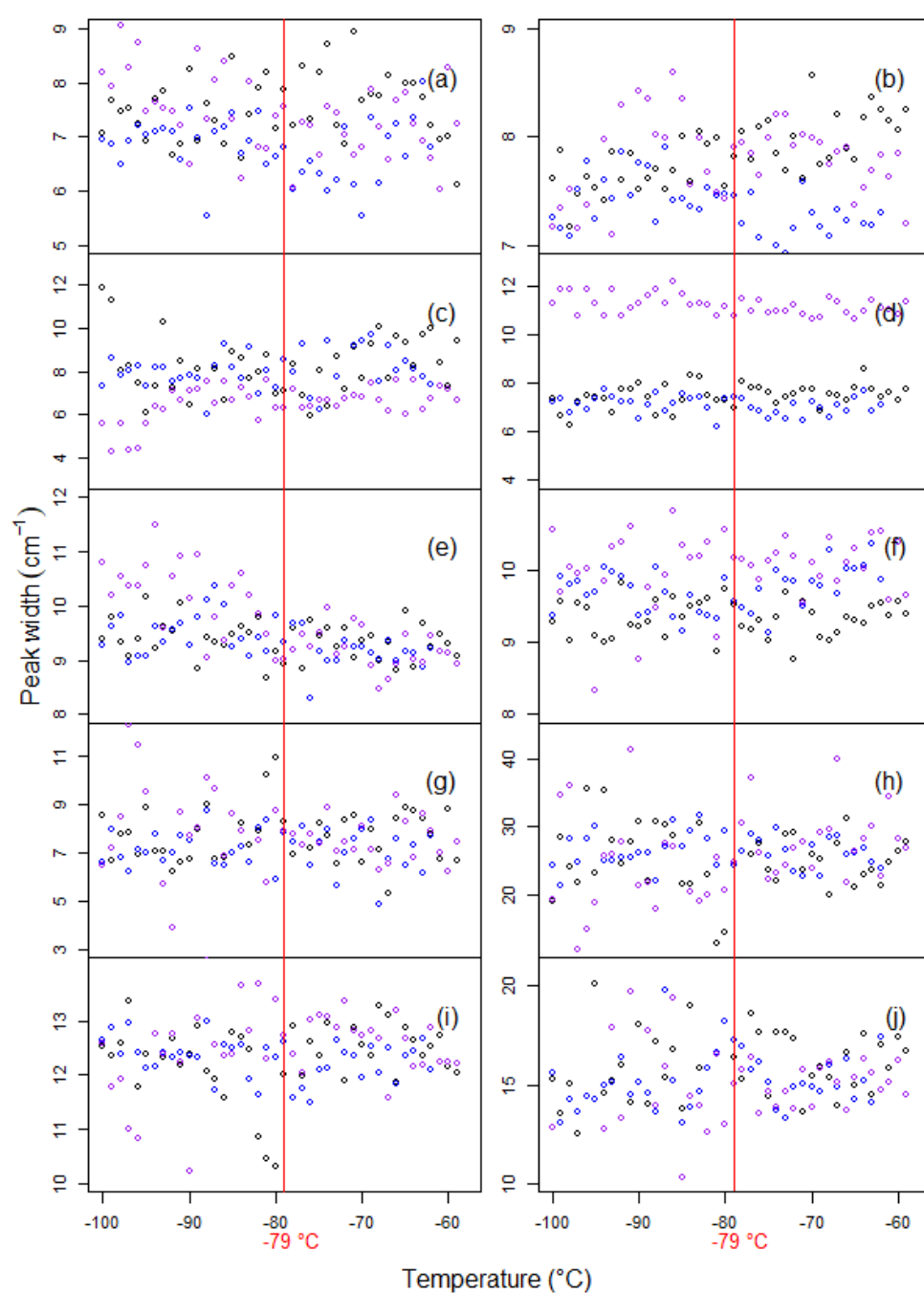


Figure 3.34. Low temperature experiment (cycle 2) - The peak fitting of width against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

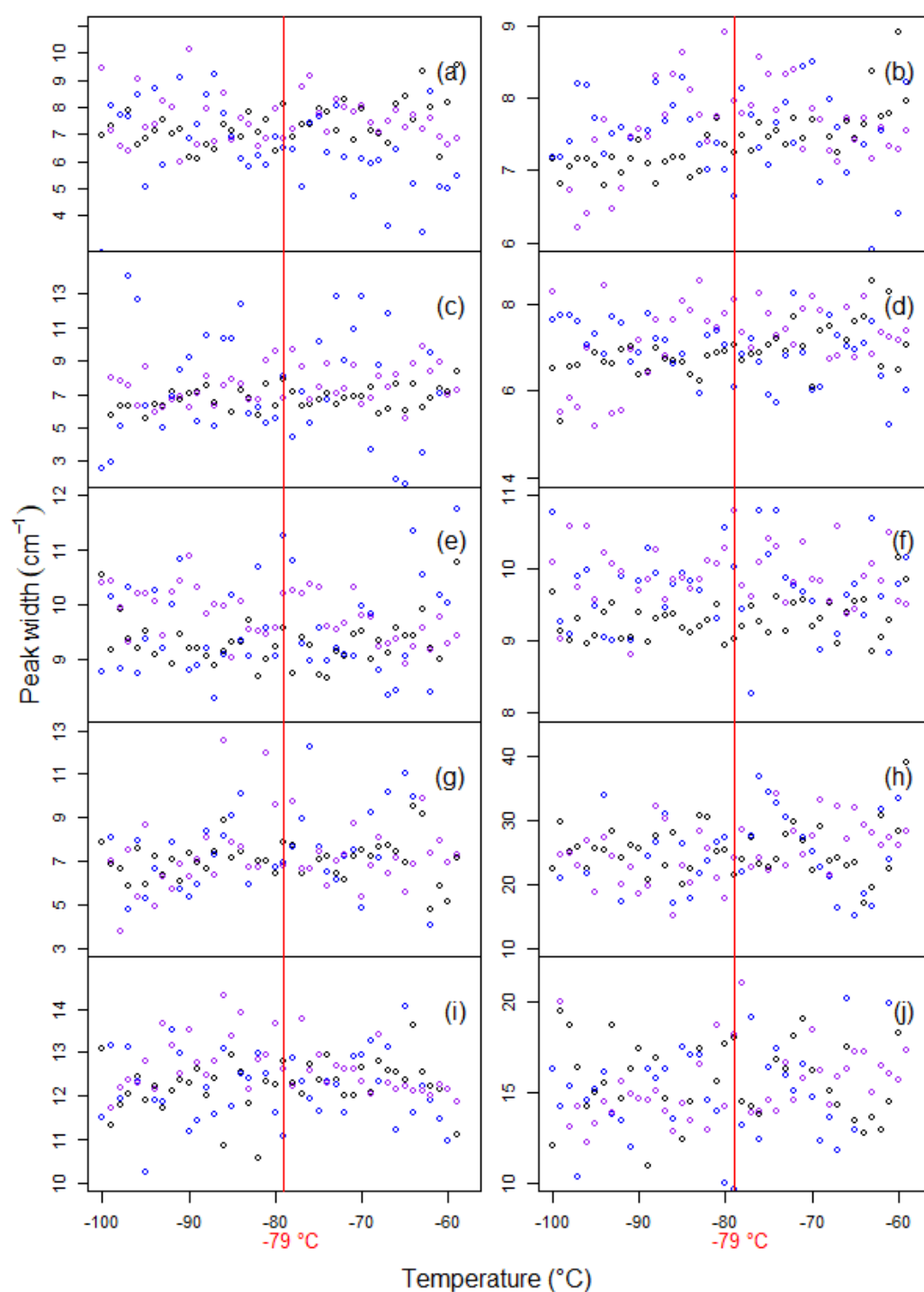


Figure 3.35. Low temperature experiment (cycle 3) - The peak fitting of width against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

In Figure 3.33, Figure 3.34 and Figure 3.35, we observe no obvious changes to peak width at around the beta transition temperature (-78.15°C). This is reasonable as we see no change to the non-crystalline region in the previous experiments (Figure 3.14). We would have to conclude that for paracetamol based upon these studies described above, beta relaxation may not be related to changes in order/disorder because the widths of the Raman peaks do not seem to change at around the beta transition temperature.

3.4.7.4 Analysis of Gaussian fit curves of peak shift in low temperature repeat tests

The Gaussian fit curves of peak position of paracetamol were plotted to investigate different Raman features caused by the beta transition temperatures in the 10 selected intramolecular vibrational modes of paracetamol (Table 3.1). Figure 3.36, Figure 3.37 and Figure 3.38 are the fit curves of peak positions of the three low temperature repeat experiments and correspond to the peaks of cycle 1, 2, and 3, respectively.

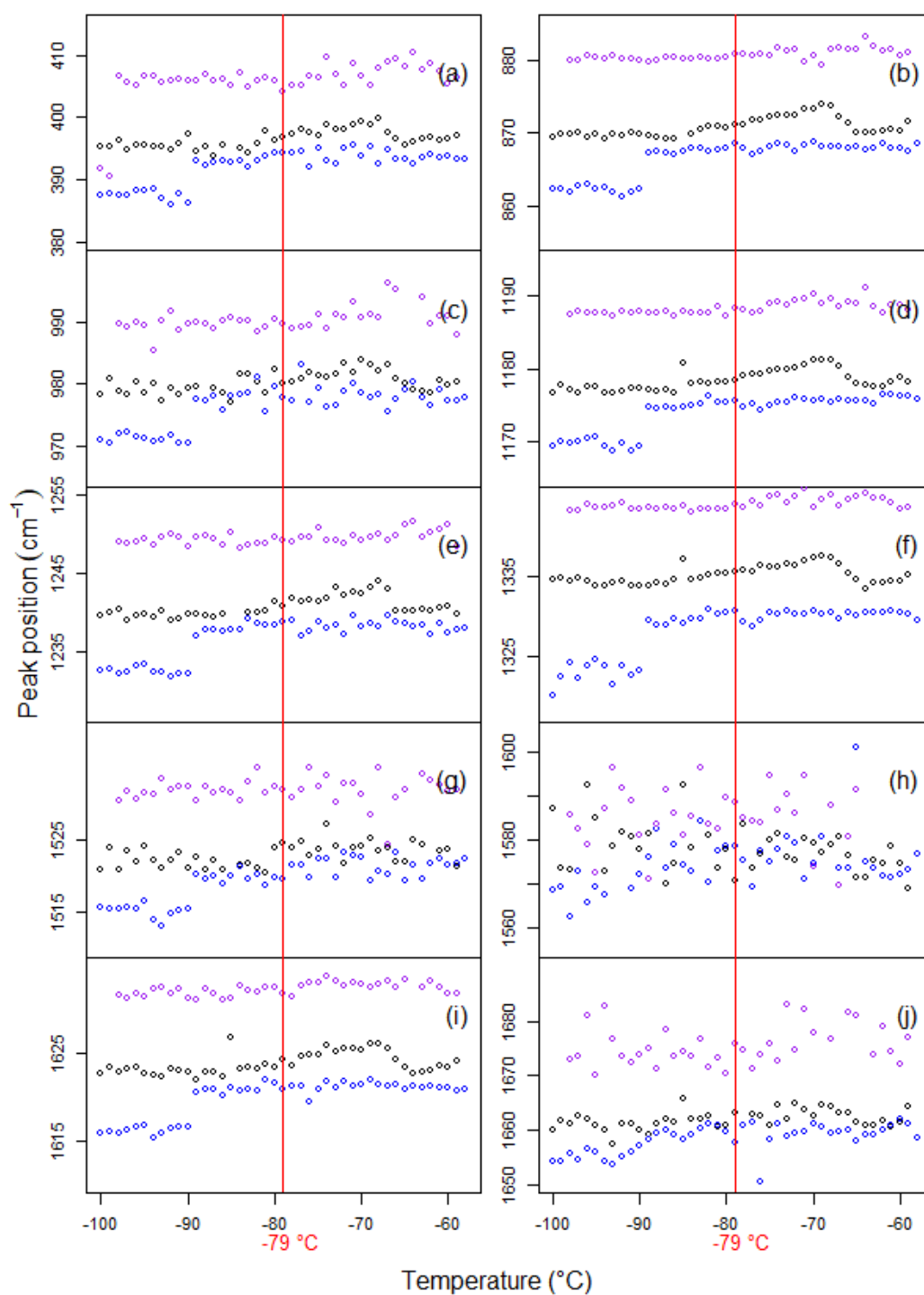


Figure 3.36. Low temperature experiment (cycle 1) - The peak fitting of position against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

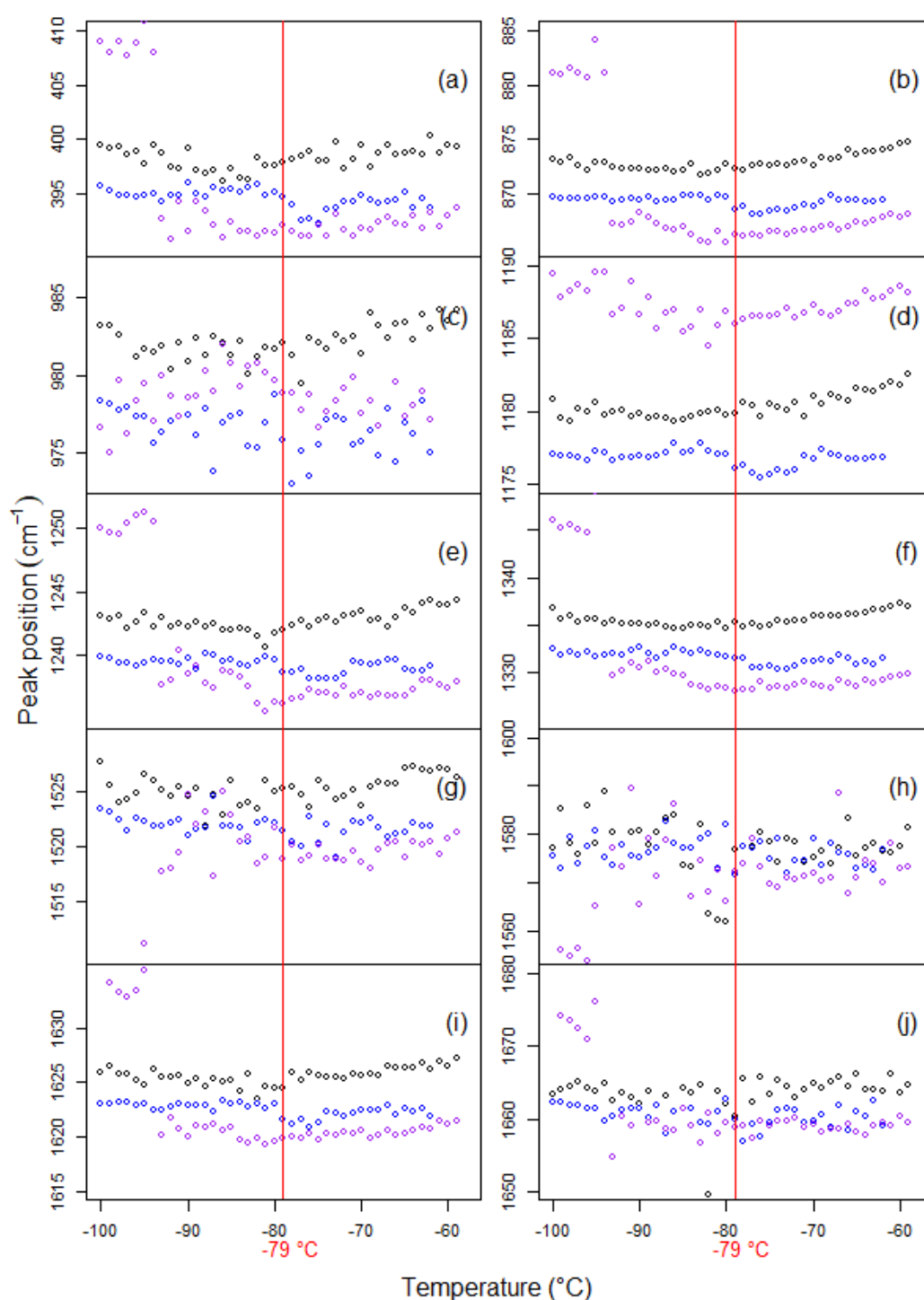


Figure 3.37. Low temperature experiment (cycle 2) - The peak fitting of position against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

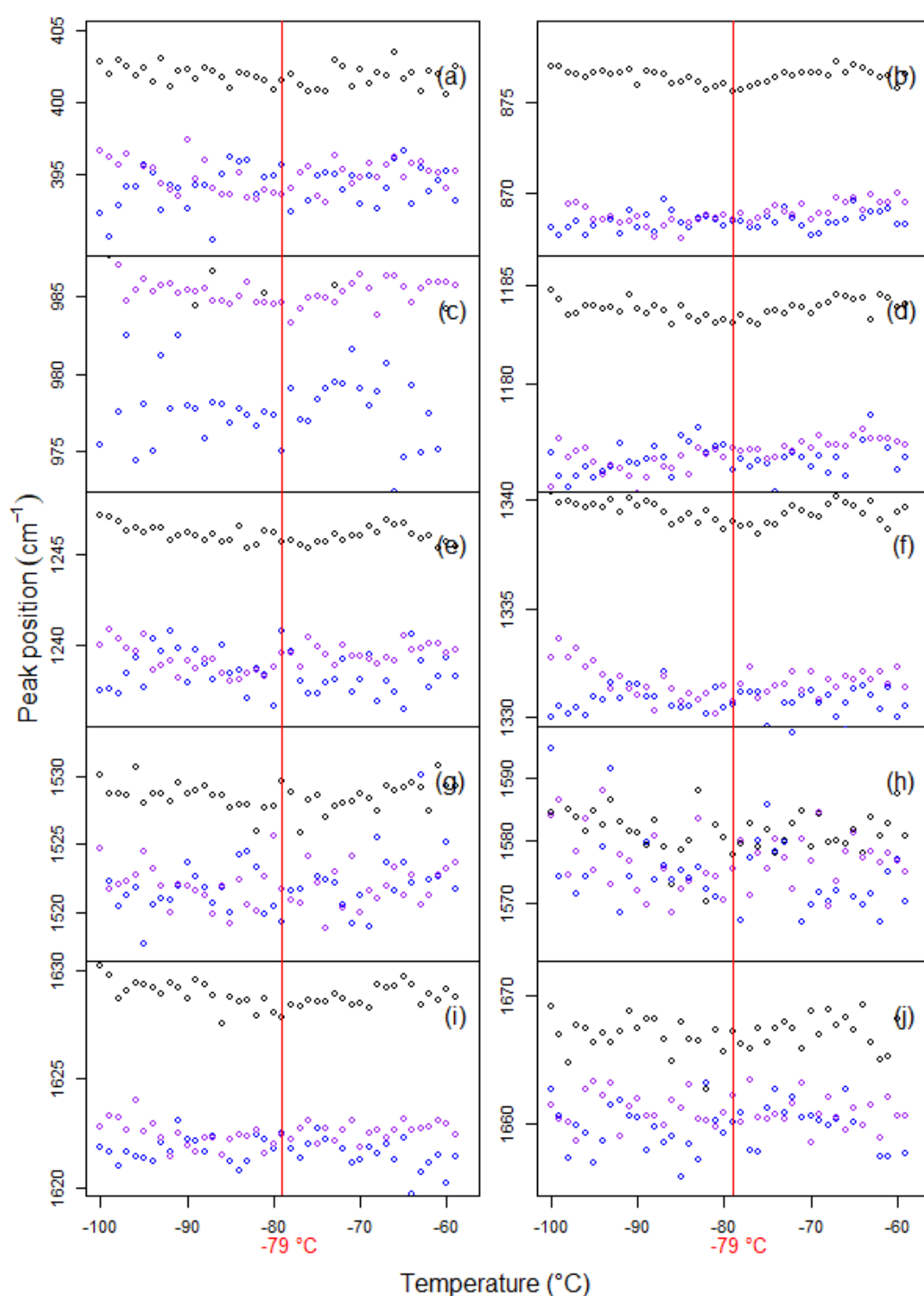


Figure 3.38. Low temperature experiment (cycle 3) - The peak fitting of position against temperature for the 10 selected peaks of paracetamol: 390 cm^{-1} , 865 cm^{-1} , 975 cm^{-1} , 1175 cm^{-1} , 1230 cm^{-1} , 1330 cm^{-1} , 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (24°C) and beta (-79°C) transition temperatures.

The Gaussian fit curves of the peak positions from cycle 1 (Figure 3.36), cycle 2 (Figure 3.37) and cycle 3 (Figure 3.38) show no obvious change to peak positions near the beta transition temperature (-78.15°C). Only in cycle 2 of the experiment 3 (Figure 3.37, purple dots), we observe an obvious change to peak position at around -95°C . These changes are not reproducible in other experiments. This contrasts with major changes in peak position that were observed at around the beta transition temperature in the previous experiments (Figure 3.15). The findings in the low temperature experiments do not support reproducibility of the data, at around beta transition temperature. We conclude that for paracetamol based upon these studies described above, structural changes do not seem to occur at around the beta transition temperature.

3.5 Conclusion

This chapter showed detailed study of alpha (42.25°C) and beta transition (-66.15°C) in heating the amorphous state of paracetamol from Raman spectroscopy. We summarised the findings from Gaussian fit curves of peak intensity, width and position of paracetamol in Table 3.3.

While we have done experiments to see any changes around alpha (23.85°C) and beta transition (-79.15°C) temperature of paracetamol, we do not see any changes that are obviously reproducible at the temperature of interest. We do not see any changes to peak width around the beta transition temperature, which is surprising in light of the JG model which predicts inhomogeneous chemical environment, but then we still do not see any change during solid to liquid transition (at around alpha transition temperature), and hence the amorphous state does not seem to be sensitive to changes in chemical environment. While some changes in peak intensity and peak position (with exception of peaks at around 1520 cm^{-1} that correspond to bending of phenyl ring and stretching of CNH group and 1560 cm^{-1} that correspond to bending of amide N-H group) were observed around the beta transitions in two of the three experiments, they were not particularly

reproducible. When we did the cycling experiment, we did not see any reproducibility. We have compared peak shifts of Raman spectra among the alpha, beta, form III and form II phase transition temperatures and we learned that peak shifts around beta transition temperature tend to be the least reproducible and consistent. Hence, we conclude that for paracetamol based upon these studies described above, there is no strong evidence for any detectable structural changes to the Raman spectra around the alpha and beta transition temperature.

Table 3.3. Summary of the results from variable temperature set up Raman experiments of paracetamol (The symbol “o” indicates presence of visual changes; “x” indicates absence of visual change)

Experiment No.		Change around beta transition temperature			Change around alpha transition temperature		
		Peak intensity	Peak width	Peak position	Peak intensity	Peak width	Peak position
Experiment 1		o	x	o	x	x	x
Experiment 2		x	x	x	x	x	x
Experiment 3		o	x	o	o	x	x
Low temperature experiment 1	Cycle 1	o	x	x			
	Cycle 2	x	x	x			
	Cycle 3	x	x	x			
Low temperature experiment 2	Cycle 1	x	x	x			
	Cycle 2	o	x	x			
	Cycle 3	x	x	x			
Low temperature experiment 3	Cycle 1	x	x	x			
	Cycle 2	x	x	x			
	Cycle 3	x	x	x			

For alpha transition, where there are massive changes in the physical properties from a solid to a liquid and it is surprising to see no changes. For paracetamol based upon these studies described above, we found no reproducible evidence of any obvious spectroscopic changes during both the alpha and beta transition and question if the results and analysis of our findings are unique characteristic of paracetamol.

It is notable that while we have not detected any changes here even around the glass transition temperature, this at first glance appears contrary

to what has been reported in the literature. For example, low frequency dielectric measurements of the JG beta-relaxation times enabled beta transition temperatures of polymers presented in table Table 1.1 (Ngai et al, 2015). Using terahertz time- domain spectroscopy, there has been a study on dielectric losses in polyalcohols that found clear spectroscopic evidence for beta relaxations (Sibik et al, 2014a). There is the obvious difference that in this study small molecules have been studied, whereas in the previous groups they looked at polymers. This could be the reason behind the lack of changes seen during beta relaxation. The relaxation time scales for polymers are vastly different to those for small molecules and furthermore, the small molecule system could not relax efficiently in the time scales available. For the polymer systems, it is possible that they are cooled much faster than the relaxation time scale, so what is seen at the glass transition temperature in the polymers is in fact the excess enthalpy that is observed in DSC (Green et al, 2007). Moreover, time-scale for studying annealing effect on glass transition temperature is a couple of hours (Pyne et al, 2003).

We will now move on to look at different molecules, nifedipine in the following chapter and felodipine (in Chapter 5), which is structurally related to nifedipine and has very similar alpha transition temperature at around 44°C, but has very different behaviour -nifedipine crystallizes more readily than felodipine from the metastable amorphous form (Marsac et al, 2006).

Chapter 4 Investigation of the applicability of Raman spectroscopy to probe the alpha and beta relaxation events in nifedipine

4.1 Introduction

Following on from the previous chapter, in which we used paracetamol as simple model molecule to study the spectra across the alpha and beta transition temperature range in detail, to see if anything happens at around beta relaxation temperature, nifedipine was selected to investigate potential changes in molecules around alpha and beta transition temperatures from low to high temperature. We are going to do cycling experiments to check the reproducibility and whether we could observe potential molecular changes in heating and cooling, or just on cooling, or just on heating the nifedipine sample.

4.2 Materials

Nifedipine was purchased by Sigma-Aldrich ($\geq 99.0\%$). It is in the form of yellow crystalline powder. The melting point, alpha transition temperature (T_g) and beta transition temperature (T_β) of nifedipine are 173°C , 42.25°C and -66.15°C , respectively (Caira et al, 2003; Kissi et al, 2018a).

4.3 Methods

4.3.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to measure the characteristic melting point of the compound to get an indication of the purity. Modulated DSC has been performed to investigate beta transition temperature of nifedipine. The experimental parameters were same as those in paracetamol experiment in Section 3.3.1.

4.3.2 X-ray diffraction (XRD)

X-ray diffraction (XRD) data was collected on a Panalytical X'pert diffractometer to determine the purity of the compound. The experimental parameters were same as those in paracetamol experiment in Section 3.3.2.

4.3.3 Fourier transform infrared (FTIR)

Fourier transform-infrared spectroscopy (FTIR) was used to assess purity of the compound. The experimental parameters were same as those in paracetamol experiment in Section 3.3.3.

4.3.4 Raman spectroscopy

Variable temperature experiments were performed to investigate any potential structural changes in amorphous nifedipine drugs at around alpha transition (42.25°C) and beta transition (-66.15°C) temperatures (Kissi et al, 2018a). Nifedipine sample slide was placed in Linkam thermal stage LTS350 to be melted up to high temperature of 180°C, which is higher than the melting point of nifedipine, i.e., 173°C, to ensure that the sample was completely melted (Caira et al, 2003). The sample was then cooled rapidly to low temperature (-100°C) in cooling rate of 30°C min⁻¹. The experimental parameters for Raman spectrometer were very similar to the variable temperature set up paracetamol experiment, but for nifedipine experiment, visible (660 nm) laser of 20 mW power was employed in the experiments and each Raman spectrum was collected for 7 seconds.

4.4 Results

4.4.1 Characterisation of the nifedipine sample

DSC, XRD, FTIR were used to determine the purity of the material. The DSC data (Figure A 8 of Appendix A) showed melting point at around 173°C. This confirmed the purity of the sample when compared to data (Figure A 9 of Appendix A) presented in the literature (Song et al, 2005), which showed good

correspondence to the experimental data. The plot of the modulated DSC thermogram could be found in the appendix (Figure A 10 of Appendix A) and does not show any obvious evidence of beta relaxation. The XRD data (Figure A 11 of Appendix A) resembled pattern of BICClZ crystal structure from the Cambridge structural database and no extra peaks were observed. This confirmed the purity of the material as crystalline form and the structure of crystalline form to be polymorph alpha. The infrared spectral data (Figure A 12 of Appendix A) confirmed the characteristic peak positions of pure nifedipine at around 3096 cm^{-1} (aromatic group) - $\nu_{\text{Ar-H}}$ (-CH) bands, 1680 cm^{-1} (aryl carboxylic group) - ν (C=O) stretching vibration, 1622 cm^{-1} (pyridine group) - ring breathing band and 1226 cm^{-1} (carbonyl group) – ν C-O/ δ O-H (Santos et al, 2014).

4.4.2 Heat map - variable temperature in Raman spectra

In order to investigate potential Raman spectral changes which can be linked with alpha and beta relaxation processes of nifedipine, contour maps were plotted. Figure 4.1 is the contour map of the normalised Raman spectra plotted as a function of temperature. The Raman spectra were collected from quench-cooled nifedipine sample prepared as outlined in Section 4.2 and the sample was heated from -100 to 150°C with step sizes of 1°C , in the wavenumber range of 0 to 2000 cm^{-1} . Figure 4.2 and Figure 4.3 are the zoomed-in version of the low (0 to 400 cm^{-1}) and high (1200 to 1700 cm^{-1}) wavenumber region of the previous contour map, Figure 4.1. These two regions were selected due to presence of high intensity peaks.

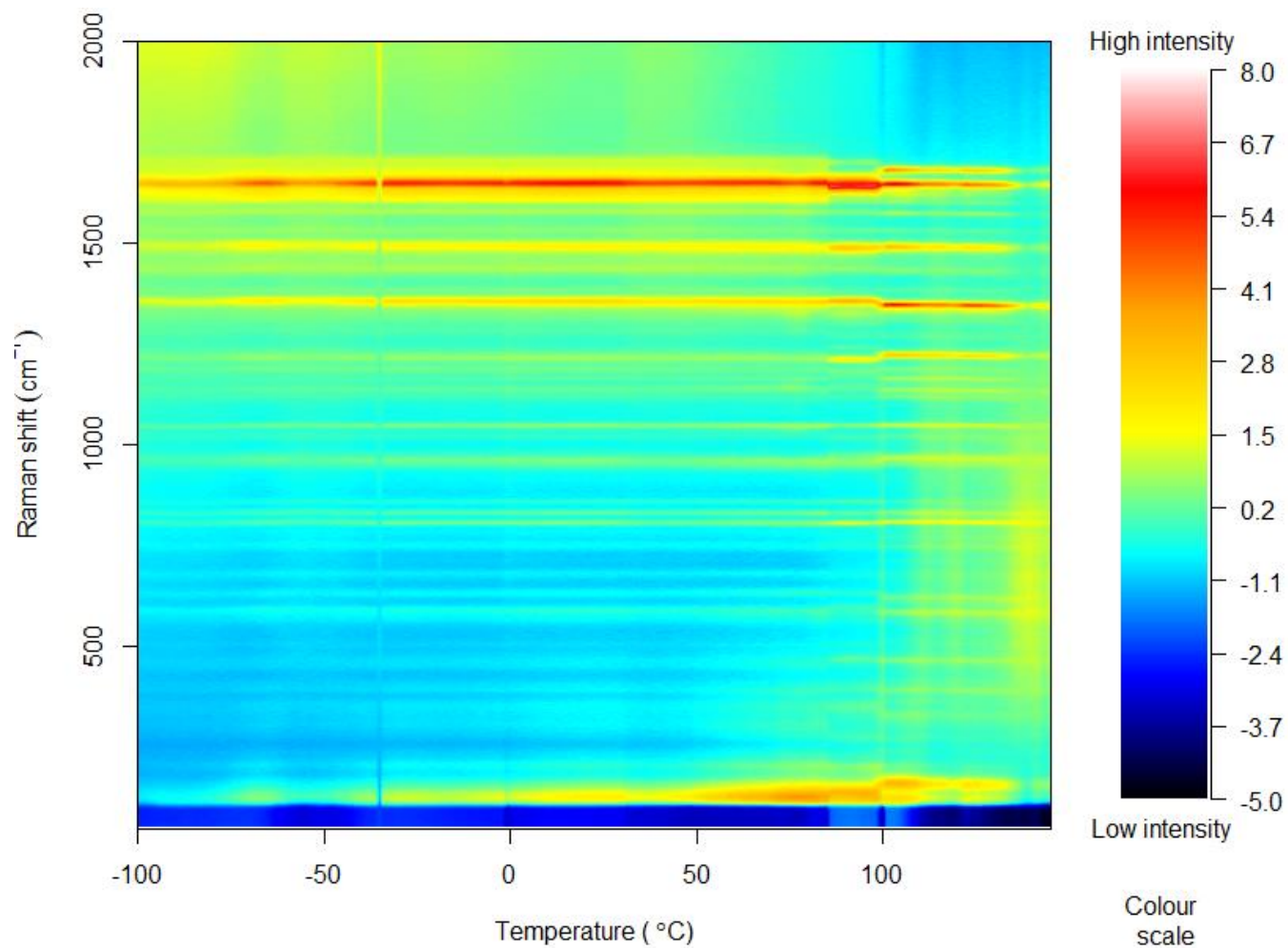


Figure 4.1. Nifedipine experiment 1 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm^{-1} . As noted in the text, the sample is heated to 180 $^{\circ}\text{C}$ and cooled to 30 $^{\circ}\text{C}/\text{min}$. It is thought to be amorphous at low temperatures.

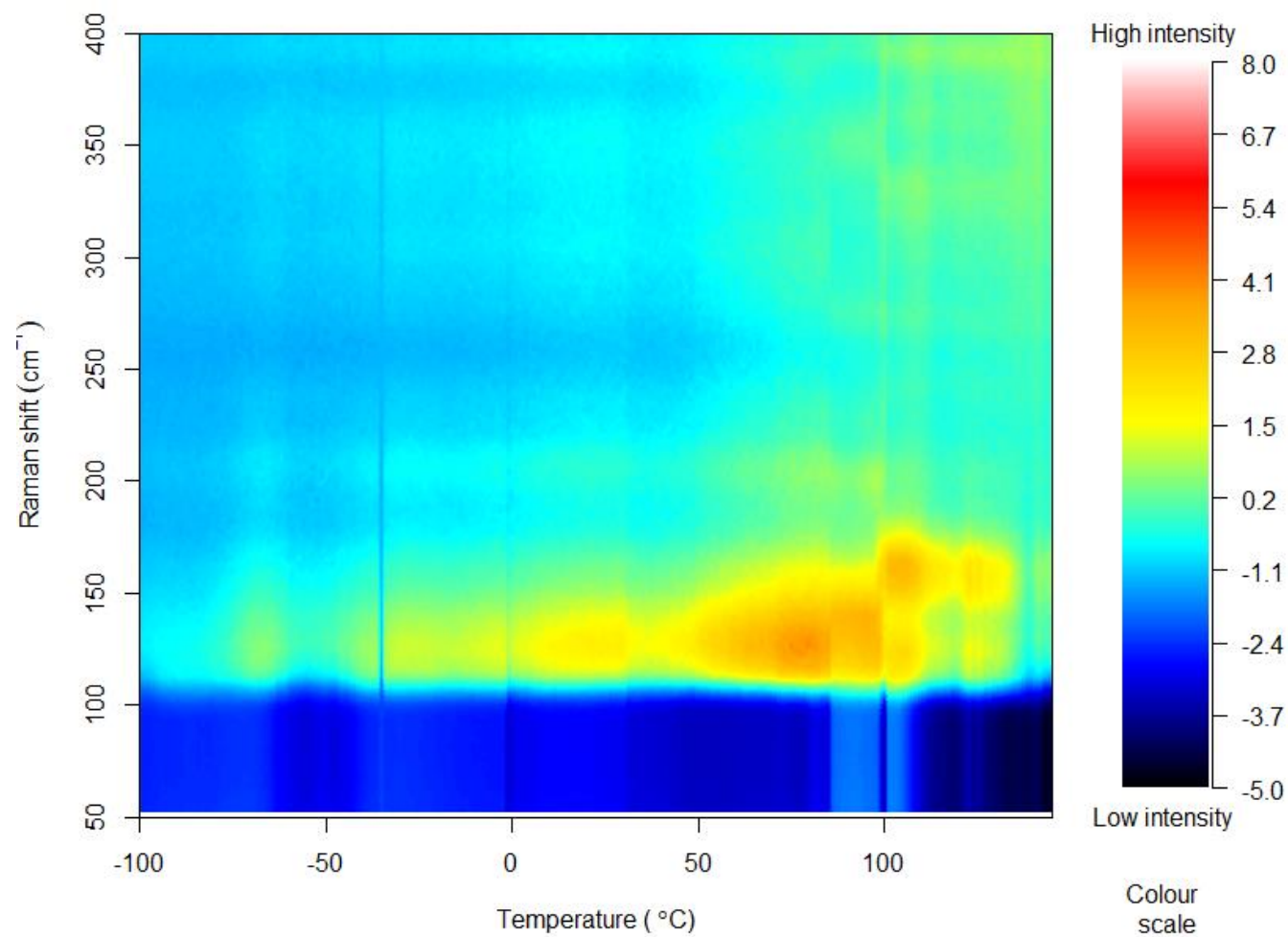


Figure 4.2. Nifedipine experiment 1 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

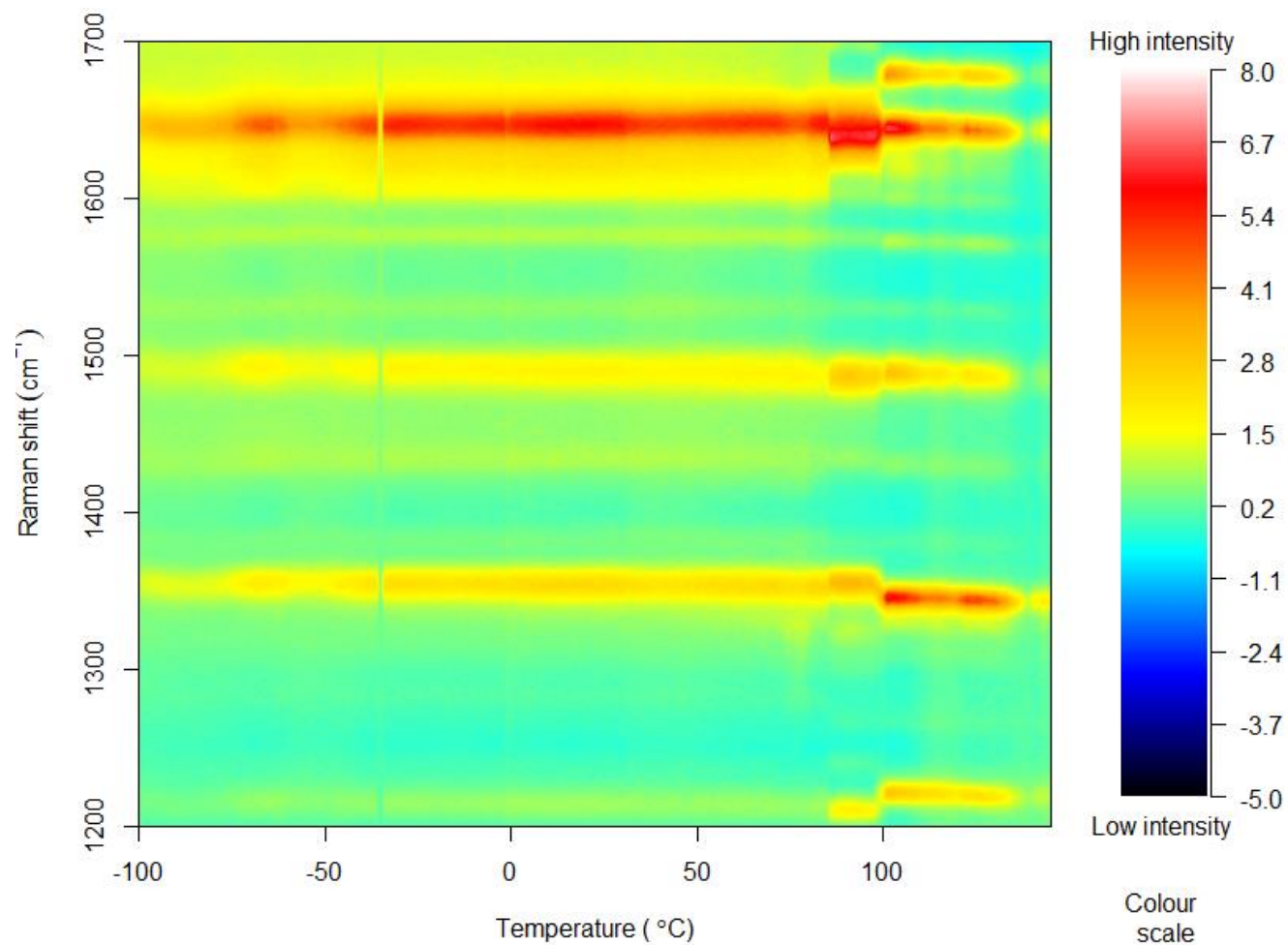


Figure 4.3. Nifedipine experiment 1 - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

In experiment 1 (Figure 4.1), the peak intensity is high at both low and high wavenumber regions. It is high at low wavenumber peaks at around 200 cm^{-1} , which are orange and yellow and at high wavenumber peaks at around 1650 cm^{-1} , which are red, orange and yellow. For clarity these regions are presented zoomed-in in the next figures, Figure 4.2. and Figure 4.3.

Figure 4.2 is the zoomed in version of the low wavenumber region of the previous contour map. The yellow peak intensity region at 200 cm^{-1} starts at around -70°C , which is not too far from the reported beta relaxation temperature of nifedipine (-66.15°C). In this region there is also a noticeable change to the background at -50°C . Slight drop in intensity is observed at 30 to 50°C , which is near the alpha transition temperature (42.25°C). The high peak intensity region starts at around 50°C . At around 90°C there is a clear abrupt change to the position and intensity of several peaks. This is apparent in low wavenumber where there is discontinuity at around 200 cm^{-1} which shifts to a slightly higher number. At around 100°C , the peak shifts up and splits.

Figure 4.3 is the zoomed in version of the high wavenumber regions of Figure 4.1. There are vertical bands that can be observed at around 1350 cm^{-1} , 1500 cm^{-1} and 1600 cm^{-1} . The broadest vertical is band at 1600 cm^{-1} . It is essentially broad up to 90°C . High intensity region, mostly in orange and yellow, is observed at around -70°C , near the beta transition temperature of nifedipine (-66.15°C). There is discontinuity at around -60°C and shifts to higher intensity again at around -50°C . Other than a slight drop in intensity observed at around 40°C , which is near around alpha transition temperature (42.25°C), there is no obvious visual change to the spectra from -50°C up to approximately 90°C , where the band becomes narrow. There are two split peaks at around 100°C . The intensity of these peaks drops very rapidly after 140°C and the peaks seem to disappear. Two more repeat experiments were carried out to test the reproducibility of the data. Figure 4.4 to Figure 4.9 are the key figures.

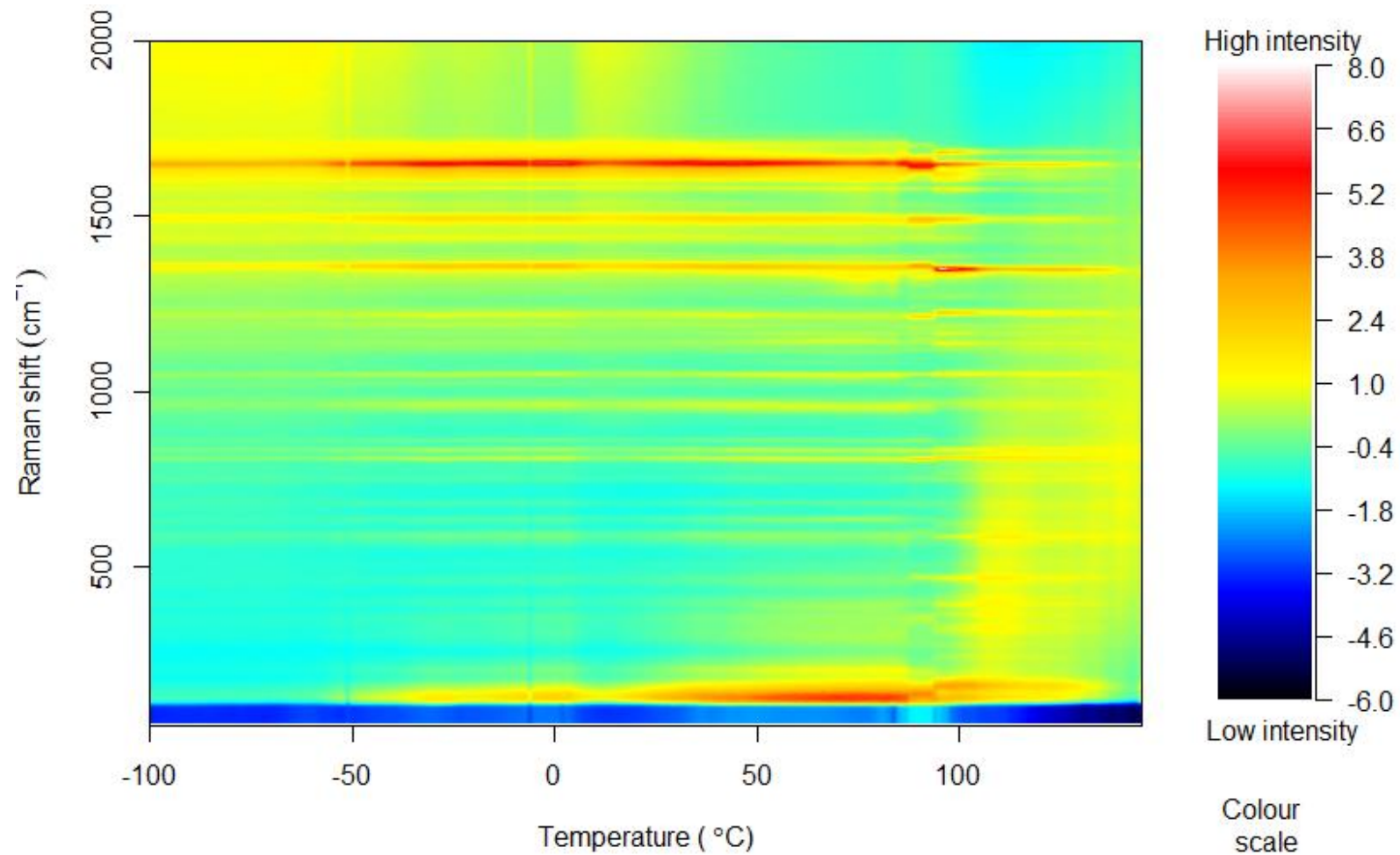


Figure 4.4. Nifedipine experiment 2 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

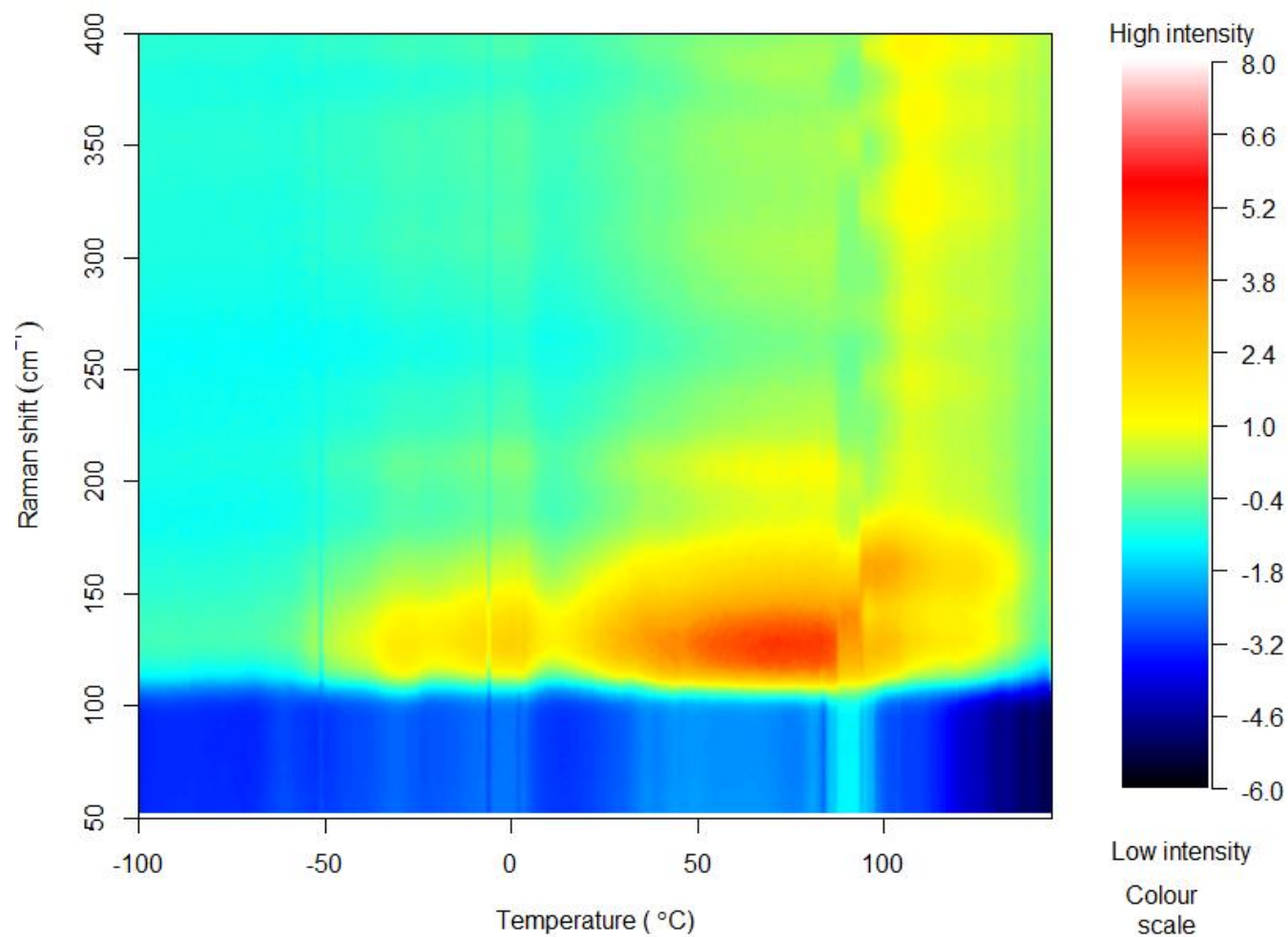


Figure 4.5. Nifedipine experiment 2 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

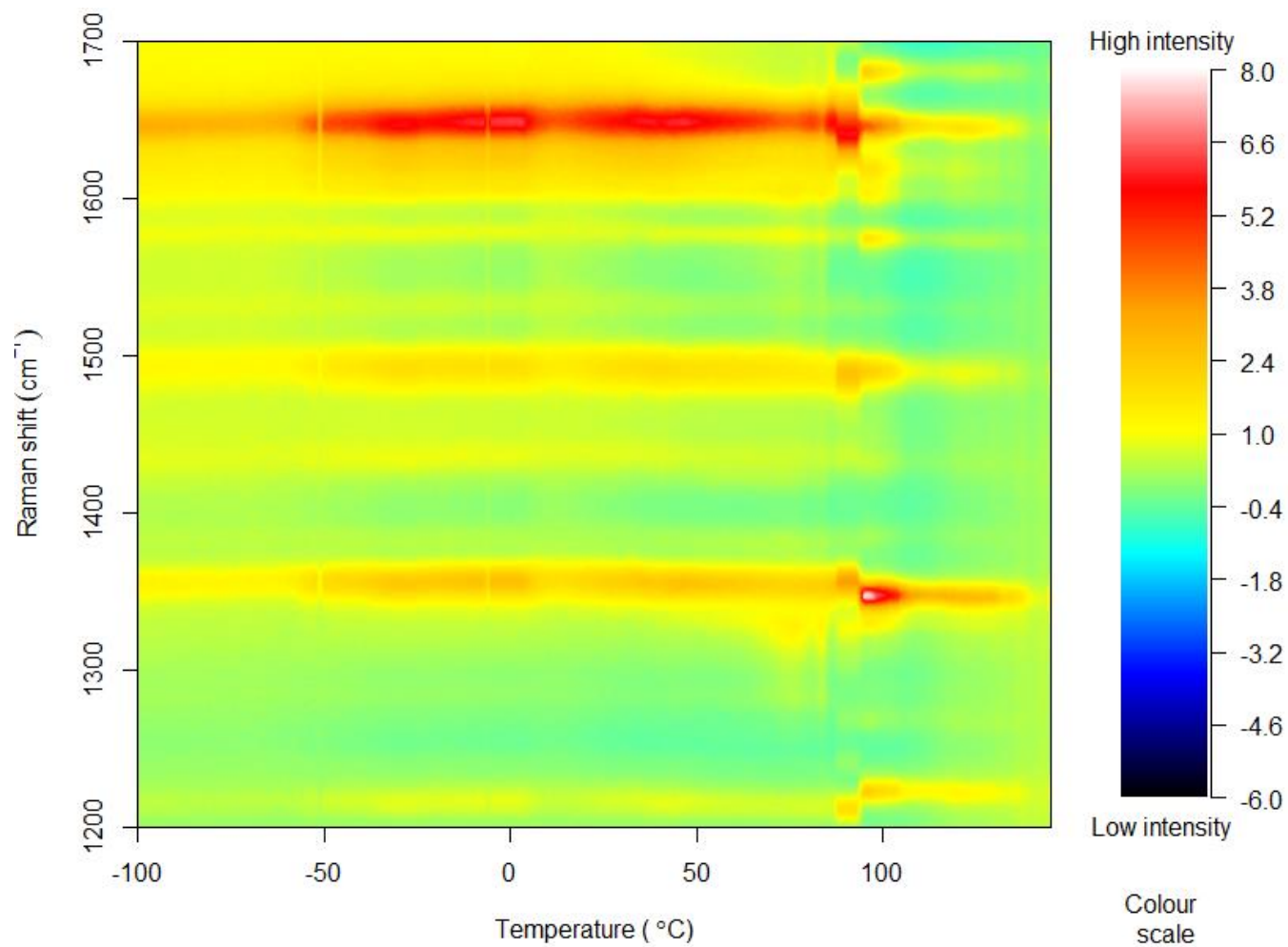


Figure 4.6. Nifedipine experiment 2 - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

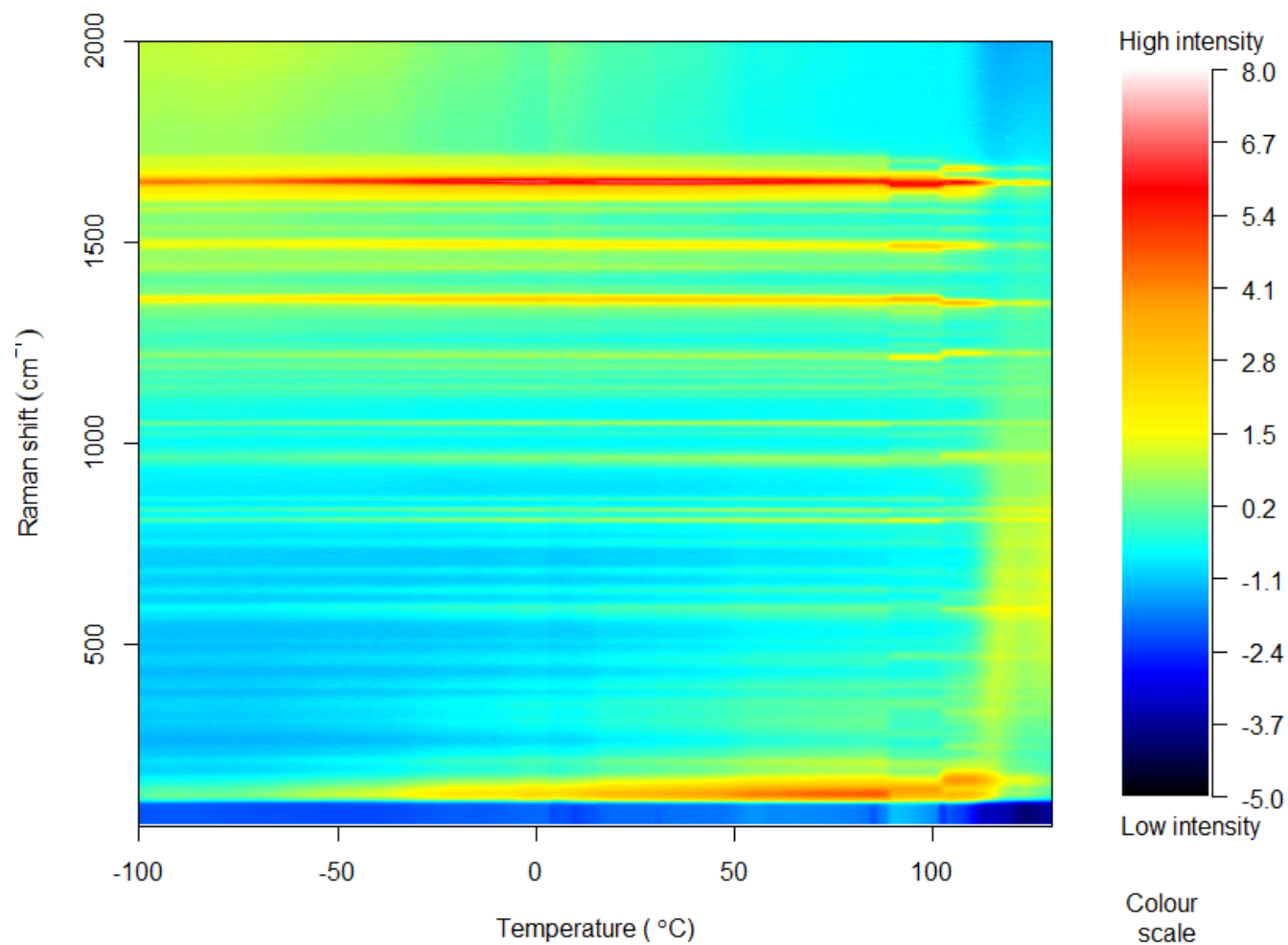


Figure 4.7. Nifedipine experiment 3 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

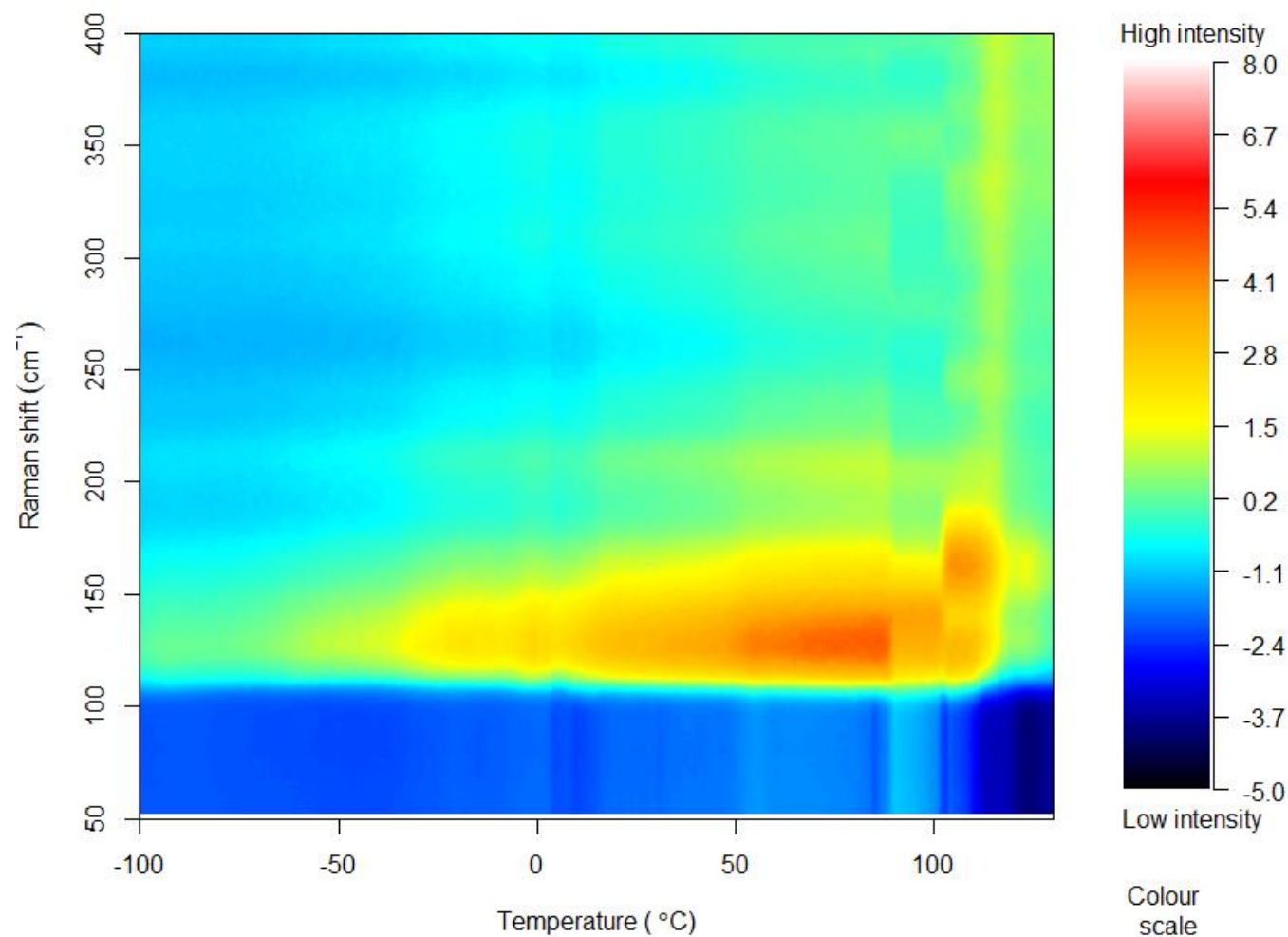


Figure 4.8. Nifedipine experiment 3 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm^{-1} . As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

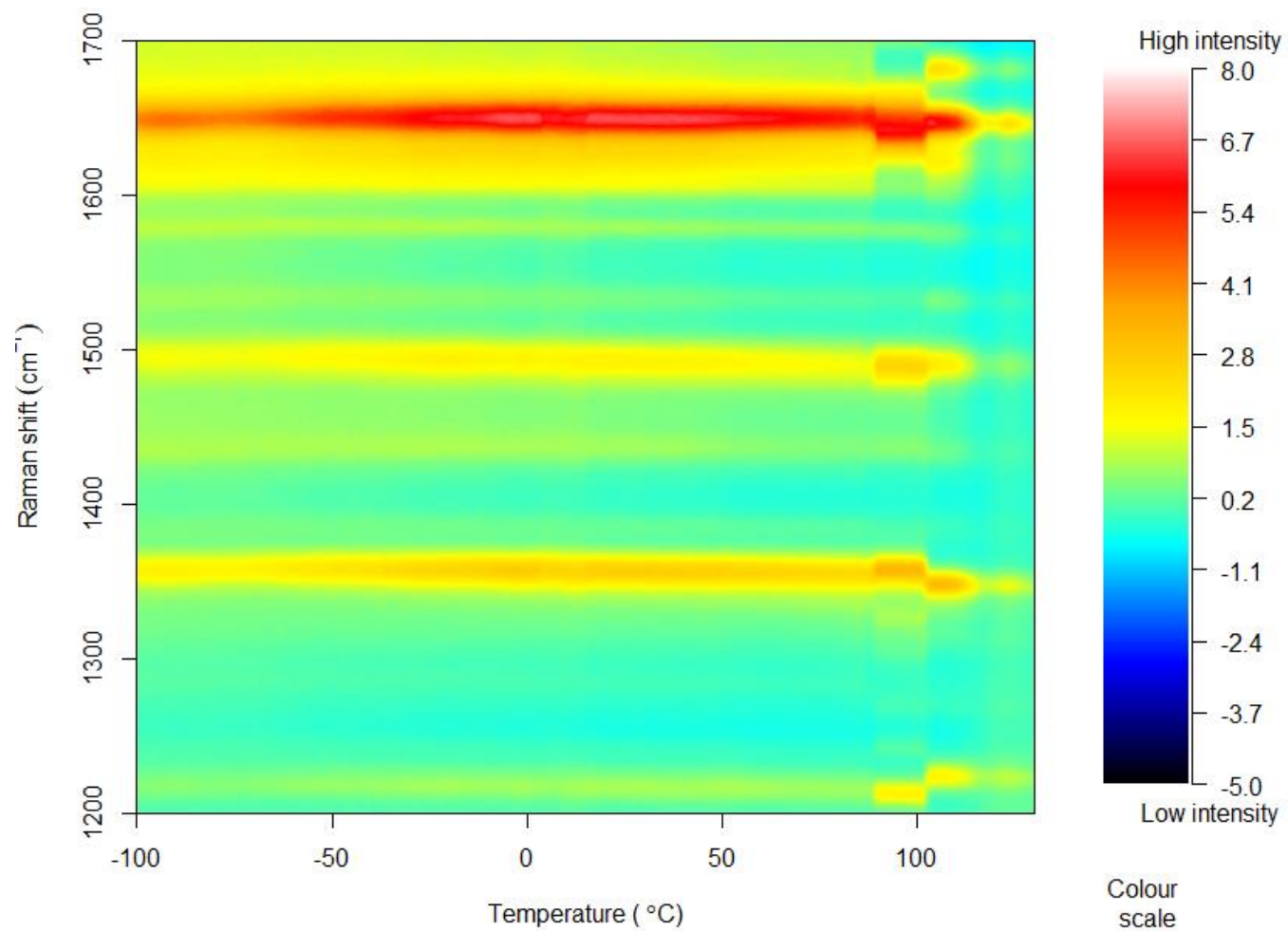


Figure 4.9. Nifedipine experiment 3 - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

Zoomed-in figures of peak intensity region at 200 cm^{-1} , Figure 4.5 and Figure 4.8, show high (yellow) peak intensity region that starts at around -50°C . The higher (orange/red) peak intensity region starts at around 30 to 50°C , which is near alpha transition temperature (42.25°C) of nifedipine. This contrasts with the zoomed in figures of peak intensity at higher wavenumbers (Figure 4.6 and Figure 4.9), which do not show any obvious change near the beta (-66.15°C) or alpha (42.25°C) transition temperatures. In all zoomed in figures the peak intensities are essentially broad up to 90°C and then they narrow as they move up in wavenumber. At around 100°C there is a clear abrupt change to the position and intensity of several peaks. At 100°C , the peak shifts up and splits. This corresponds to the previous study of amorphous nifedipine obtained from the supercooled melt crystallized to a metastable state (form B) at 90°C , and then converted to the thermodynamically stable form (form A) from 110°C (Aso et al, 1995). Visual inspection of the Raman bands possibly indicates phase transformation between different forms of nifedipine at various temperatures.

The visual changes occurring at around the alpha and beta transition temperature in the contour maps are not reproducible. Hence, we cannot fully conclude that for nifedipine based upon the studies described above, we see changes to Raman intensity around the alpha or beta transition temperature. In order to better characterise differences in Raman features caused by temperature changes, particularly around the reported alpha and beta transition temperatures, quantitative peak shape analysis has been taken for Raman peak characterisation as shown in the next section.

4.4.3 Gaussian peak fitting

In order to characterise differences in Raman features caused by alpha and beta relaxation in nifedipine, quantitative peak shape analysis has been taken for peak characterisation of intramolecular vibrational modes. Gaussian peak fitting was undertaken on 10 selected intramolecular vibrational modes. The Raman band positions with corresponding wavenumbers are summarised in Table 4.1. They were assigned from the Raman features determined from

a previous study on polymorphism and devitrification of nifedipine using FT-Raman, IR and Raman microscopy (Chan et al, 2004). We intend to diagnose these Raman peaks with three specific parameters – peak intensity, peak width and peak position - that were mentioned in Section 2.2.4.

Table 4.1. Wavenumbers (cm^{-1}) and suggested assignments derived from Raman spectra of glassy and crystalline nifedipine

Wavenumbers (cm^{-1})	Assignment
810	Ring CH
836	
859	
954	Dihydropyridine ring
1049	1,2 substituted ring
1224	Ester
1348	$\text{Vs}(\text{NO}_2)$
1492	$\text{C}=\text{C}$ (Aromatic)
1648	$\text{C}=\text{C}$ stretching
1680	$\text{C}=\text{O}$ stretching

4.4.3.1 Analysis of Gaussian fit curves of peak intensity

We now review Raman data captured across all temperatures. Figure 4.10 shows Gaussian fit curves of peak intensities of nifedipine plotted as a function of temperature at around 10 selected vibrational modes (Table 4.1). The spectral peaks were fitted in to study different Raman features caused by alpha (42.25°C), beta (-66.15°C).

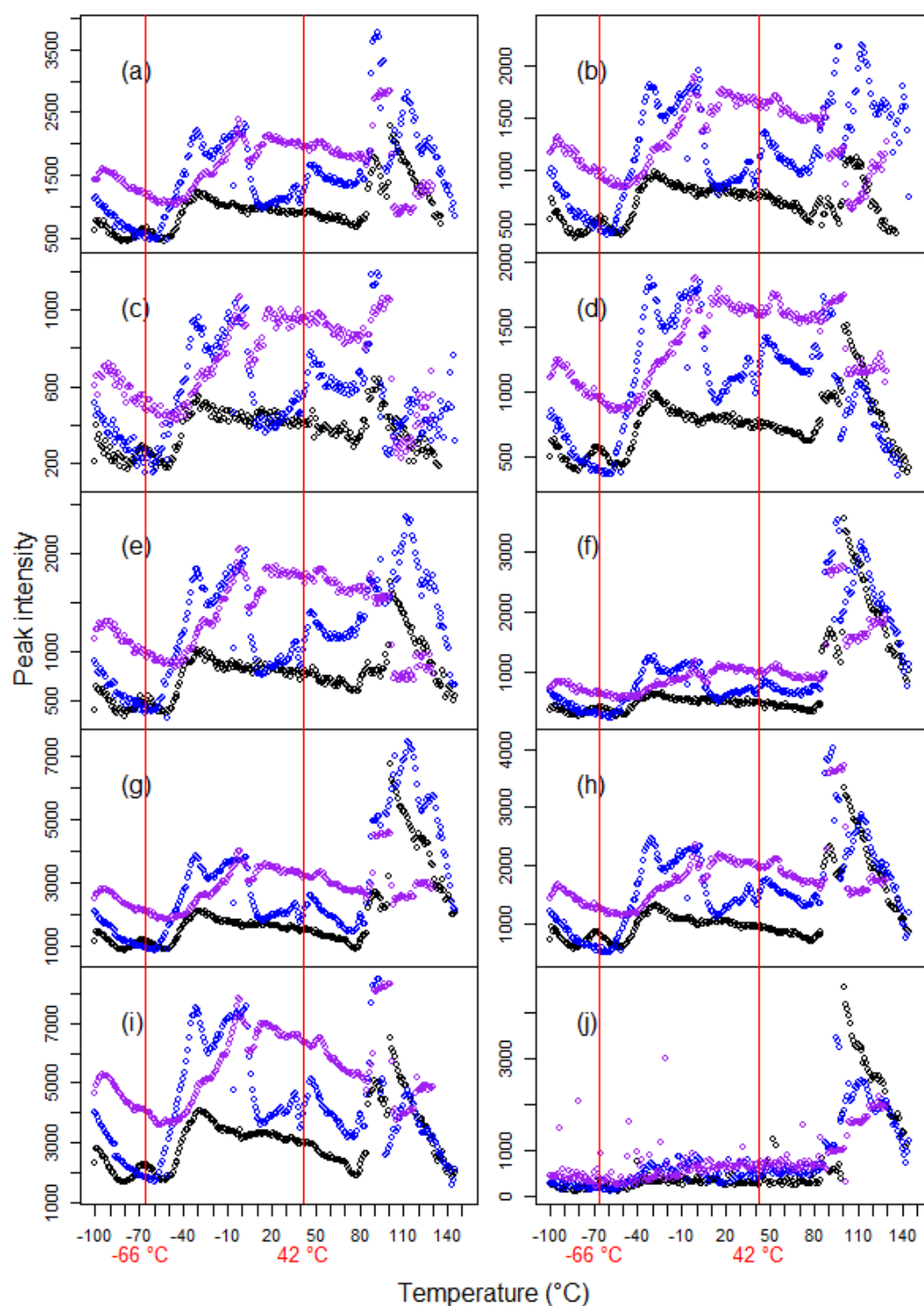


Figure 4.10. The peak fitting of intensity against temperature for the 10 selected peaks of nifedipine: 810 cm^{-1} , 836 cm^{-1} , 859 cm^{-1} , 954 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1348 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (42°C) and beta (-66°C) transition temperatures.

In experiment 1 (Figure 4.10, black dots), there are obvious changes to peak intensities in most peaks around the beta transition temperature (-66.15°C), but not around the alpha transition temperature (42.25°C). The change around peak intensity is not as obvious in peaks at around 1224 cm^{-1} that corresponds to ester and 1680 cm^{-1} that correspond to C=O stretching (Figure 4.10 (f) and Figure 4.10 (j)). In experiment 2 (Figure 4.10, blue dots), obvious changes in peak intensity are observed at both alpha and beta transition temperatures. The peak intensity drops at around the beta transition temperature. Again, the change around the peak intensity is not obvious in peaks at around 1224 cm^{-1} that corresponds to ester and 1680 cm^{-1} that corresponds to C=O stretching. There is no obvious change to peak intensities around the beta or alpha transition temperature in experiment 3 (Figure 4.10, purple dots). In all three experiments, the major changes in peak intensity and discontinuities are observed at around 90 and 110°C , which correspond to the temperatures at which there were clear abrupt change to the position and intensity of several peaks in Figure 4.2.

In the first two experiments, experiment 1 and 2, there seemed to be obvious changes to peak intensities at around the beta transition temperature of nifedipine (-66.15°C). In order to check that the result from the peak fitting is reasonable, we plotted spectra either side of both the beta (-66.15°C) and alpha (42.25°C) transition temperatures of nifedipine, in Figure 4.11 and Figure 4.12, respectively. The spectra at 5°C below and above the temperature of interest were plotted as a function of temperature. The subplots from the experiment 1, 2 and 3 were enumerated with (a), (b), and (c), respectively.

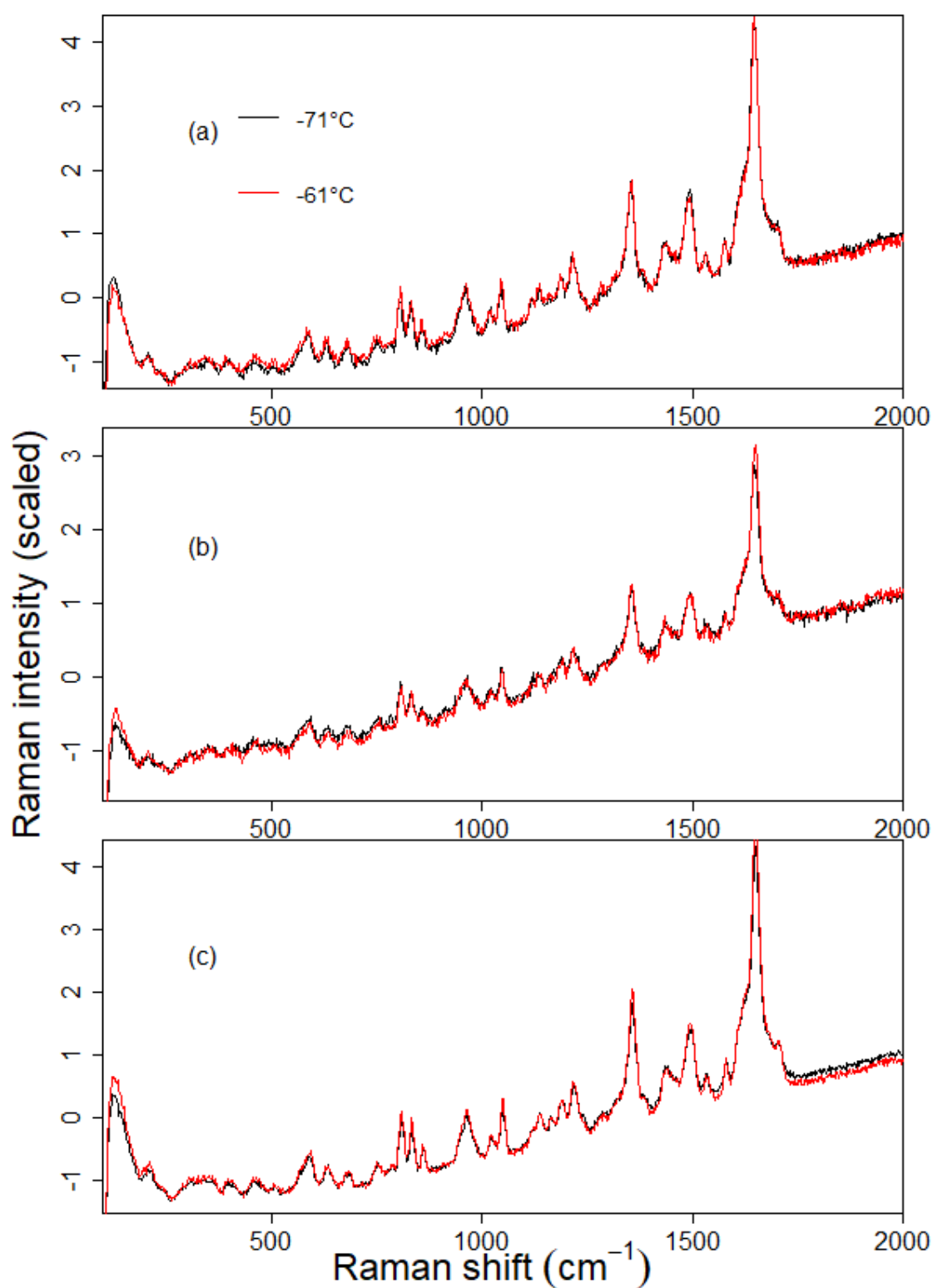


Figure 4.11. The Raman spectra of nifedipine 5°C below and above beta transition temperature (-66.15°C): -61°C (black line) and -71°C (red line), (a) experiment 1 (b) experiment 2 (c) experiment 3

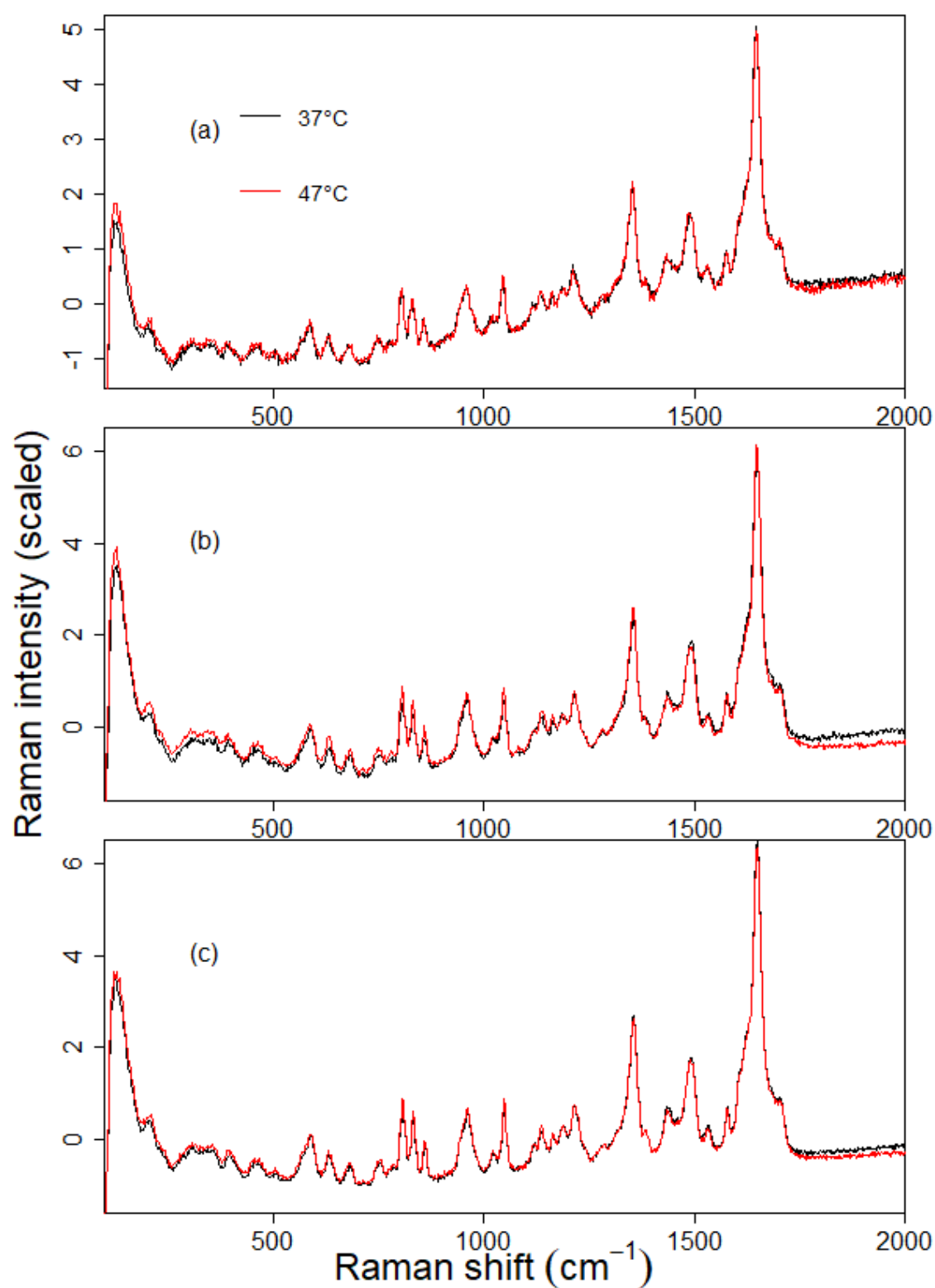


Figure 4.12. The Raman spectra of nifedipine 5°C below and above alpha transition temperature (42.25°C): 37°C (black line) and 47°C (red line), (a) experiment 1 (b) experiment 2 (c) experiment 3

Experiment 1 and 3 (Figure 4.11 (a) and Figure 4.11 (c)) show some changes before and after beta transition temperature at -66.15°C. Experiment 2 (Figure 4.11 (b)), seems to have the most obvious changes below and above beta transition temperature. On the other hand, in Figure 4.12, all the experiments show no significant changes 5°C below and above alpha transition temperature. For nifedipine based upon these studies, we cannot fully conclude that Raman intensity as a function of temperature could lead to detectable change in Raman scattering of functional groups that are associated with alpha or beta relaxation of nifedipine.

4.4.3.2 Analysis of Gaussian fit curves of peak width

Figure 4.13 shows Gaussian fit curves of peak widths plotted as a function of temperature at around 10 selected vibrational modes (Table 4.1). We will proceed to review changes in peak width of nifedipine sample captured across all temperatures to see change in disorder of the system at beta (-66.15°C) and alpha (42.25°C) transition temperatures of nifedipine.

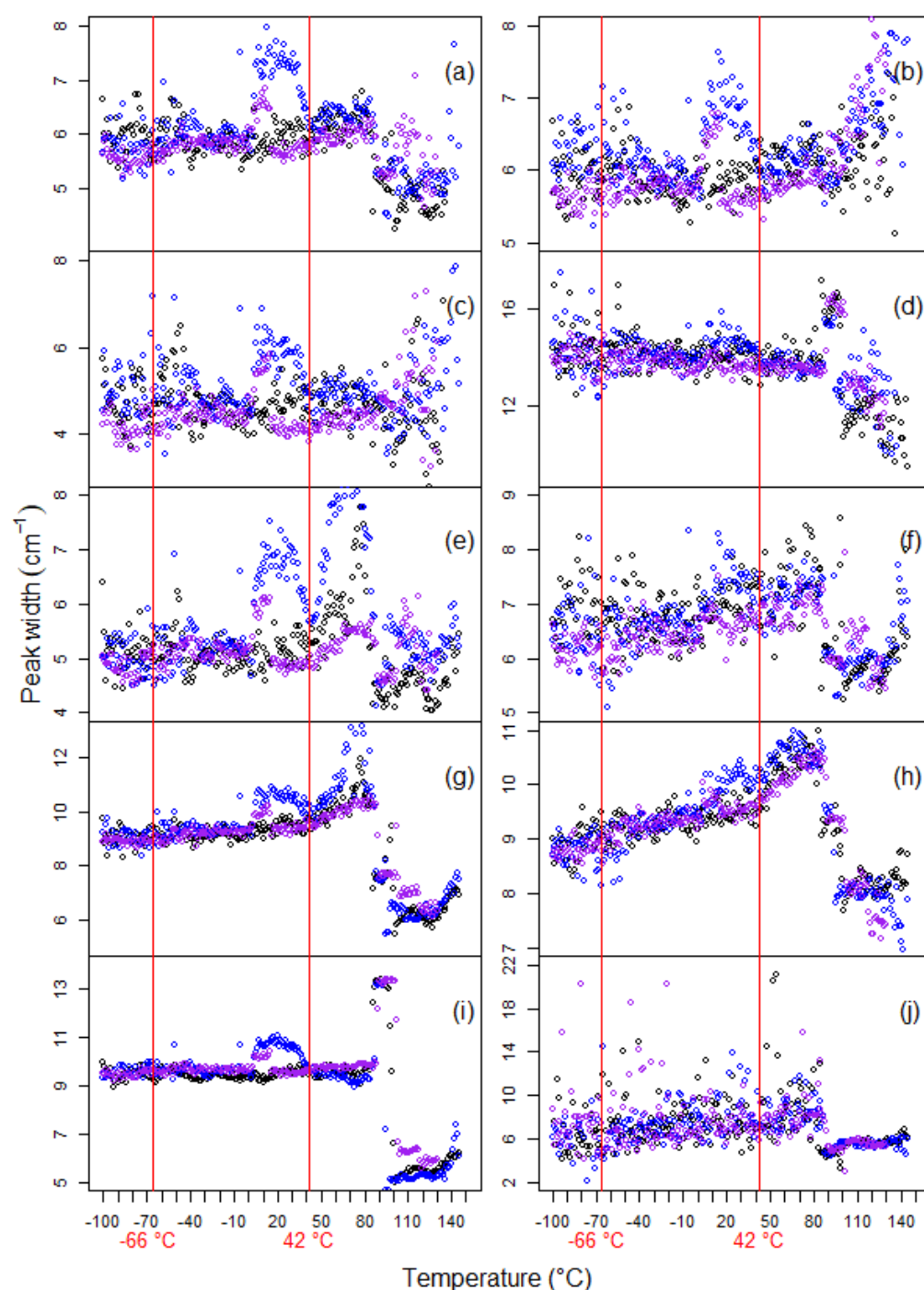


Figure 4.13. The peak fitting of width against temperature for the 10 selected peaks of nifedipine: 810 cm^{-1} , 836 cm^{-1} , 859 cm^{-1} , 954 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1348 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (42°C) and beta (-66°C) transition temperatures.

Experiments 1, 2 and 3 in Figure 4.13 show no obvious change to peak width in the non-crystalline region. No obvious change is observed at around beta (-66.15°C) transition temperature. In experiment 2 (Figure 4.13, blue dots), we observe change in peak width at around alpha transition temperature (42.25°C). Most peaks have unevenly distributed broad peaks in area from 0 to 40°C and we observe drop of peak width around that temperature. The change in peak width is not as obvious in peaks at around 1492 cm⁻¹ (aromatic C=C) and 1680 cm⁻¹ (C=O stretching) that are found in Figure 4.13 (h) and Figure 4.13 (j), respectively. At 80°C, we observe changes in peak width and discontinuity in all peaks, which correspond to the reported phase transformation during crystallization of amorphous nifedipine to a metastable state (form β) at around 90°C. The changes in peak width are reasonable as we expect nifedipine to go from amorphous to liquid at around 42.25°C, and liquid to crystal transition at around 90°C. Hence, for nifedipine based upon these studies, Raman band width as a function of temperature shows no evidence of detectable changes in order/disorder of nifedipine structure that are associated with alpha or beta relaxation.

4.4.3.3 Analysis of Gaussian fit curves of peak shift

Figure 4.14 presents Gaussian fit curves of peak positions plotted as a function of temperature at around 10 selected vibrational modes (Table 4.1). We will proceed to review changes to peak position of nifedipine sample captured across all temperatures to investigate potential structural changes caused by alpha (42.25°C), beta (-66.15°C) and other phase transition temperatures.

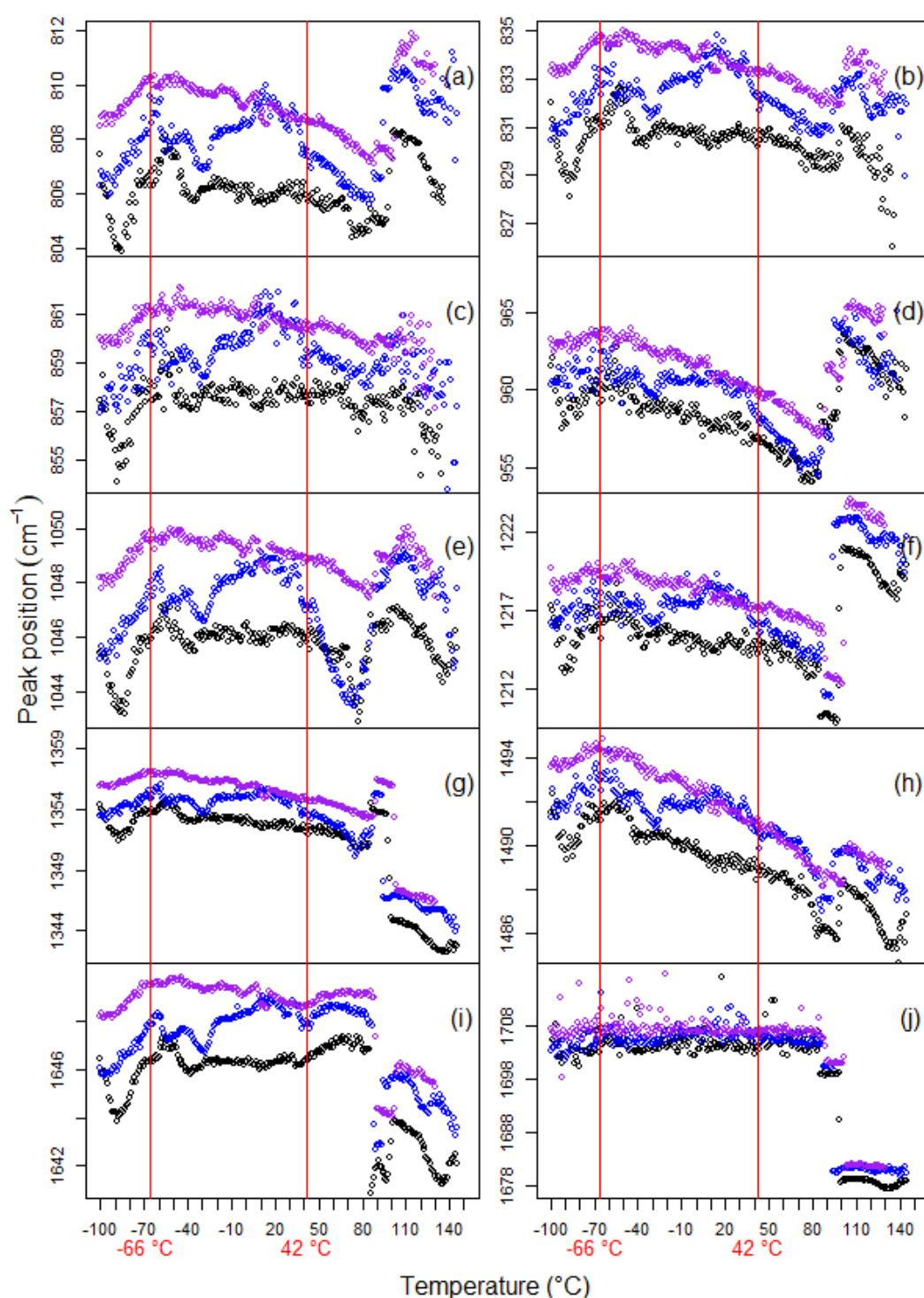


Figure 4.14. The peak fitting of position against temperature for the 10 selected peaks of nifedipine: 810 cm⁻¹, 836 cm⁻¹, 859 cm⁻¹, 954 cm⁻¹, 1049 cm⁻¹, 1224 cm⁻¹, 1348 cm⁻¹, 1492 cm⁻¹, 1648 cm⁻¹, and 1680 cm⁻¹. The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (42°C) and beta (-66°C) transition temperatures.

In experiment 1 (Figure 4.14, black dots) and 2 (Figure 4.14, blue dots), the peaks show obvious change to peak position at around -70°C , which is not too far from the beta relaxation temperature of nifedipine (-66.15°C). No obvious change to peak position is observed at around the alpha transition temperature (42.25°C). In all three experiments, the most obvious changes in peak position are observed at around 90°C , at which the peaks either show discontinuity or shift to different wavenumber. These findings on peak shifts were promising for the temperature at around the beta relaxation but not around the alpha transition temperature. We could conclude that these results support the reproducibility of the data and that they exhibit changes around the beta transition temperature, but not the alpha transition temperature.

From inspection of the Gaussian peak fit curves, for nifedipine based upon the studies described above, we could immediately conclude that only the peak intensity and peak position (and not the peak width) of the Raman spectra exhibits reproducible changes around the beta transition (-66.15°C) temperature, but not the alpha transition temperature (42.25°C). There are detectable changes in peak position when nifedipine goes through phase transition from supercooled melt crystallized to a metastable state (form B) at 90°C .

4.4.4 Low temperature repeat tests

From inspection of the contour maps and Gaussian peak fit curves from the previous section, some visual changes were observed for Raman spectra at around the beta (-66.15°C) but not around the alpha transition temperature (42.25°C) of nifedipine. These results were not obviously reproducible in terms of the beta transition temperature. There seemed to be sample to sample variation in each of the experiments which was not expected given the samples were prepared in the same way, so the temperature cycling experiments of a single sample (three repeats for each sample) were repeated at low temperature (-100 to -30°C) to test the reproducibility of the data around the beta transition temperature. Three sets of experiments were repeated, and

each experiment consisted of three heat - cool - heat cycling experiments at low temperature.

4.4.4.1 Heat map – low temperature repeat tests

Figure 4.15 to Figure 4.23 are the key figures of the contour maps of the normalised Raman spectra collected for a quench-cooled nifedipine in low temperature repeat experiments. The figures were plotted as a function of temperature similar to Section 4.4.2, but during heating of the sample from -100 to -30°C. The scaled intensities are plotted on the colour scale, similar to those in Section 3.4.2. Figure 4.16 and Figure 4.17 are the zoomed-in version of the low (0 to 400 cm^{-1}) and high (1200 to 1700 cm^{-1}) wavenumber region of the previous contour map, Figure 4.15. These two regions were selected due to presence of high intensity peaks.

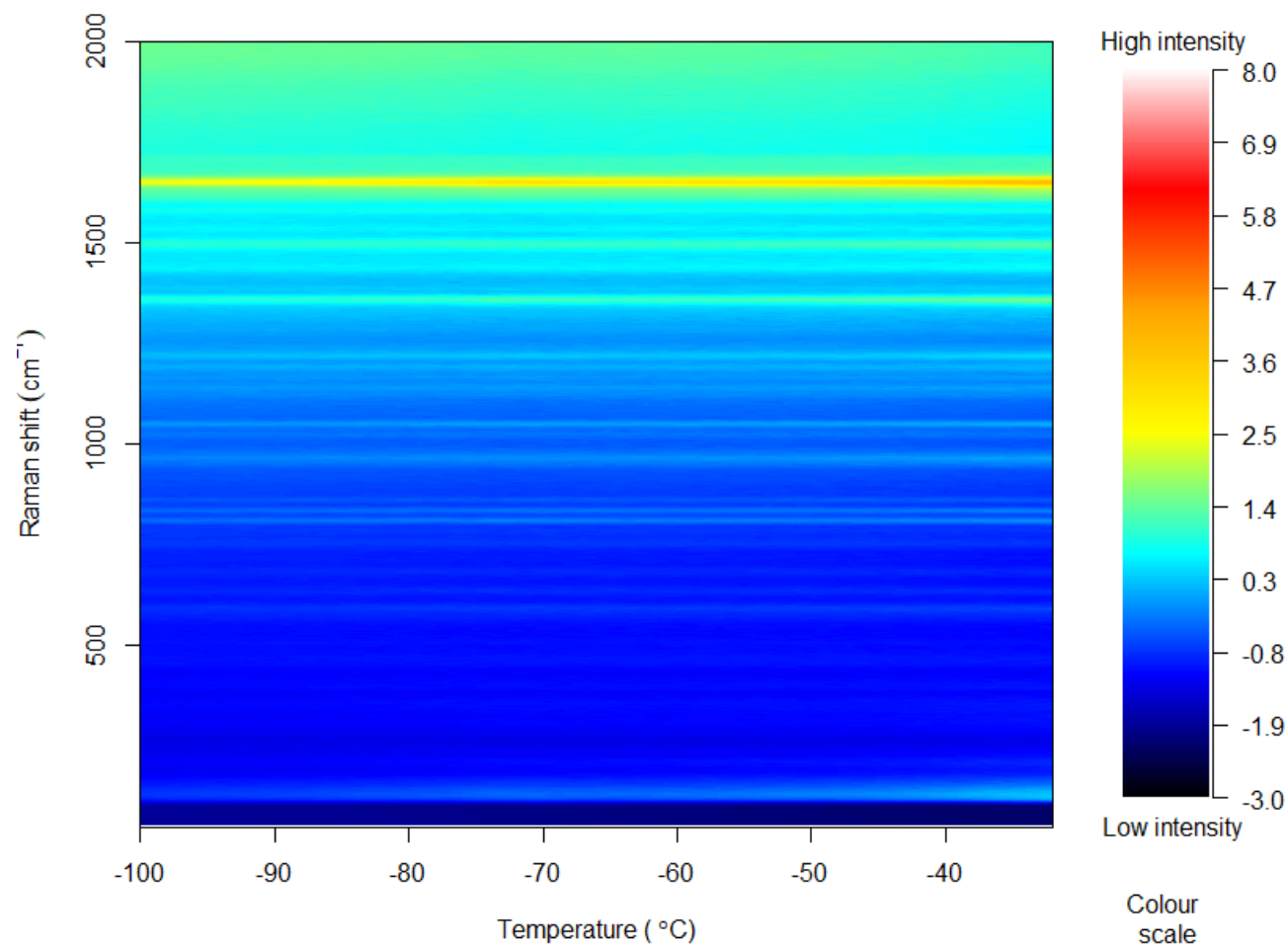


Figure 4.15. Low temperature nifedipine repeat experiment 1 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

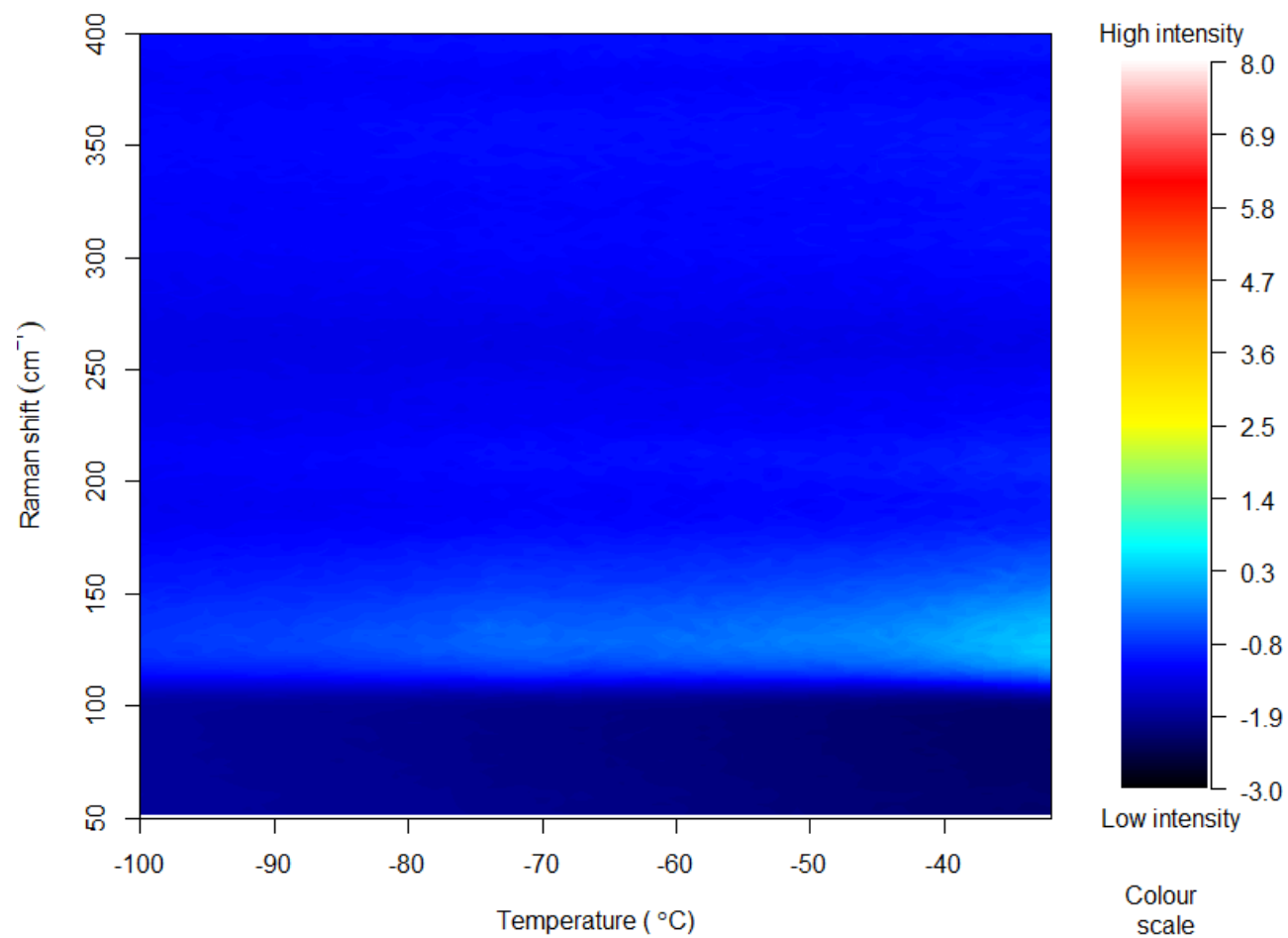


Figure 4.16. Low temperature nifedipine repeat experiment 1 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

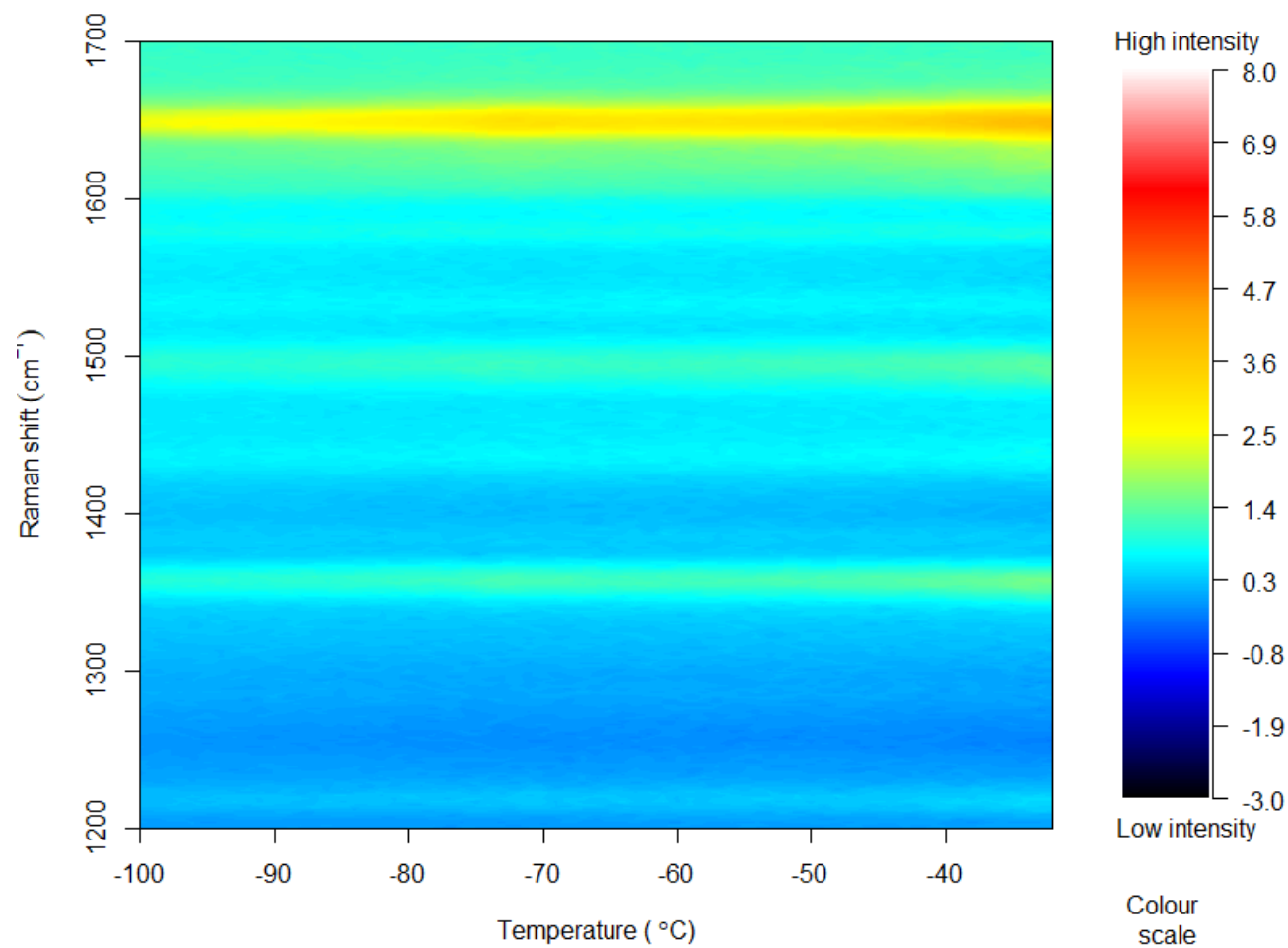


Figure 4.17. Low temperature nifedipine repeat experiment 1 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

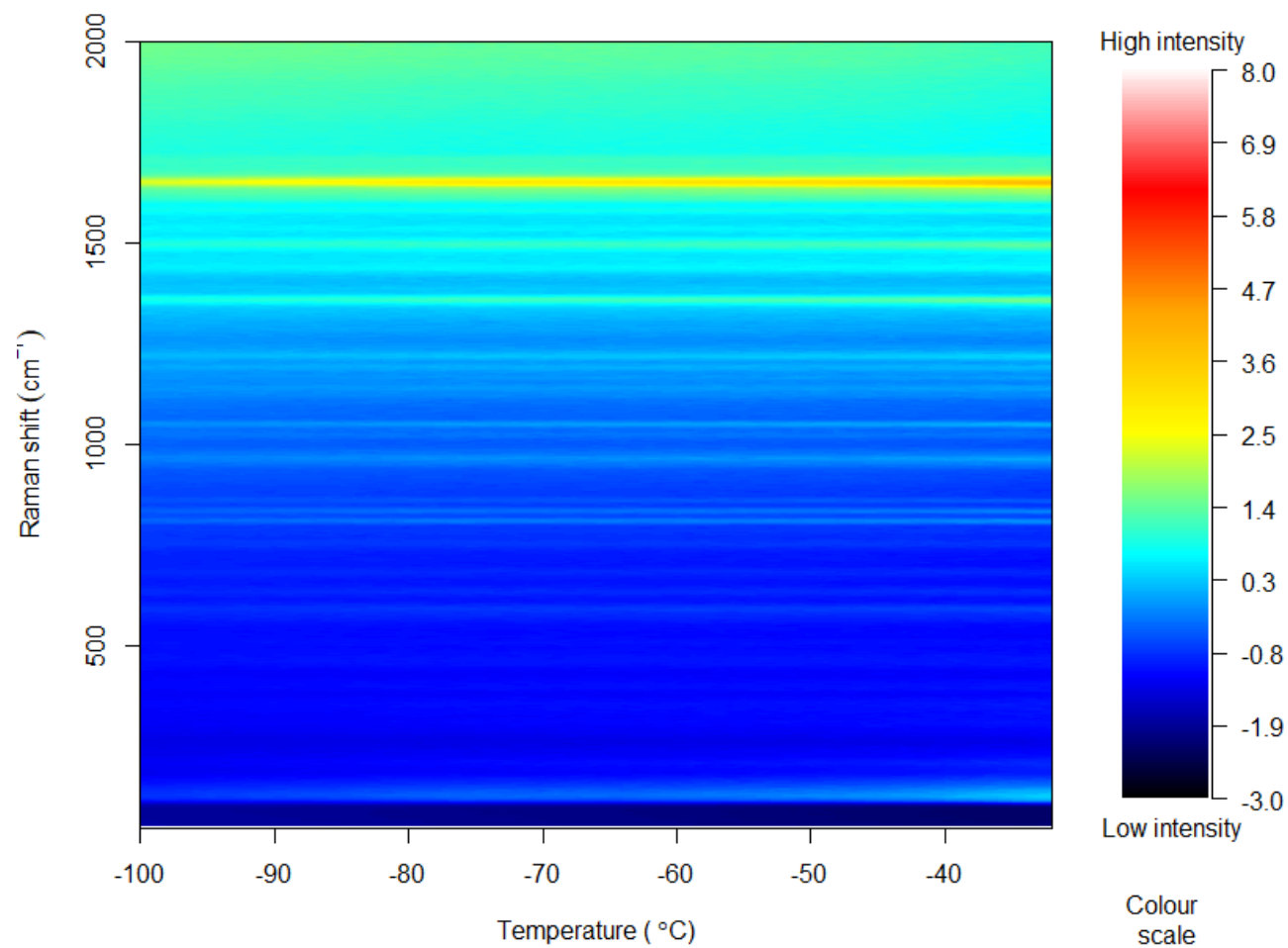


Figure 4.18. Low temperature nifedipine repeat experiment 1 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ cooled from -30 to -100°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

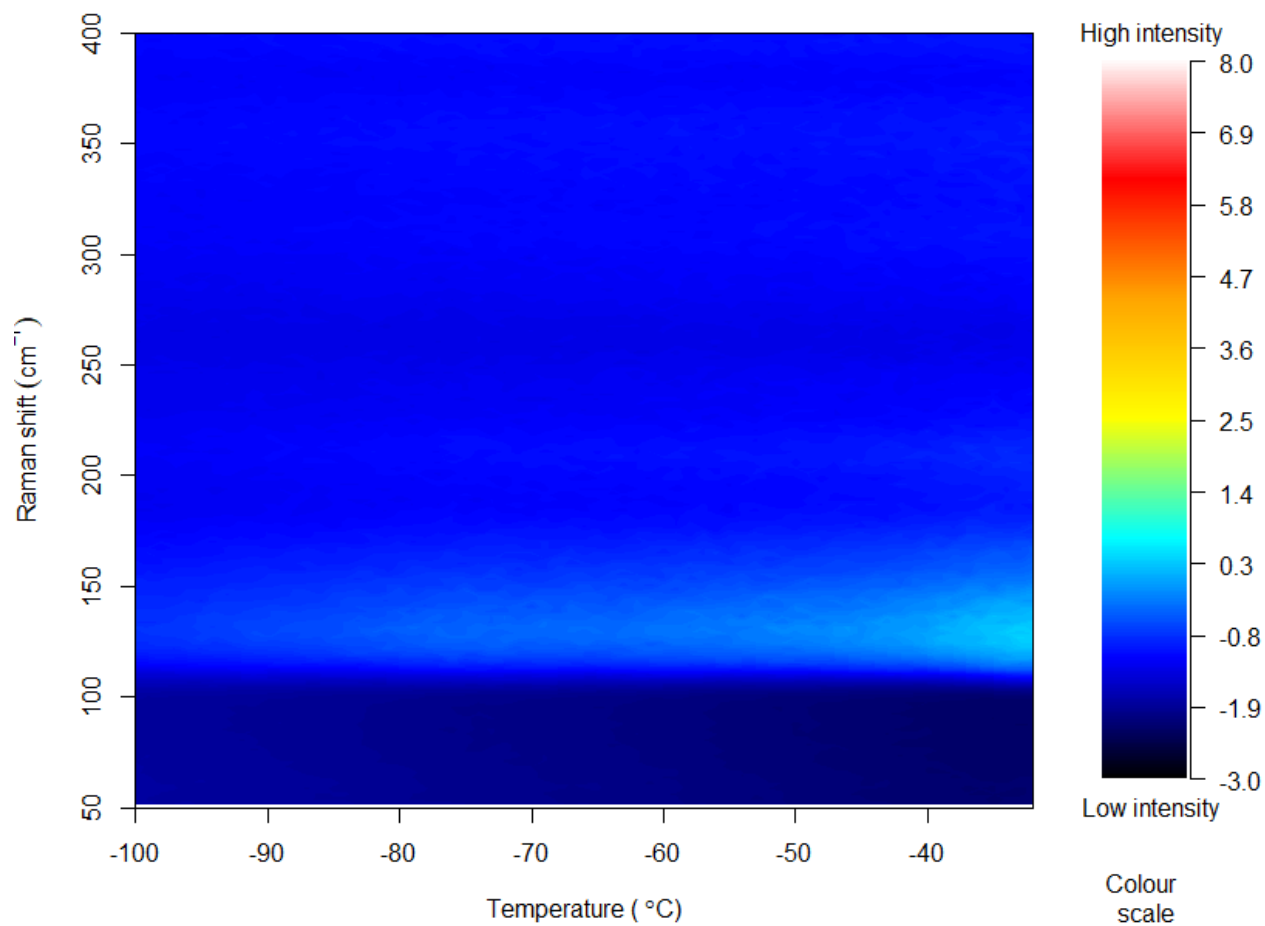


Figure 4.19. Low temperature nifedipine repeat experiment 1 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ cooled from -30 to -100°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

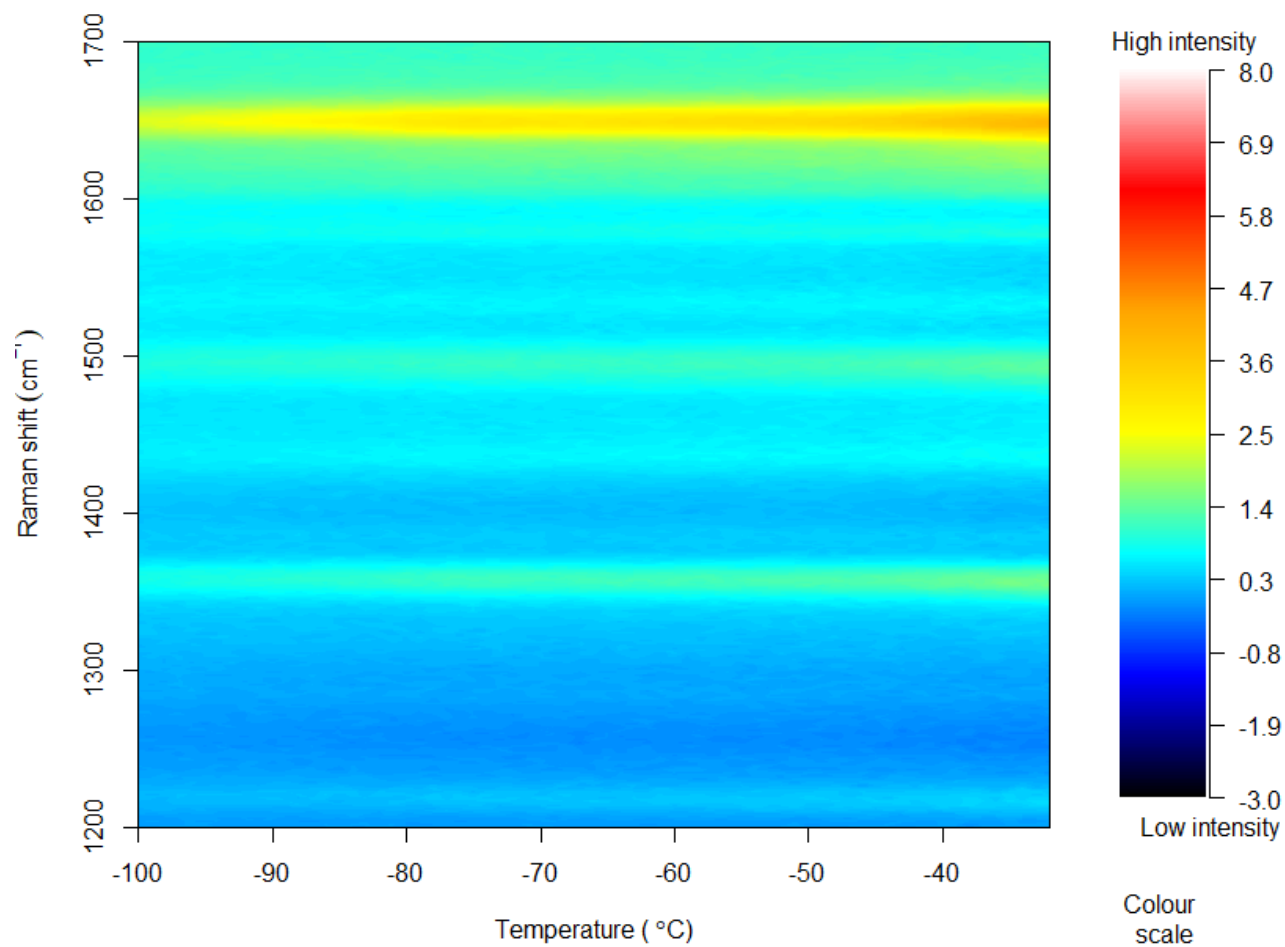


Figure 4.20. Low temperature nifedipine repeat experiment 1 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ cooled from -30 to -100°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

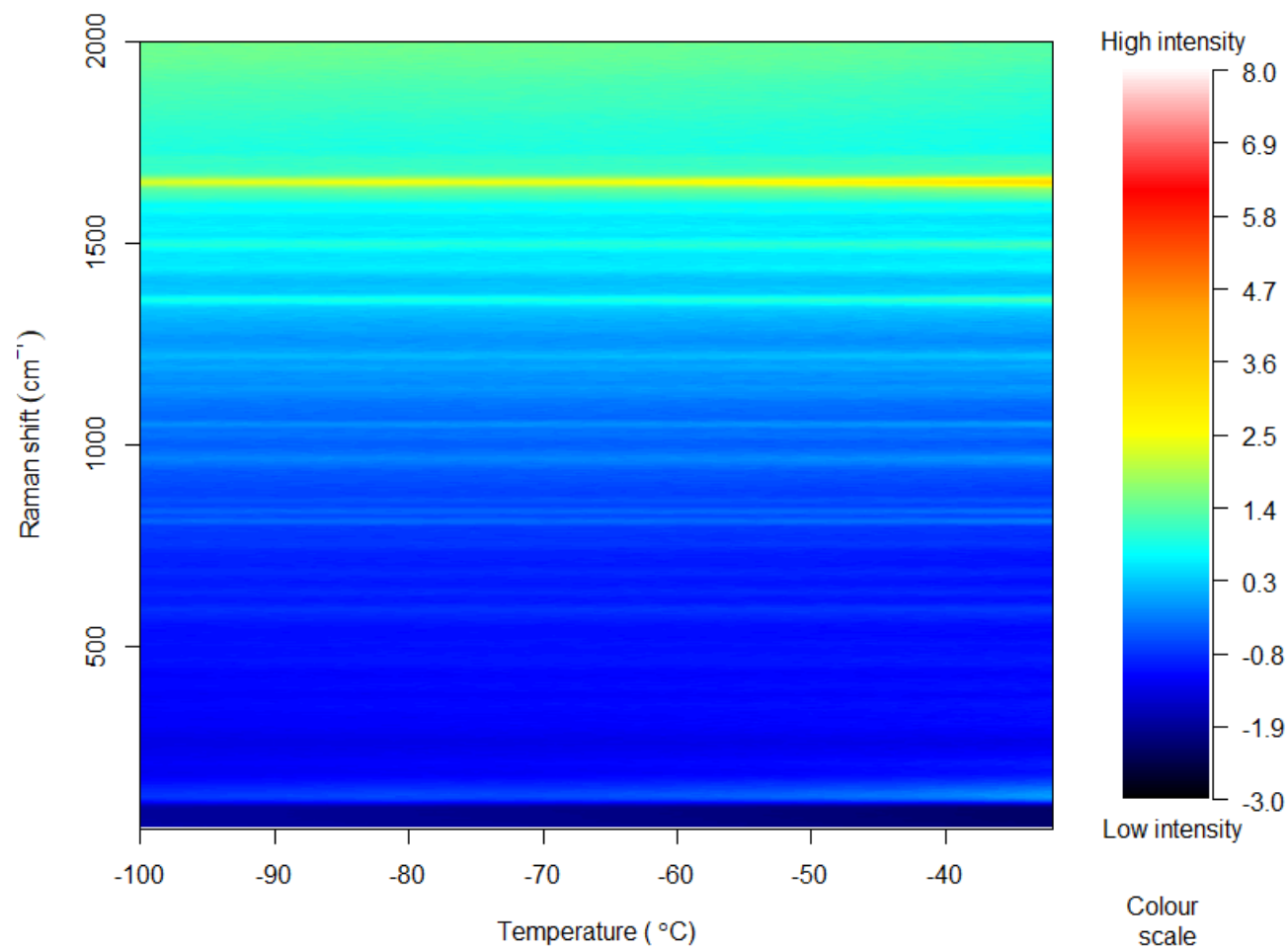


Figure 4.21. Low temperature nifedipine repeat experiment 1 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm^{-1} heated from -100 to -30 $^{\circ}\text{C}$. As noted in the text, the sample is heated to 180 $^{\circ}\text{C}$ and cooled to 30 $^{\circ}\text{C}/\text{min}$. It is thought to be amorphous at low temperatures.

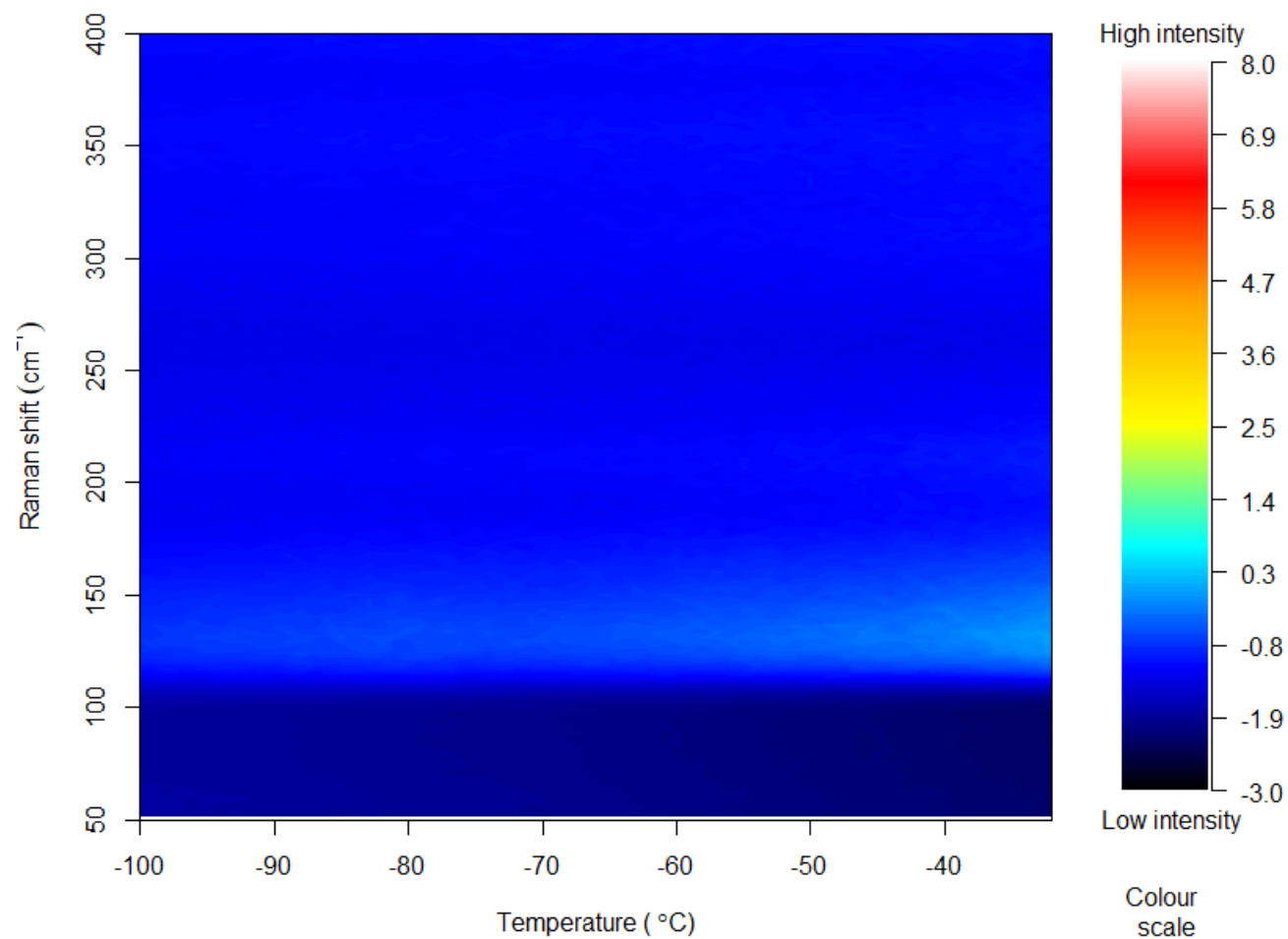


Figure 4.22. Low temperature nifedipine repeat experiment 1 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

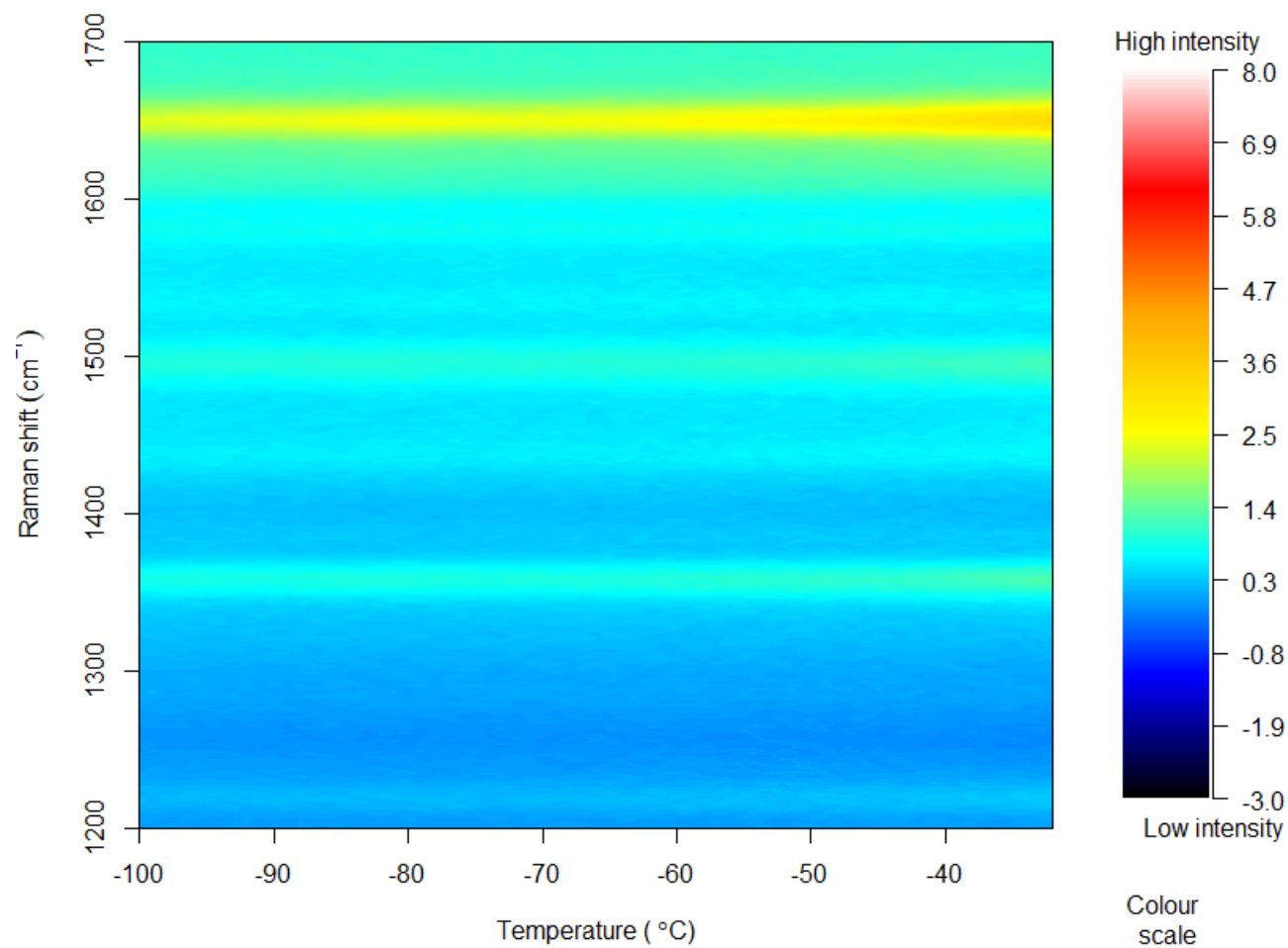


Figure 4.23. Low temperature nifedipine repeat experiment 1 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

In cycle 1 of experiment 1 (Figure 4.15, Figure 4.16 and Figure 4.17), there is no obvious change around the beta transition temperature of nifedipine (-66.15°C) observed in peak positions, therefore repeated the experiment of the same quench-cooled nifedipine sample by re-freezing and reheating, for the second (Figure 4.18, Figure 4.19 and Figure 4.20) and the third (Figure 4.21, Figure 4.22 and Figure 4.23) time. No obvious changes around the beta transition temperature were observed in the second and third repeat cycle. We repeated two more sets of the experiments but did not see obvious changes around the beta transition temperature. The plots of the experiment 2 (Figure C 1 to Figure C 9 of Appendix C) and experiment 3 (Figure C 10 to Figure C 18 of Appendix C) are found in the appendix. Hence, for nifedipine based upon these studies, we cannot fully conclude that temperature could lead to detectable changes in Raman scattering of functional groups that are associated with beta relaxation.

4.4.4.2 Analysis of Gaussian fit curves of peak intensity in low temperature repeat tests

We have seen the contour maps of the low temperature repeat experiments and we proceed to test the reproducibility of the data around the beta transition temperature of nifedipine (-66.15°C). The spectral peaks from the low temperature repeat experiments were analysed to see different Raman features caused by the beta transition temperature in the 10 selected intramolecular vibrational modes (Table 4.1). Figure 4.24, Figure 4.25 and Figure 4.26 correspond to the peak fit curves of cycle 1, 2, and 3 of the low temperature experiments, respectively. Each dot on the scatterplot represents peak intensity of selected peaks in the range of -100 to -30°C . The black, blue and purple dots represent low temperature experiment 1, 2 and 3, respectively.

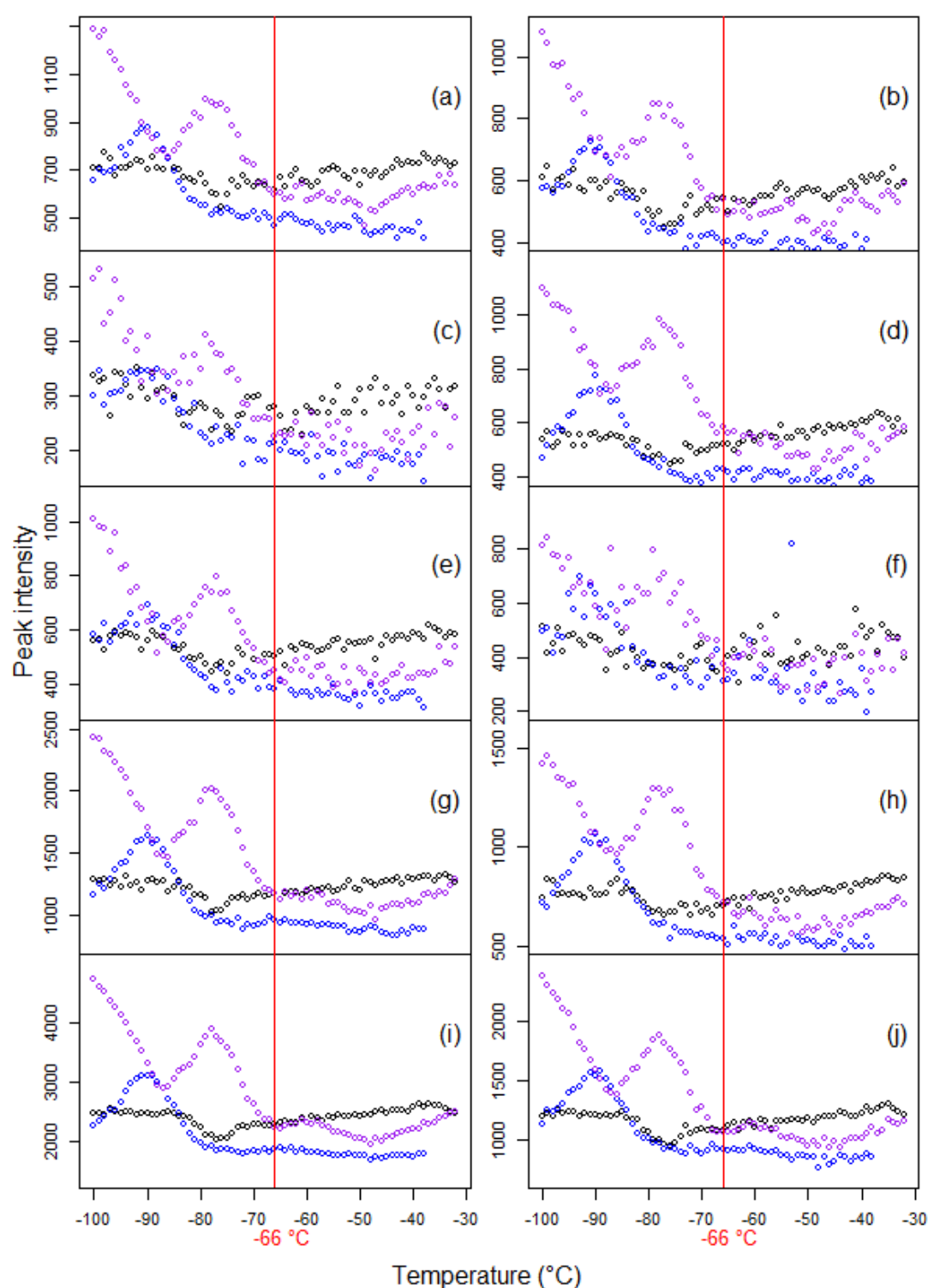


Figure 4.24. Low temperature experiment (cycle 1) - The peak fitting of intensity against temperature for the 10 selected peaks of nifedipine: 810 cm^{-1} , 836 cm^{-1} , 859 cm^{-1} , 954 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1348 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

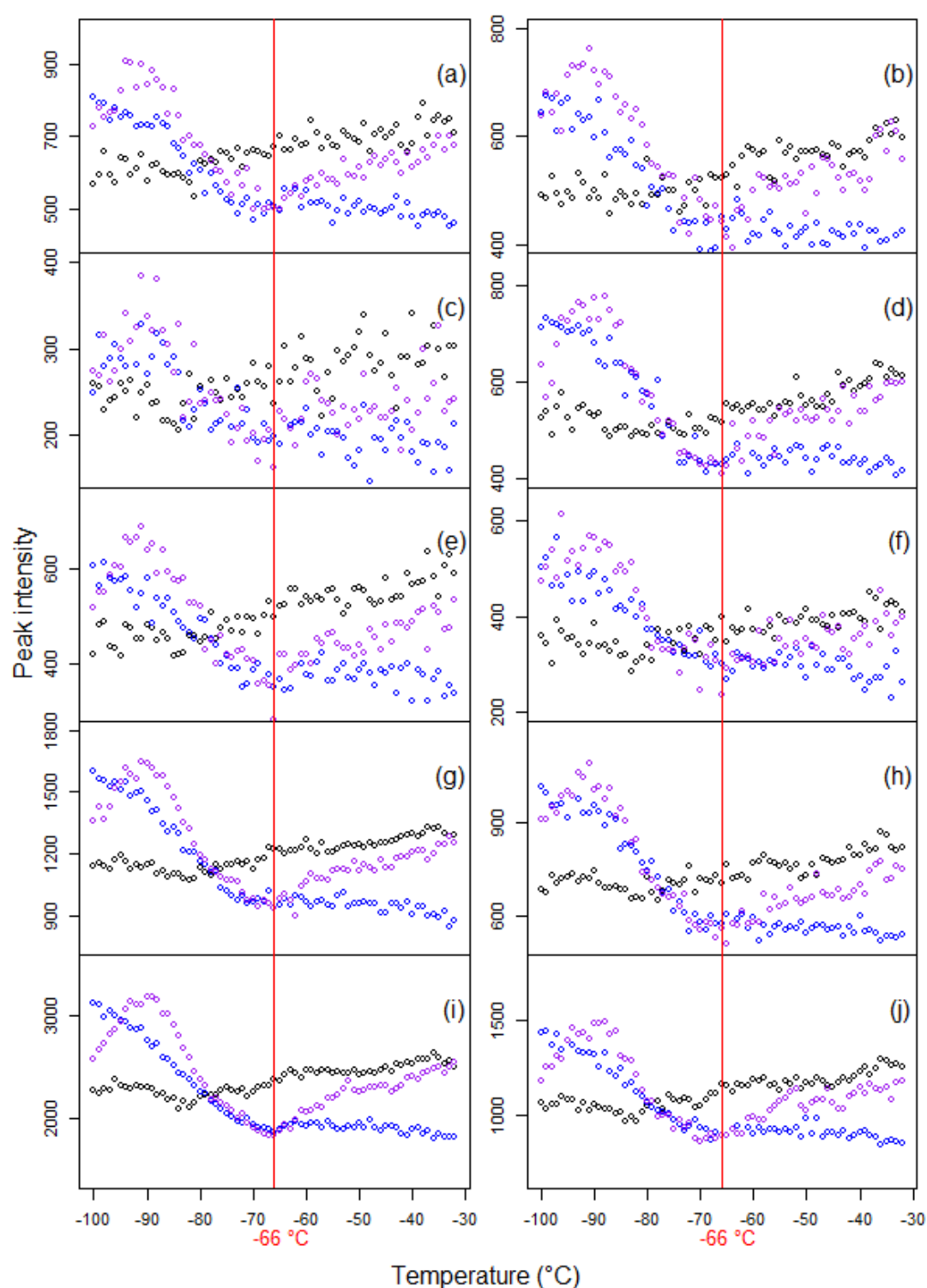


Figure 4.25. Low temperature experiment (cycle 2) - The peak fitting of intensity against temperature for the 10 selected peaks of nifedipine: 810 cm^{-1} , 836 cm^{-1} , 859 cm^{-1} , 954 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1348 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

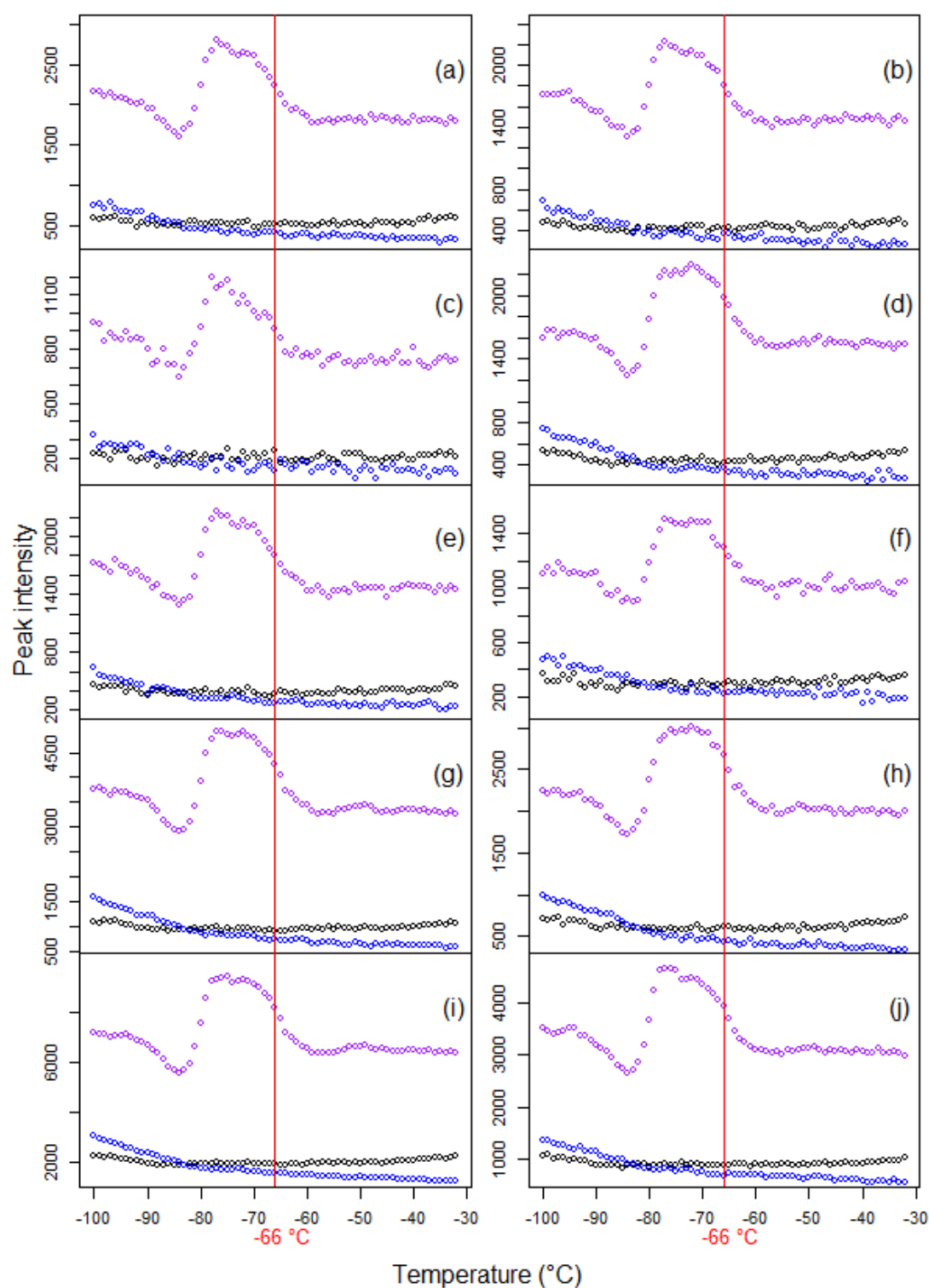


Figure 4.26. Low temperature experiment (cycle 3) - The peak fitting of intensity against temperature for the 10 selected peaks of nifedipine: 810 cm^{-1} , 836 cm^{-1} , 859 cm^{-1} , 954 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1348 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

In cycle 1 (Figure 4.24), the peak intensity shows detectable change in peak intensities at around -80 and -90°C, but we observe no obvious changes around the beta transition temperature. In cycle 2, the change to peak intensity around the beta transition temperature is obvious in experiment 2 (Figure 4.25, blue dots) and experiment 3 (Figure 4.25, purple dots). In cycle 3 of experiment 3 (Figure 4.26, purple dots), the peak intensities appear to initially increase from 0 to -80°C and then decrease after -80°C. Overall, we observe some changes in peak intensities around the beta transition temperatures in the low temperature experiments, but we question the reproducibility of these changes. Given the figures are not reproducible, we conclude that for nifedipine based upon these studies, the beta temperature does not appear to have detectable changes in Raman scattering of functional groups in the experiments described above.

4.4.4.3 Analysis of Gaussian fit curves of peak width in low temperature repeat tests

The Gaussian fit curves of peak width were plotted to investigate different Raman features caused by the beta transition temperature (-66.15°C) in the 10 selected intramolecular vibrational modes of nifedipine (Table 4.1). Figure 4.27, Figure 4.28 and Figure 4.29 correspond to the peak fit curves of cycle 1, 2, and 3 of the low temperature experiments, respectively.

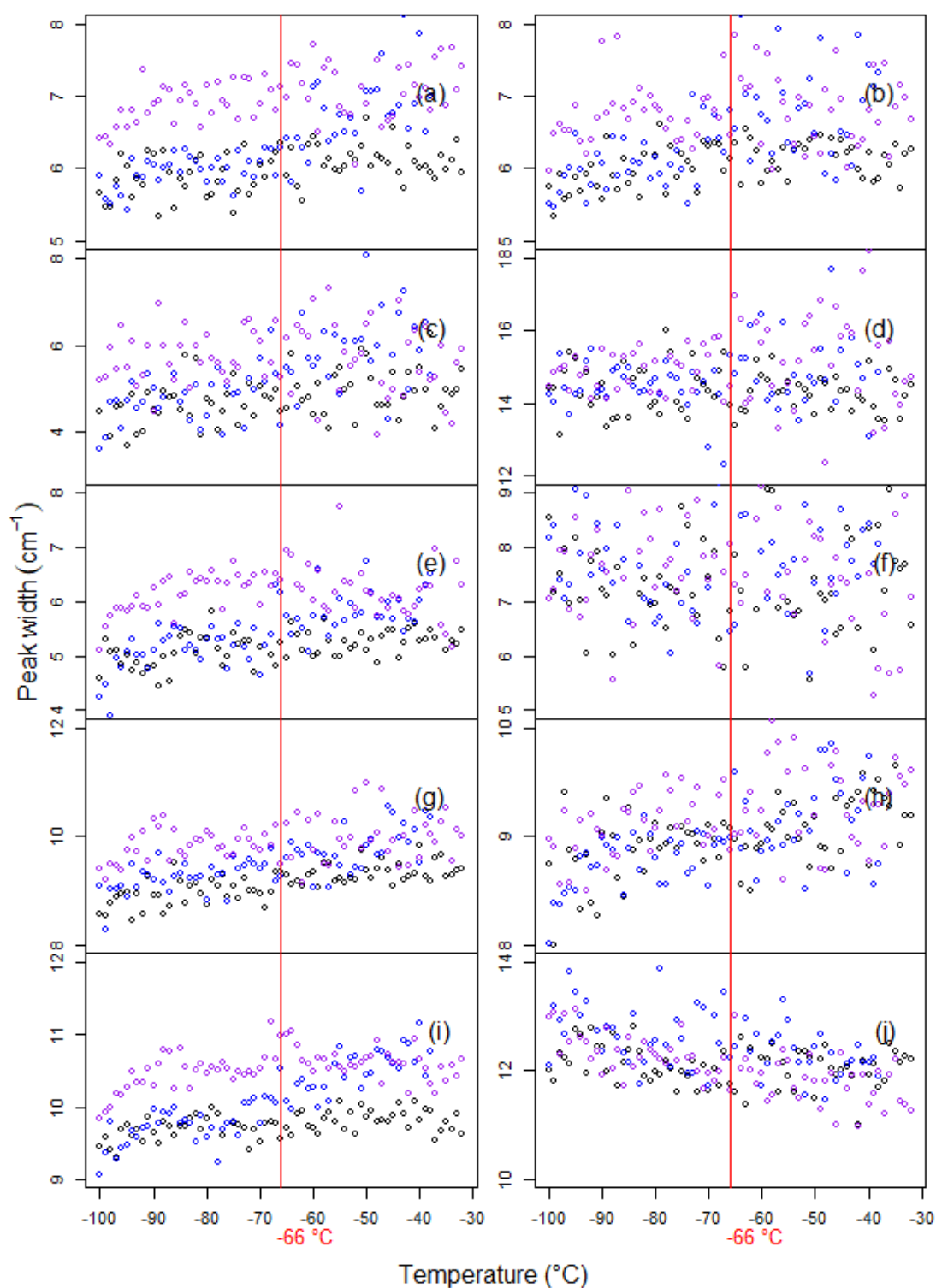


Figure 4.27. Low temperature experiment (cycle 1) - The peak fitting of width against temperature for the 10 selected peaks of nifedipine: 810 cm⁻¹, 836 cm⁻¹, 859 cm⁻¹, 954 cm⁻¹, 1049 cm⁻¹, 1224 cm⁻¹, 1348 cm⁻¹, 1492 cm⁻¹, 1648 cm⁻¹, and 1680 cm⁻¹. The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

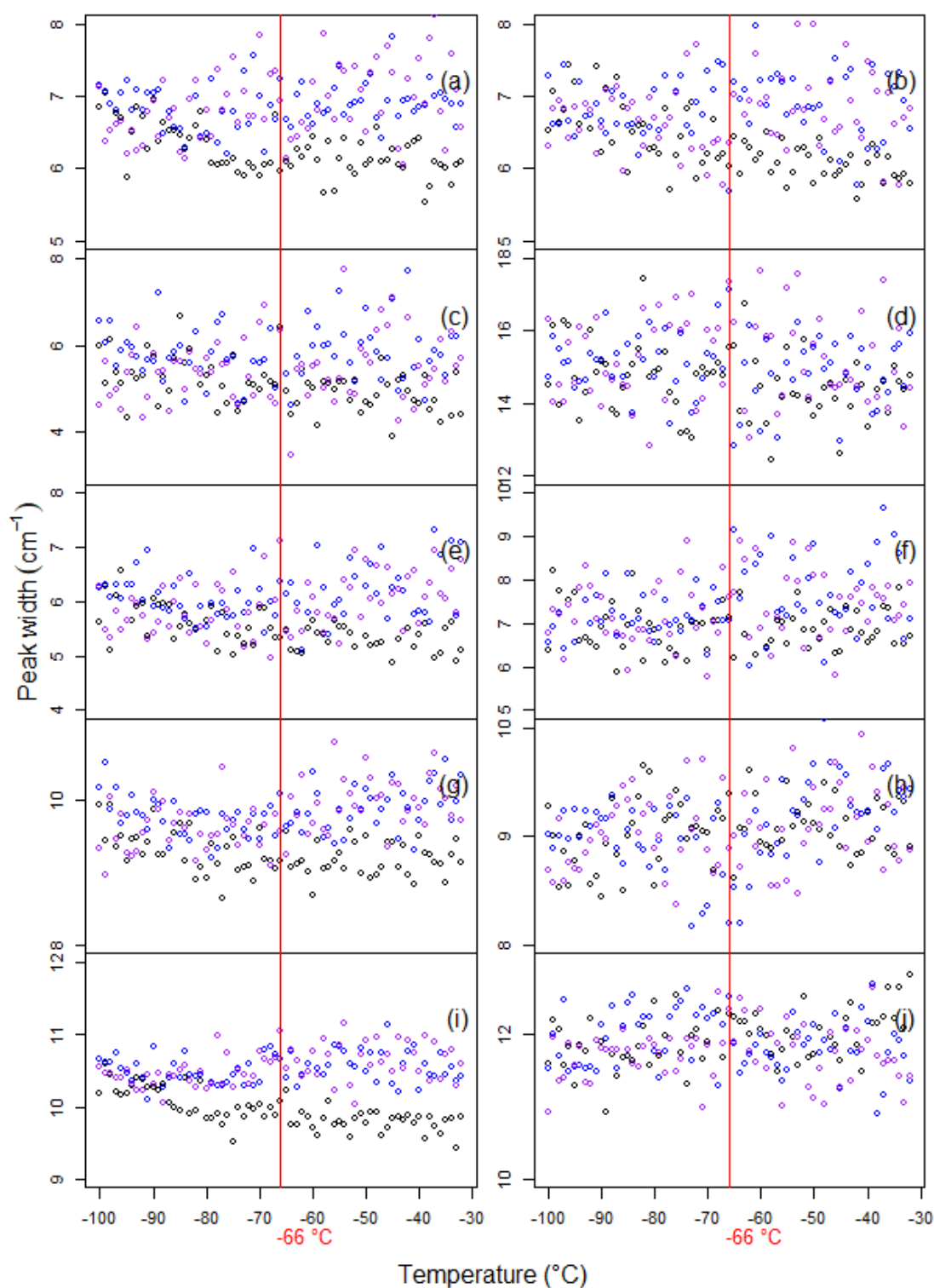


Figure 4.28. Low temperature experiment (cycle 2) - The peak fitting of width against temperature for the 10 selected peaks of nifedipine: 810 cm^{-1} , 836 cm^{-1} , 859 cm^{-1} , 954 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1348 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

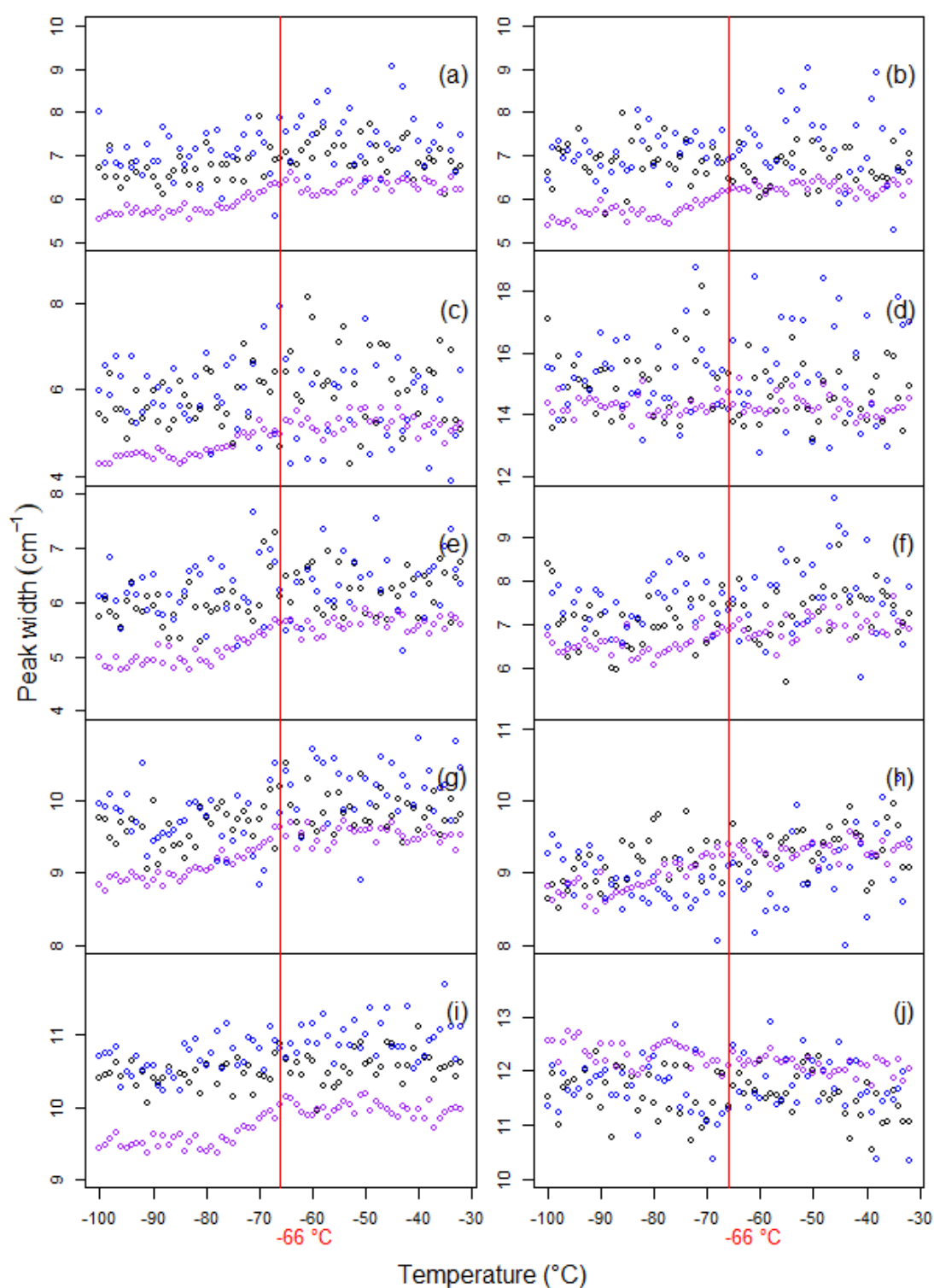


Figure 4.29. Low temperature experiment (cycle 3) - The peak fitting of width against temperature for the 10 selected peaks of nifedipine: 810 cm^{-1} , 836 cm^{-1} , 859 cm^{-1} , 954 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1348 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

In cycle 1 (Figure 4.27) and cycle 2 (Figure 4.28), we observe no obvious change in peak width. This is reasonable as we see no change to the non-crystalline region in the previous experiments (Figure 4.13). Cycle 3 of the experiment 3 (Figure 4.29, purple dots) have peak widths that changes at around -75°C, which is not far from the beta transition temperature (-66.15°C). This happens only in peaks at around 810 cm⁻¹(ring CH), 1049 cm⁻¹ (1,2 substituted ring) and 1648 cm⁻¹ (C=C stretching), thus we question the reproducibility of these changes. We conclude that for nifedipine based upon these studies, beta relaxation may not be related to changes in order/disorder because the widths of the Raman peaks do not seem to change at around the beta transition temperature in all of the low temperature repeat experiments.

4.4.4.4 Analysis of Gaussian fit curves of peak shift in low temperature repeat tests

The Gaussian fit curves of peak position of nifedipine were plotted to investigate different Raman features caused by the beta transition temperature (-66.15°C) in the 10 selected intramolecular vibrational modes (Table 4.1). Figure 4.30, Figure 4.31 and Figure 4.32 correspond to the peak fit curves of cycle 1, 2, and 3 of the low temperature experiments, respectively.

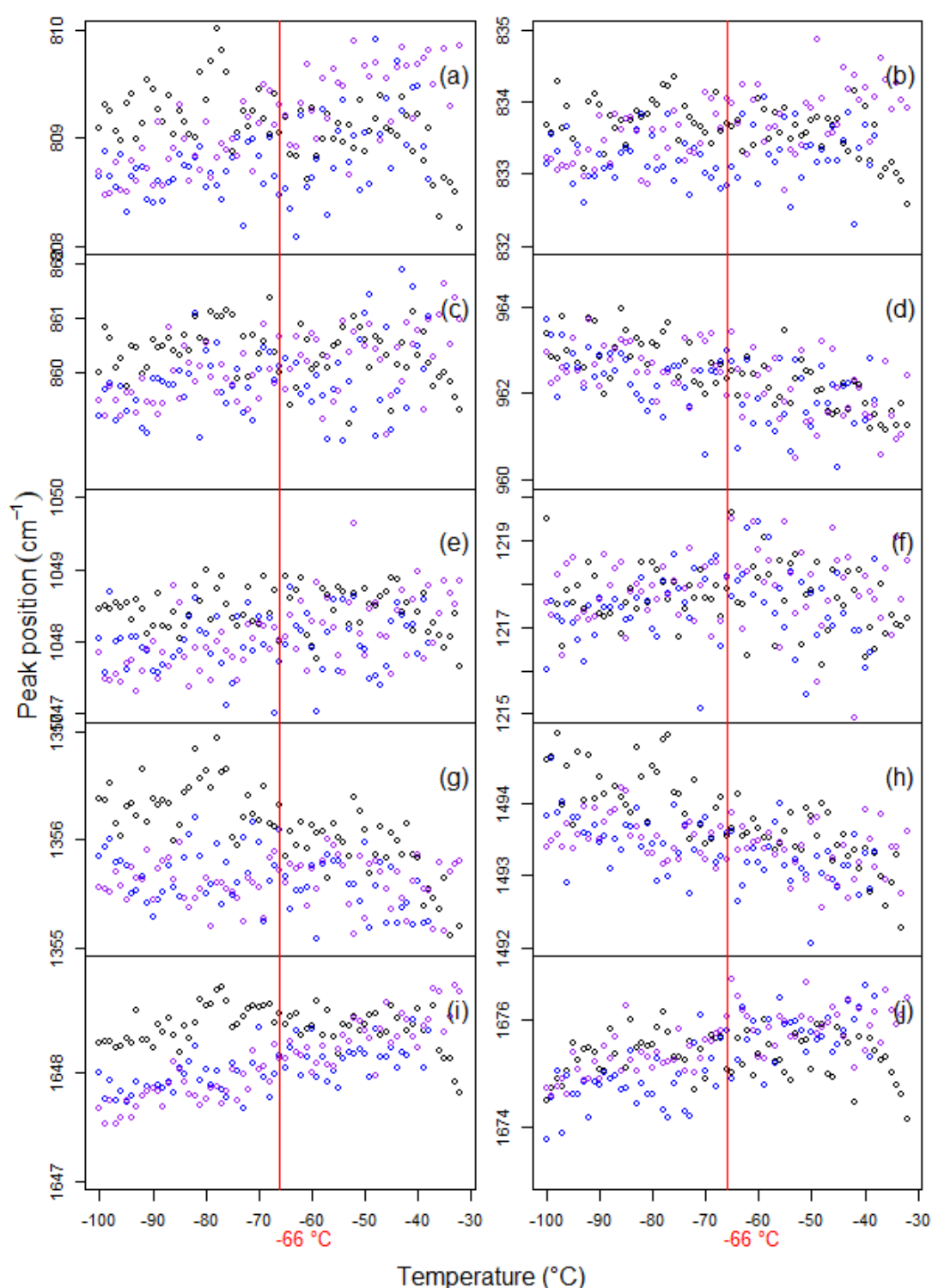


Figure 4.30. Low temperature experiment (cycle 1) - The peak fitting of position against temperature for the 10 selected peaks of nifedipine: 810 cm^{-1} , 836 cm^{-1} , 859 cm^{-1} , 954 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1348 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

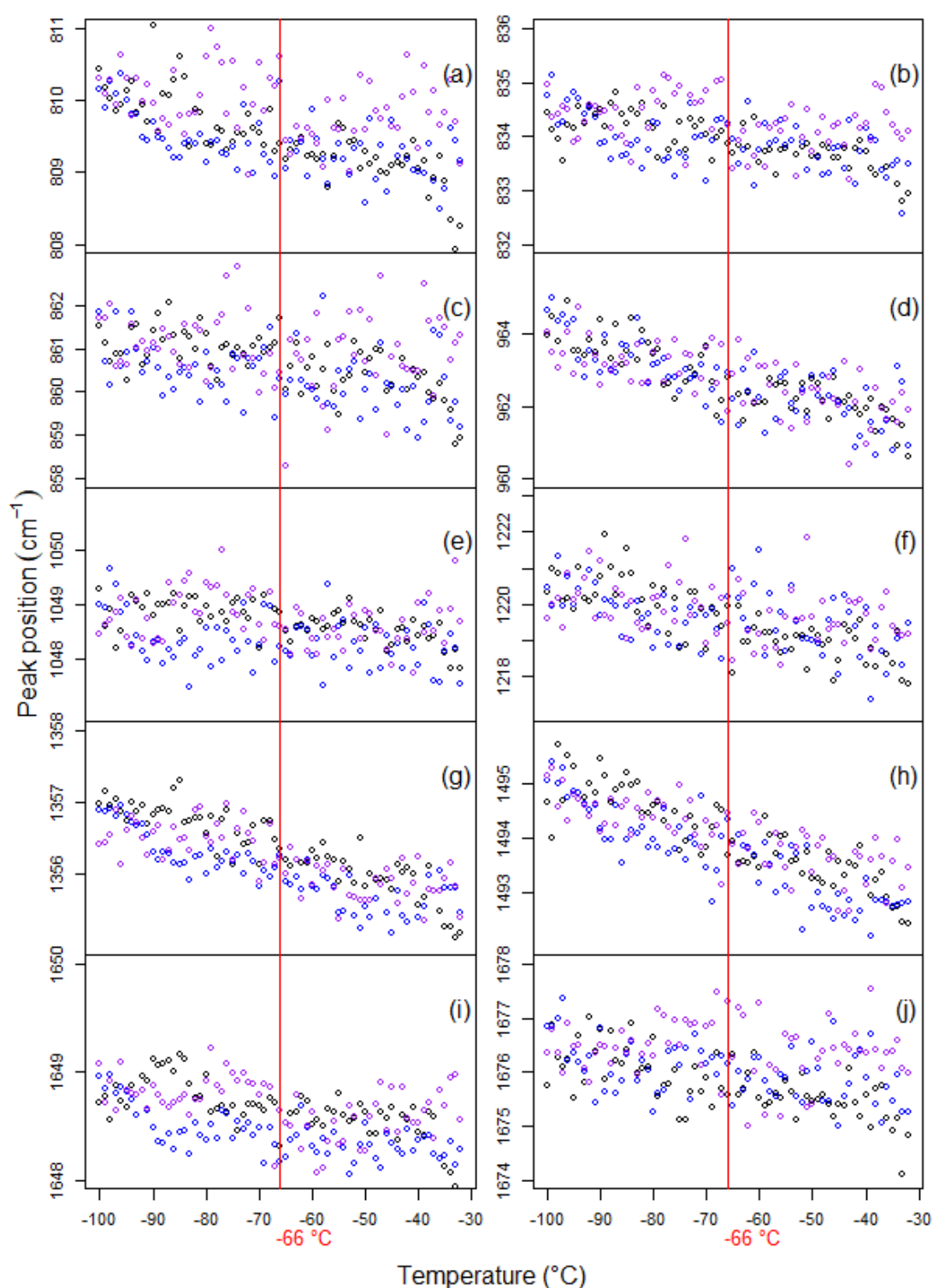


Figure 4.31. Low temperature experiment (cycle 2) - The peak fitting of position against temperature for the 10 selected peaks of nifedipine: 810 cm⁻¹, 836 cm⁻¹, 859 cm⁻¹, 954 cm⁻¹, 1049 cm⁻¹, 1224 cm⁻¹, 1348 cm⁻¹, 1492 cm⁻¹, 1648 cm⁻¹, and 1680 cm⁻¹. The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

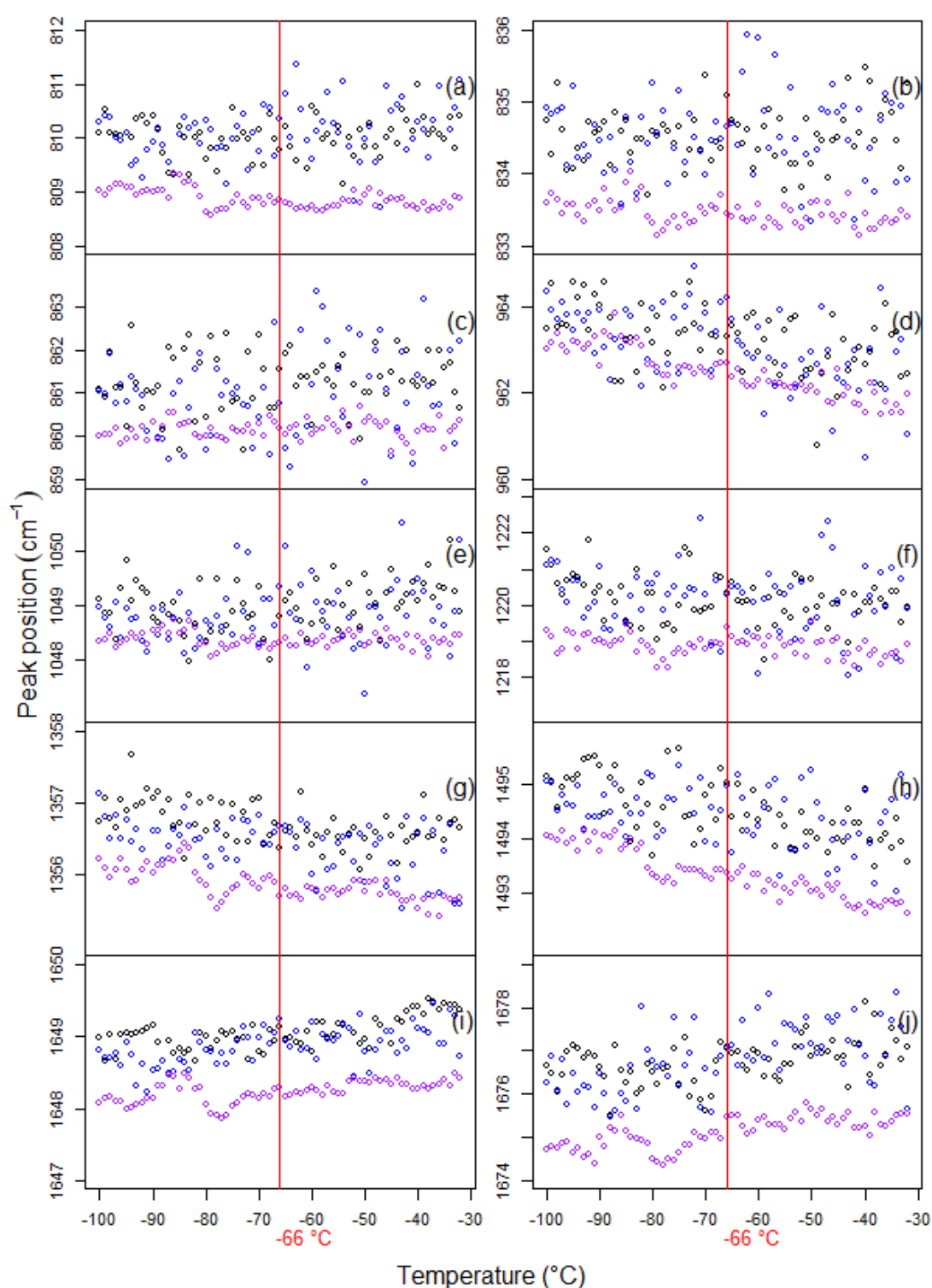


Figure 4.32. Low temperature experiment (cycle 3) - The peak fitting of position against temperature for the 10 selected peaks of nifedipine: 810 cm⁻¹, 836 cm⁻¹, 859 cm⁻¹, 954 cm⁻¹, 1049 cm⁻¹, 1224 cm⁻¹, 1348 cm⁻¹, 1492 cm⁻¹, 1648 cm⁻¹, and 1680 cm⁻¹. The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

In experiments 1, 2, 3 of cycle 1, 2 and 3 (Figure 4.30, Figure 4.31 and Figure 4.32), the peak fit position of the low temperature repeat cycling experiments does not show any obvious changes near the beta transition temperature (-66.15°C). This contrasts with major changes in peak position that are observed at around -66.15°C in the previous experiments (Figure 4.14). The peak shift figures of the low temperature experiments do not support reproducibility of the data, at beta transition temperature. We conclude that for nifedipine based upon these studies, structural changes do not seem to occur at around the beta transition temperature.

4.5 Conclusion

This chapter showed detailed study to see any changes around alpha (42.25°C) and beta transition (-66.15°C) of nifedipine in heating the amorphous state from Raman spectroscopy. We summarised the findings from Gaussian fit curves of peak intensity, width and position of nifedipine in Table 4.2.

We do see some obvious changes in peak intensity and position that are reproducible in peaks at around 810 cm^{-1} (ring CH), 836 cm^{-1} (ring CH), 859 cm^{-1} (ring CH), 954 cm^{-1} (dihydropyridine ring) 1049 cm^{-1} (1,2 substituted ring), 1348 cm^{-1} ($\text{Vs}(\text{NO}_2)$), 1492 cm^{-1} (aromatic $\text{C}=\text{C}$) and 1648 cm^{-1} ($\text{C}=\text{C}$ stretching) near the beta transition temperature. The change around peak intensity is not obvious in peaks at 1680 cm^{-1} that correspond to $\text{C}=\text{O}$ stretching (Figure 4.10 (j)). While some changes in peak intensity and position were observed around the beta transitions, they were not particularly reproducible when we did the low temperature cycling experiment.

We observe detectable change in peak width at around the alpha, but not around the beta transition temperature in experiment 2. The peaks at around 810 cm^{-1} (ring CH), 836 cm^{-1} (ring CH), 859 cm^{-1} , 1049 cm^{-1} , 1348 cm^{-1} and 1648 cm^{-1} have unevenly distributed broad peaks in area from 0 to 40°C . The change around peak width is not as obvious in peaks at 1680 cm^{-1} that correspond to $\text{C}=\text{O}$ stretching (Figure 4.13 (j)).

These findings are not particularly reproducible in that we only see detectable changes in one out of the three repeated experiments. Therefore, for nifedipine based upon the studies described above, there is no evidence for detectable change to the Raman spectra around the alpha and beta transition temperatures.

We will now move on to look at a different molecule, felodipine, which is structurally related to nifedipine and has very similar alpha transition temperature at around 44°C, but has very different behaviour - nifedipine crystallizes more readily than felodipine from the metastable amorphous form (Marsac et al, 2006)

Table 4.2. Summary of the results from variable temperature set up Raman experiments of paracetamol (The symbol “o” indicates presence of visual changes; “x” indicates absence of visual change)

Experiment No.		Change around beta transition temperature			Change around alpha transition temperature		
		Peak intensity	Peak width	Peak position	Peak intensity	Peak width	Peak position
Experiment 1		o	x	o	x	x	x
Experiment 2		o	x	o	o	o	x
Experiment 3		x	x	o	x	x	x
Low temperature experiment 1	Cycle 1	x	x	x			
	Cycle 2	x	x	x			
	Cycle 3	x	x	x			
Low temperature experiment 2	Cycle 1	x	x	x			
	Cycle 2	x	x	x			
	Cycle 3	x	x	x			
Low temperature experiment 3	Cycle 1	x	x	x			
	Cycle 2	x	x	x			
	Cycle 3	x	x	x			

Chapter 5 Investigation of the applicability of Raman spectroscopy to probe the alpha and beta relaxation events in felodipine

5.1 Introduction

Following on from the previous chapter, in which we investigated potential structural changes of nifedipine involved in alpha and beta relaxation events, felodipine was selected to investigate potential changes in molecules around alpha and beta transition temperatures from low to high temperature. Felodipine makes a good model system because it is structurally related to nifedipine and have very similar alpha transition temperature (44°C), but have very different behaviours - nifedipine crystallized more readily than felodipine from the metastable amorphous form (Marsac et al, 2006). We are going to do cycling experiments to check the reproducibility and whether we could observe potential molecular changes in heating and cooling, or just on cooling, or just on heating the felodipine sample.

5.2 Materials

Felodipine was purchased by Sigma-Aldrich ($\geq 99.0\%$). It is in the form of white crystalline powder. The melting point and alpha transition temperature (T_g) of nifedipine are 145°C and $44.7 \pm 1.8^\circ\text{C}$, respectively (Rollinger & Burger, 2001; Saboo et al, 2021).

5.3 Methods

5.3.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to measure the characteristic melting point of the compound to get an indication of the purity. Thermal analysis was performed using DSC Q2000. Nitrogen flow was set at

20 mL/min and samples were heated from 20 to 150°C at a rate of 5°C/min. Modulated DSC has been performed to investigate beta transition temperature of nifedipine. The DSC was set up to modulate ± 1.00 °C at every 60 seconds with ramp of 0.50 °C/min from -100 to 160.00 °C. TRIOS software was used for viewing and analysing of the data.

5.3.2 X-ray diffraction (XRD)

X-ray diffraction (XRD) data was collected on a Panalytical X'pert diffractometer to determine the purity of the compound. The experimental parameters were same as those in paracetamol experiment in Section 3.3.2.

5.3.3 Fourier transform infrared (FTIR)

Fourier transform-infrared spectroscopy (FTIR) was used to assess purity of the compound. The experimental parameters were same as those in paracetamol experiment in Section 3.3.3.

5.3.4 Raman spectroscopy

For the variable temperature experiments, felodipine sample slide was placed in Linkam thermal stage LTS350 to be melted up to high temperature of 155°C, which is higher than the melting point of felodipine, i.e. 145°C, to ensure that the sample was completely melted (Rollinger & Burger, 2001). The sample was then cooled rapidly to low temperature (-100°C) in cooling rate of 30°C min⁻¹. The experimental parameters for Raman spectrometer were very similar to the variable temperature set up nifedipine experiment (Section 4.3.4), but in felodipine experiment, each Raman spectrum was collected for 8 seconds.

5.4 Results

5.4.1 Characterisation of felodipine sample

DSC, XRD, FTIR were used to determine the purity of the material. The DSC data showed (Figure A 13 of Appendix A) melting point at around 147°C. This confirmed the purity of the sample when compared to data (Figure A 14 of Appendix A) presented in the literature (Abiad et al, 2010), which showed good correspondence to the experimental data. The plot of the modulated DSC thermogram could be found in the appendix (Figure A 15 of Appendix A) and does not show any obvious evidence of beta relaxation. The XRD data (Figure A 16 of Appendix A) resembled pattern of DONTIJ crystal structure from the Cambridge structural database and no extra peaks were observed. This confirmed the purity of the material as crystalline form and the structure of crystalline form to be polymorph I. The infrared spectral data (Figure A 17 of Appendix A) confirmed the characteristic peak positions of pure felodipine at around 1697 cm⁻¹ (C=O group) and 3372 cm⁻¹ (N-H group) (Jana et al, 2014).

5.4.2 Heat map - variable temperature in Raman spectra

In order to investigate any potential spectral changes which can be linked with to alpha ($44.7 \pm 1.8^\circ\text{C}$) and beta relaxation processes, contour maps were plotted (Saboo et al, 2021). The beta transition temperatures are commonly considered to typically occur at around $0.75T_g$ (reportedly actual values may span from 0.65 to $0.85T_g$) (Boyer, 1976). Hence, we assign the beta relaxation of felodipine to occur at around -66°C , which is not far from the beta relaxation of nifedipine (-66.15°C). Figure 5.1 is the contour map of the normalised Raman spectra plotted as a function of temperature. The Raman spectra were collected from quench-cooled felodipine sample prepared as outlined in Section 5.2 and the sample was heated from -100 to 150°C with step sizes of 1°C , in the wavenumber range of 0 to 2000 cm^{-1} . Figure 5.2 and Figure 5.3 are the zoomed-in version of the low (0 to 400 cm^{-1}) and high (1200 to 1700 cm^{-1}) wavenumber region of the previous contour map, Figure 5.1.

The high (1200 to 1700 cm^{-1}) region was selected due to presence of high intensity peaks. On the other hand, the low region (0 to 400 cm^{-1}) was zoomed-in in an effort to make comparison with other plots of similar wavenumber range in the previous chapter on nifedipine.

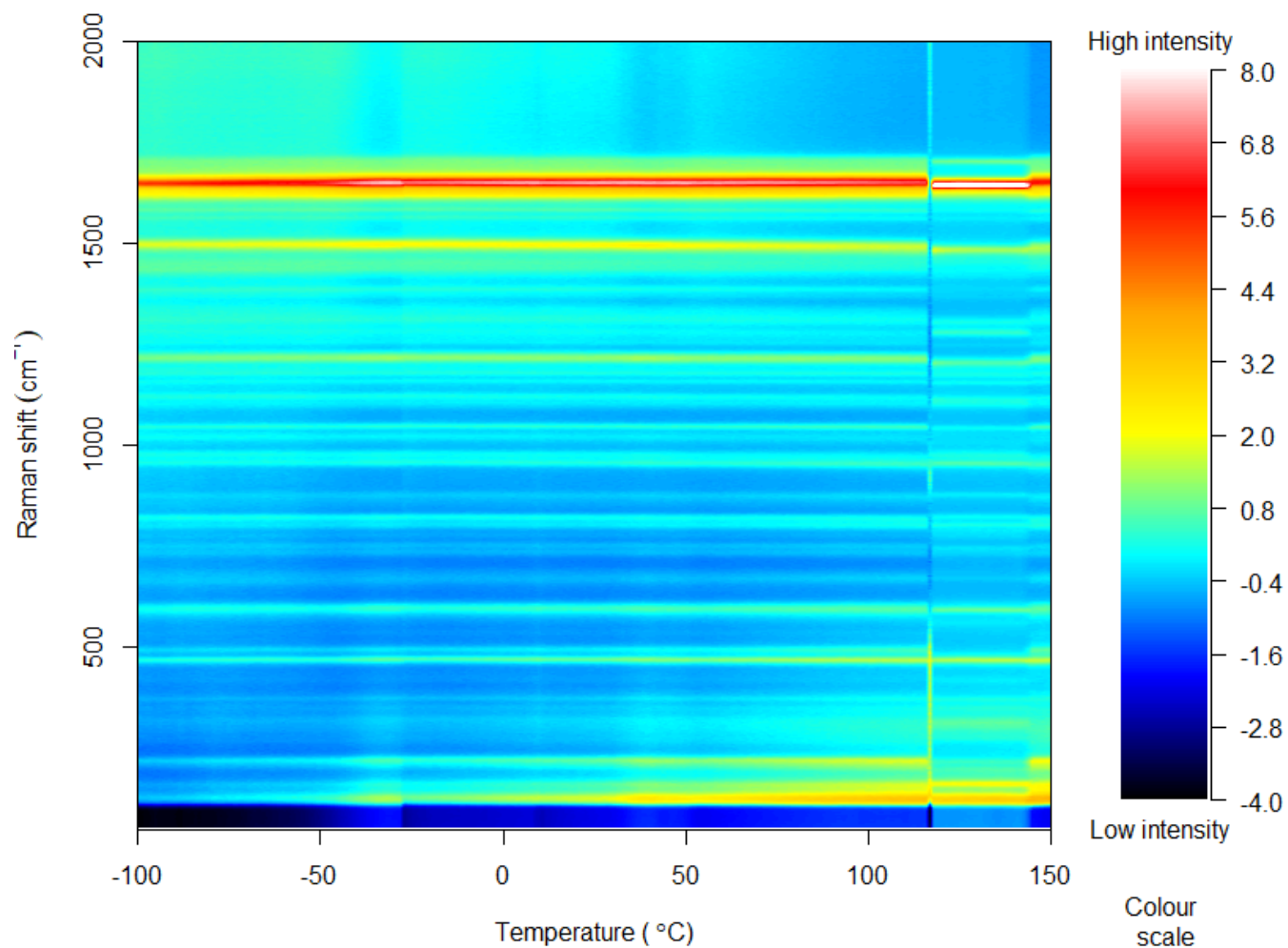


Figure 5.1. Felodipine experiment 1 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

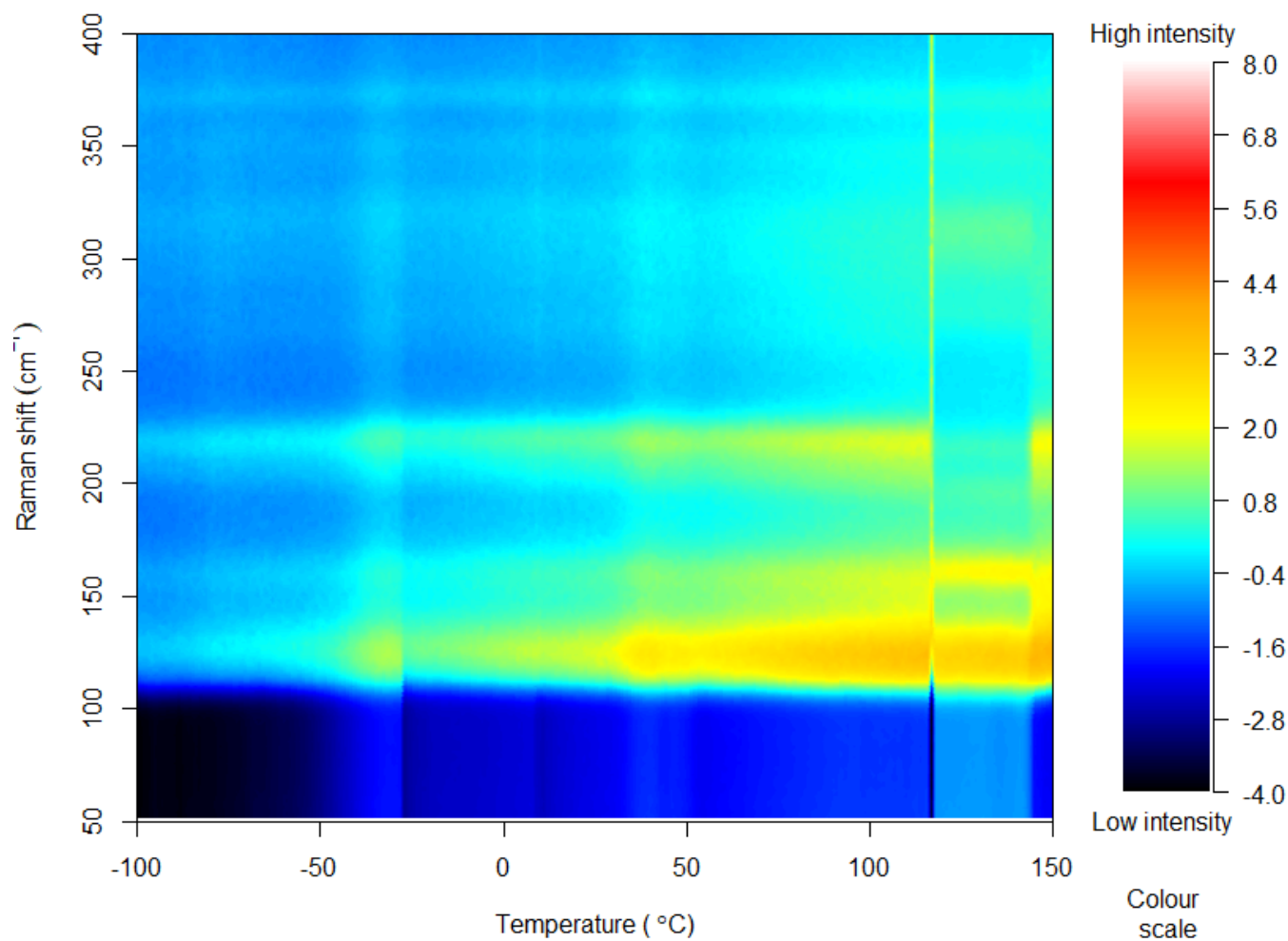


Figure 5.2. Felodipine experiment 1 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

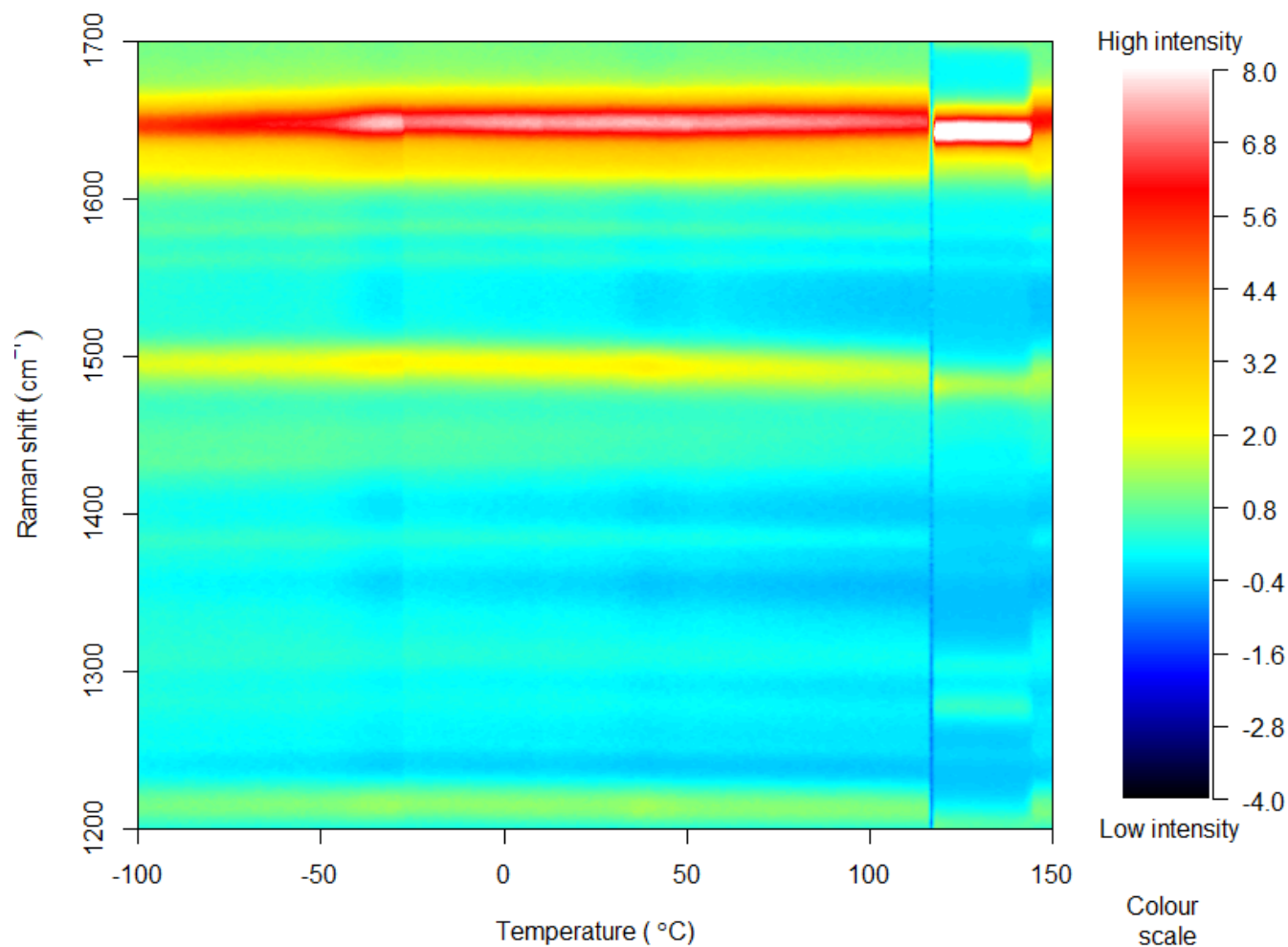


Figure 5.3. Felodipine experiment 1 - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

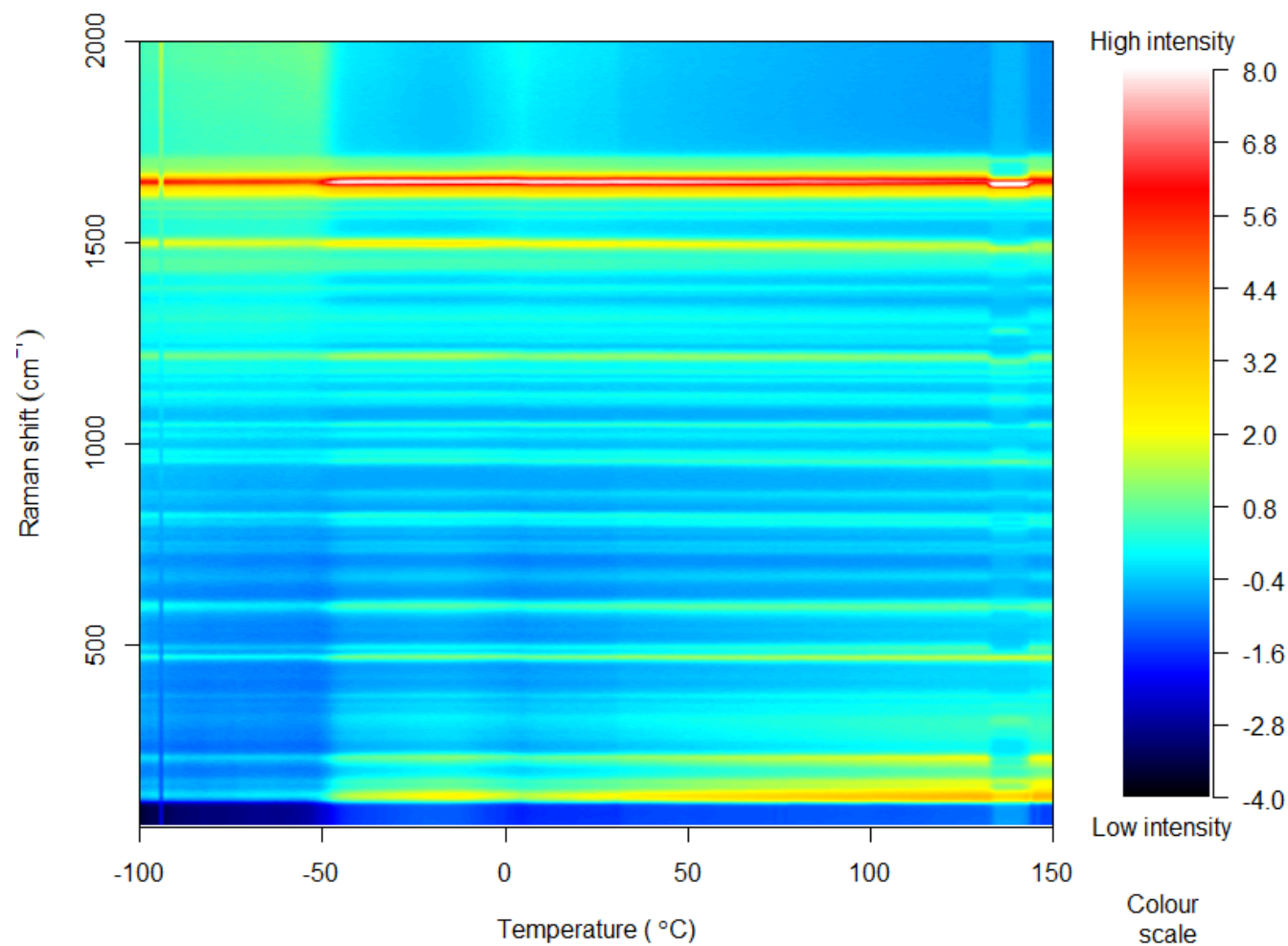


Figure 5.4. Felodipine experiment 2 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

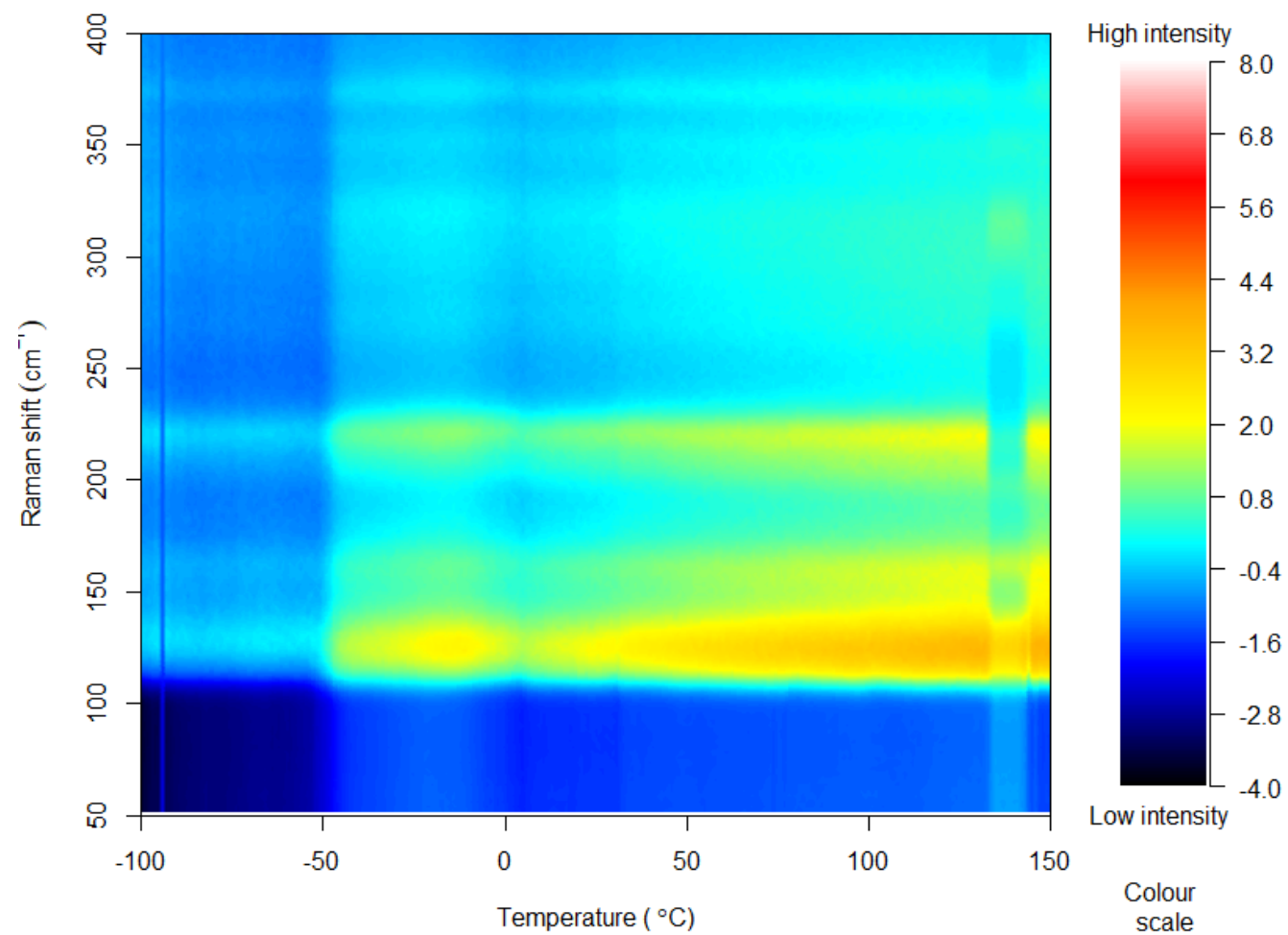


Figure 5.5. Felodipine experiment 2 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

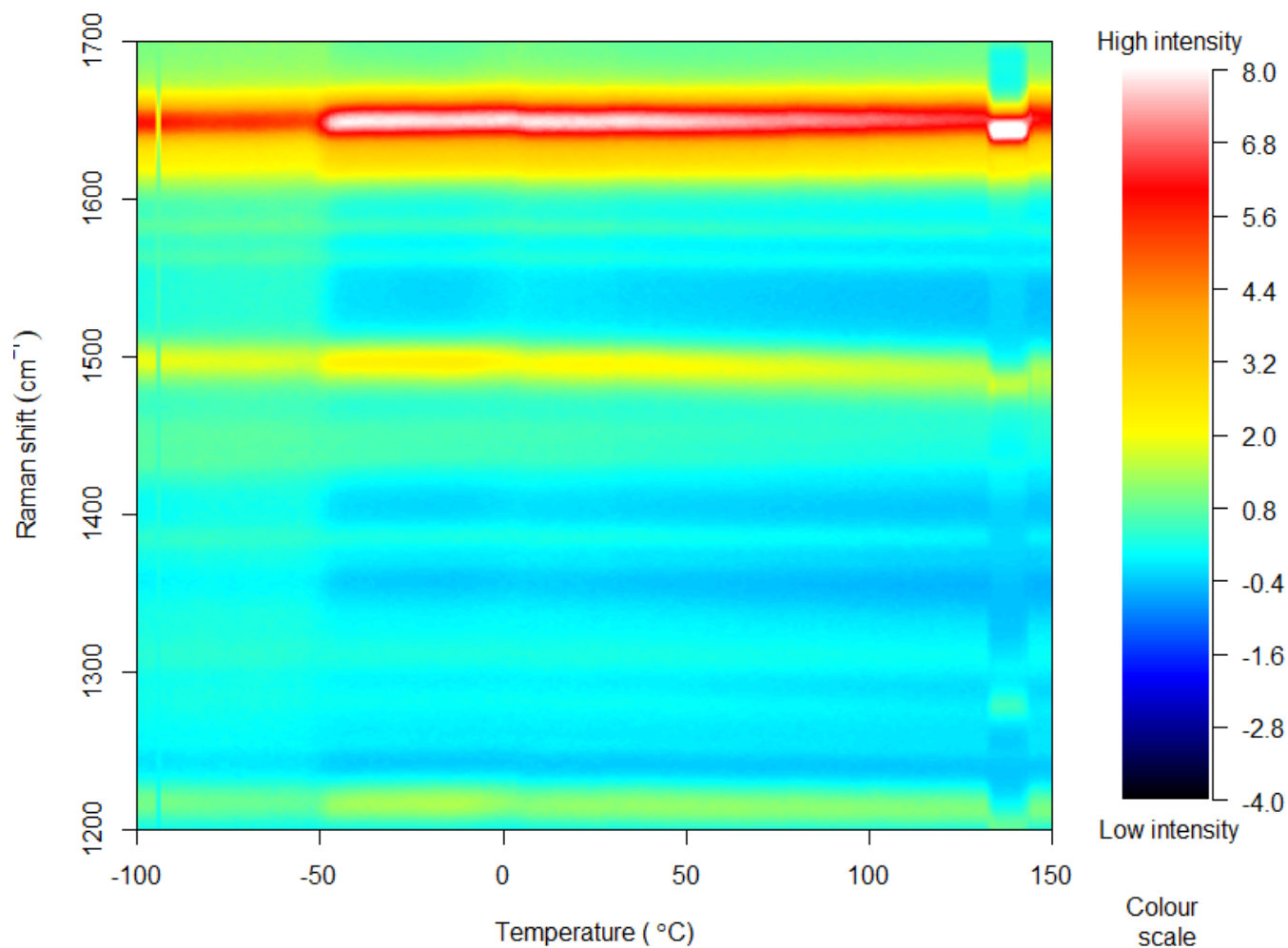


Figure 5.6. Felodipine experiment 2 - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

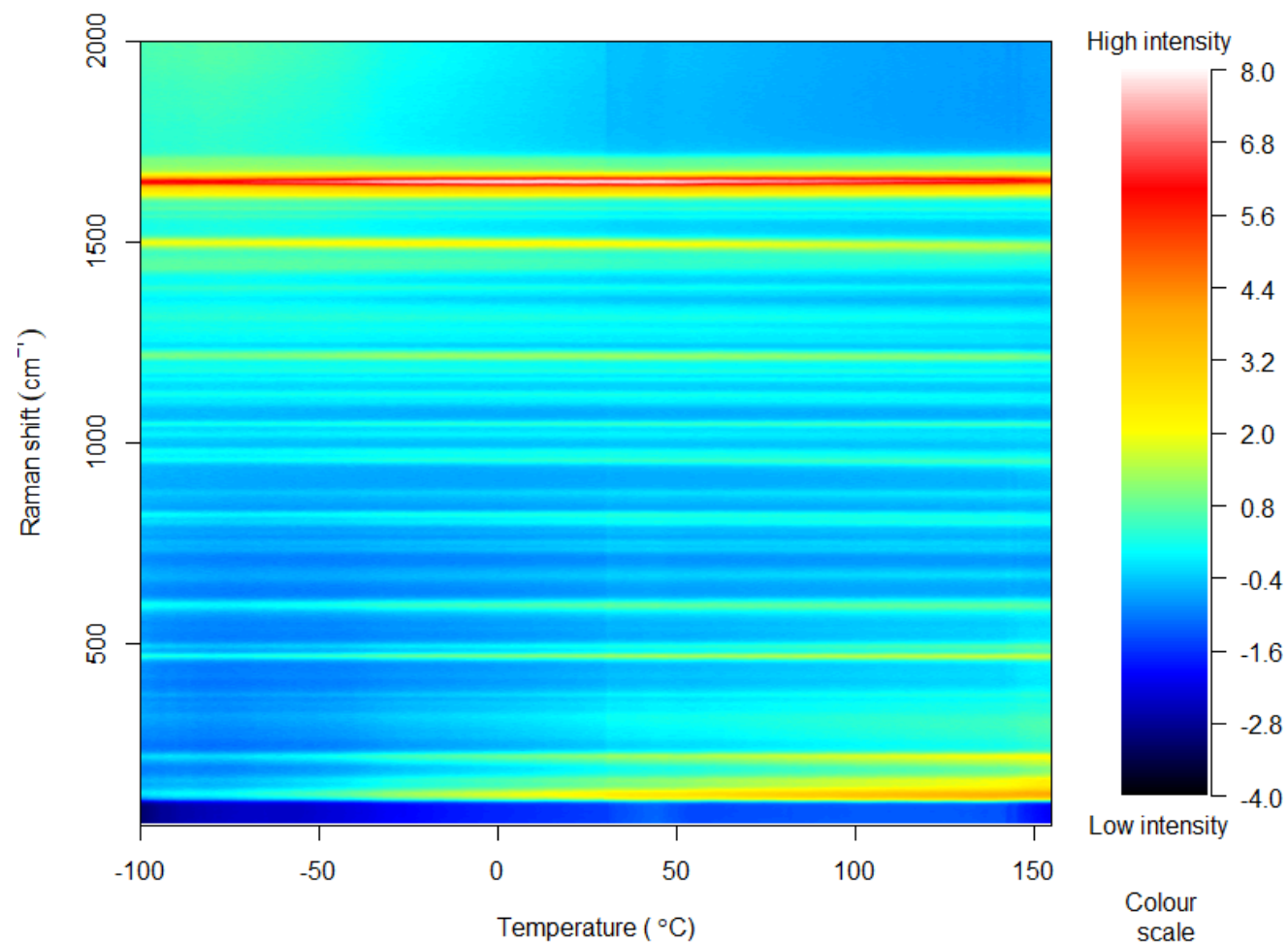


Figure 5.7. Felodipine experiment 3 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

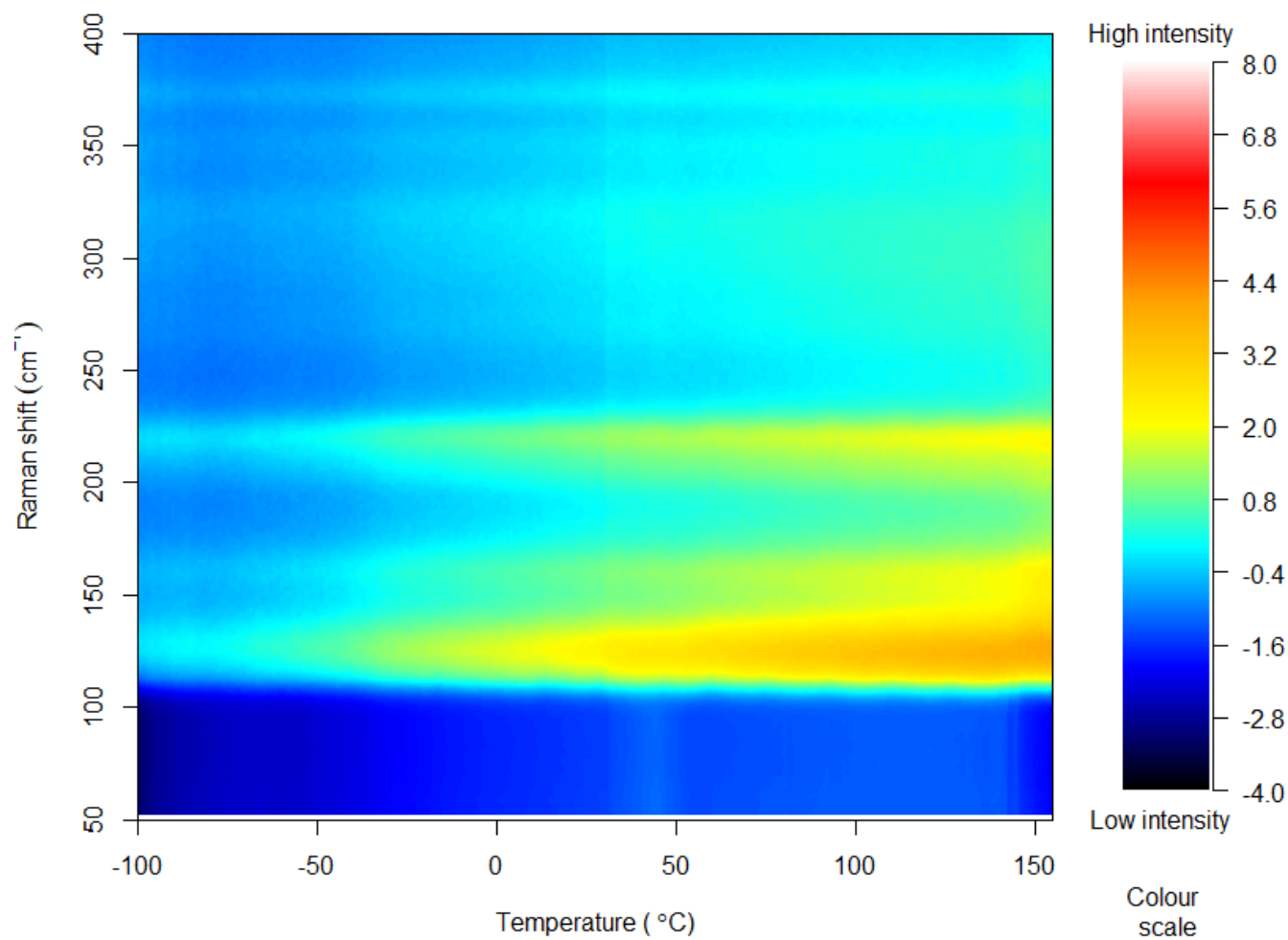


Figure 5.8. Felodipine experiment 3 - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

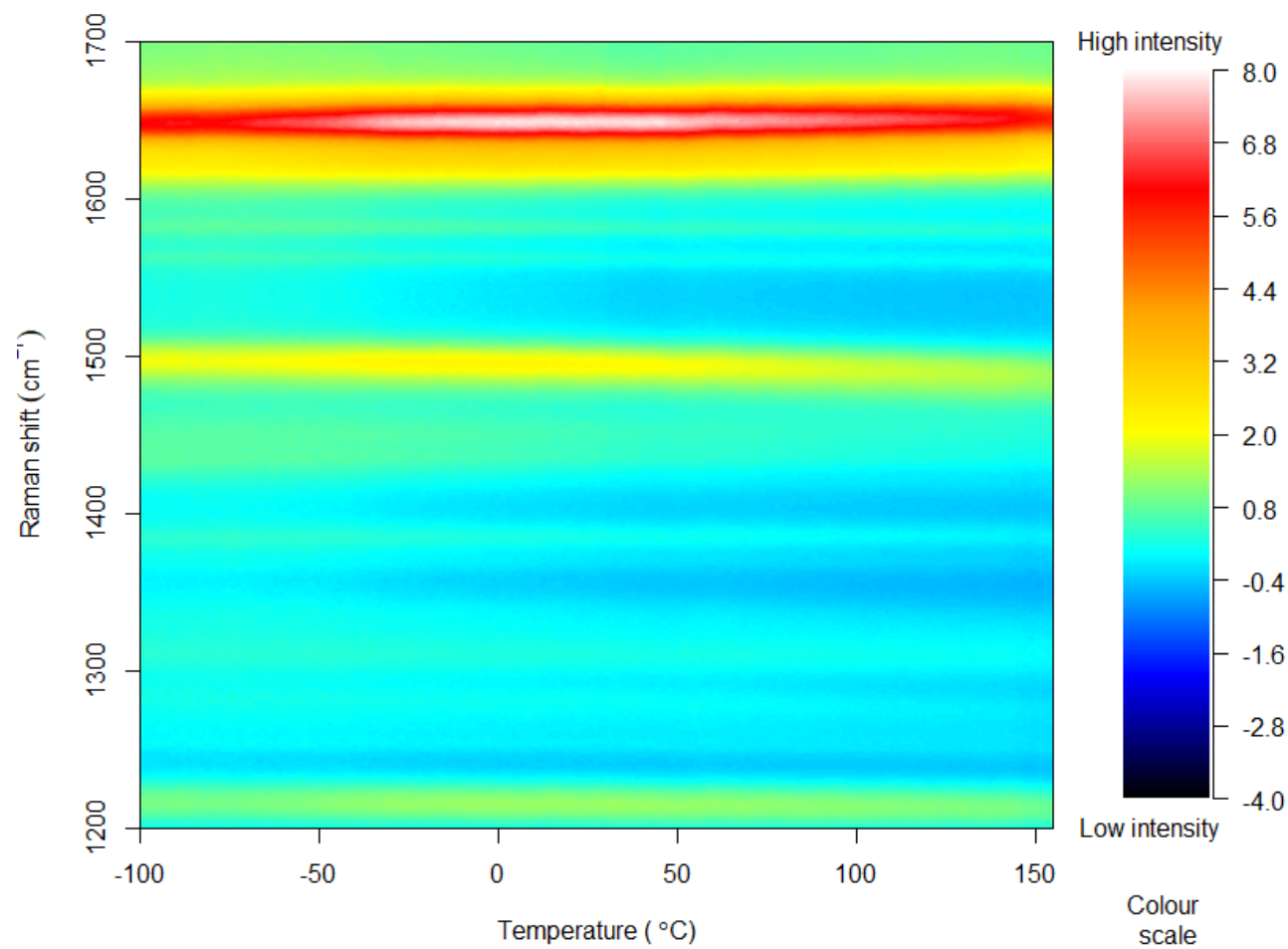


Figure 5.9. Felodipine experiment 3 - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

In experiment 1 (Figure 5.1), experiment 2 (Figure 5.4) and experiment 3 (Figure 5.7) - the peak intensity is high at around 200 cm^{-1} , which are yellow, and at around 1650 cm^{-1} , which are white, red, and yellow. For clarity, the low wavenumber regions of experiment 1, 2 and 3 are presented zoomed-in in Figure 5.2, Figure 5.5 and Figure 5.8, respectively. Likewise, the zoomed-in high wavenumber regions of experiment 1, 2 and 3 are presented in Figure 5.3, Figure 5.6 and Figure 5.9, respectively.

Figure 5.2, Figure 5.5 and Figure 5.8 are the zoomed-in version of the low wavenumber region of the contour map in experiment 1, 2 and 3. The yellow peak intensity region at 200 cm^{-1} starts at around -50°C , which is not far from the reported beta relaxation temperature of nifedipine (-66.15°C). Slight drop in intensity is observed at around 30 to 40°C , which is near the alpha transition temperature (42.25°C). The high peak intensity region restarts at around 40°C . There are clear abrupt changes to the position and intensity of several peaks at 110°C for experiment 1 (Figure 5.2) and at 140°C for experiment 2 (Figure 5.5). There are two split peaks at around these temperatures. These changes are not observed in experiment 3 (Figure 5.8).

Figure 5.3, Figure 5.6 and Figure 5.9 are the zoomed-in plots of the high wavenumber region of the contour map in experiment 1, 2 and 3. There is a vertical band that can be observed at around 1600 cm^{-1} . High intensity region, mostly in white, red and yellow, is observed at around -50°C , near the beta transition temperature of nifedipine (-66.15°C). There is no obvious visual change to the spectra from -50°C up to approximately 100°C . There are clear abrupt changes to the position and intensity of several peaks at 110°C for experiment 1 (Figure 5.3) and at 140°C for experiment 2 (Figure 5.6). At these temperatures, the bands shift and split into two peaks. This corresponds to the previous study of amorphous felodipine obtained from the supercooled melt crystallized to form I at $143.8 \pm 0.2^{\circ}\text{C}$ (Surov et al, 2012). We observe no such changes in experiment 3 (Figure 5.9). Visual inspection of the Raman bands possibly indicates phase transformation between different form of felodipine at various temperatures. The visual changes occurring at around the beta

transition temperature in the contour maps are not reproducible. Hence, for felodipine described in these studies, we cannot fully conclude that we see detectable changes to Raman intensity around the beta transition temperature. In order to better characterise differences in Raman features caused by temperature changes, particularly around the reported alpha and beta transition temperatures, quantitative peak shape analysis has been taken for Raman peak characterisation as shown in the next section.

5.4.3 Gaussian peak fitting

In order to characterise differences in Raman features caused by alpha and beta relaxation in felodipine, quantitative peak shape analysis has been taken for peak characterisation of intramolecular vibrational modes. Gaussian peak fitting was undertaken on 8 selected intramolecular vibrational modes as discussed in the 2.2.5. data analysis section. The Raman band positions with corresponding wavenumbers are summarised in Table 5.1. We performed basic analysis of Raman spectra in the Knowitall informatic system to obtain functional group information from the felodipine spectra by browsing the chemical classes included in knowledge bases (Sons, 2001). We intend to diagnose these Raman peaks with three specific parameters – peak intensity, peak width and peak position - that were mentioned in Section 2.2.4.

Table 5.1. Wavenumbers (cm^{-1}) and suggested assignments derived from Raman spectra of glassy and crystalline felodipine

Wavenumbers (cm^{-1})	Assignment
470	C-O-C bend
595	In-plane ring deformation
1049	1,2 substituted ring
1224	Ester
1380	$\text{Vs}(\text{NO}_2)$
1492	C=C (Aromatic)

1648	C=C stretching
1680	C=O stretching

5.4.3.1 Analysis of Gaussian fit curves of peak intensity

We now review Raman data captured across all temperatures. Figure 5.10 shows Gaussian fit curves of peak intensities plotted as a function of temperature at around 8 selected vibrational modes (Table 5.1). Each dot on the scatterplot represents peak intensity of selected peaks in the range of -100 to 150°C. The black, blue and purple dots represent low temperature experiment 1, 2 and 3, respectively.

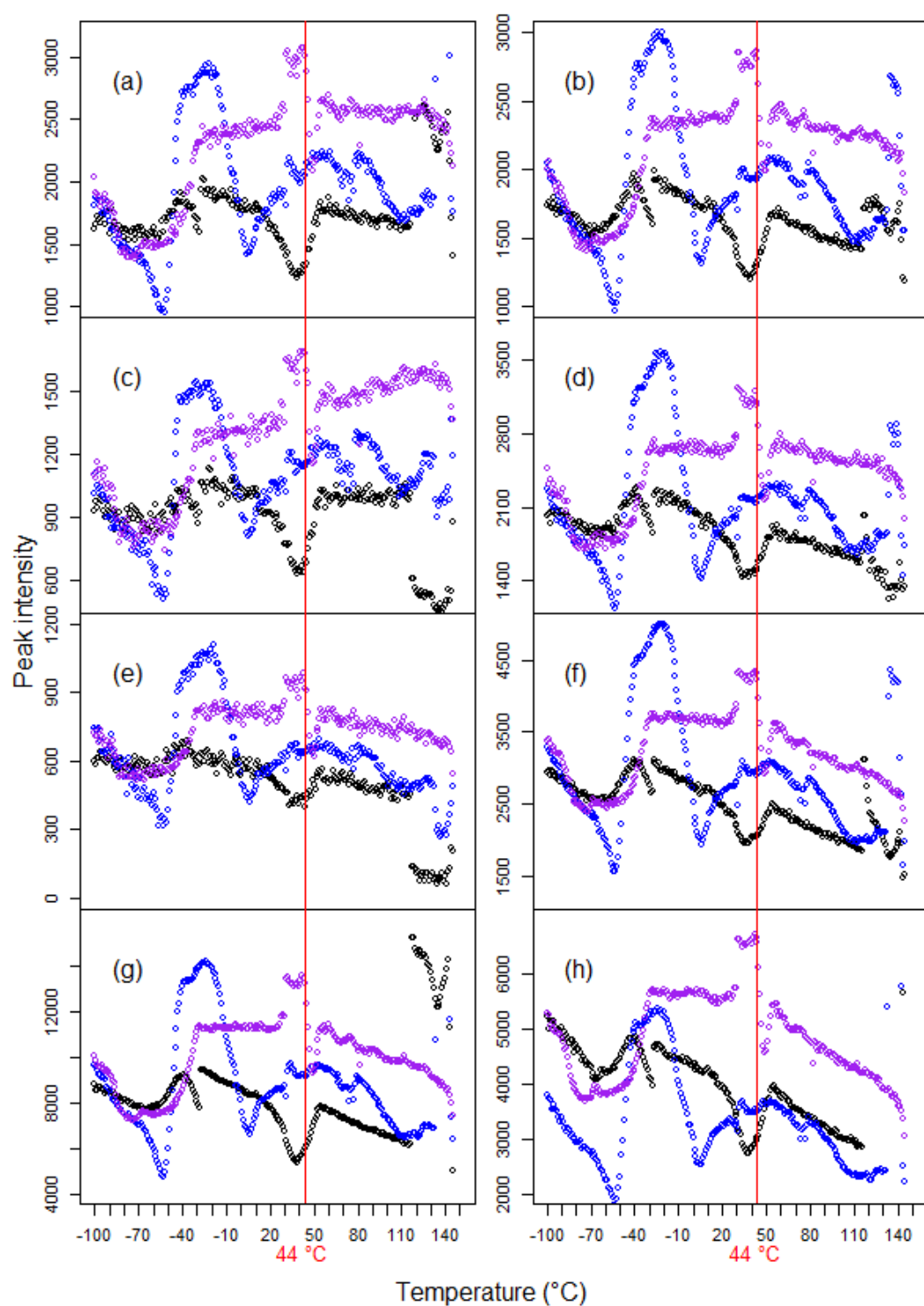


Figure 5.10. The peak fitting of intensity against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (44°C) transition temperature.

In experiment 1 (Figure 5.10, black dots), there appears to be increase in peak intensities at around -70°C , and discontinuities at around -30°C . We observe obvious changes at around the alpha (44°C) transition temperature. In experiment 2 (Figure 5.10, blue dots), obvious changes in peak intensity are observed at around -60 and 0°C . Discontinuity is observed at around 30°C , which is not far from the alpha transition temperature (44°C). In experiment 3 (Figure 5.10, purple dots), we see some obvious changes in peak intensities in the non-crystalline region at around -70 and -30°C . We observe discontinuity at around 40°C , which are not far from the alpha transition temperature (44°C). In experiment 1 and 2, there seems to be obvious changes at around 140°C which correspond to the temperatures at which there are clear abrupt change to the position and intensity of several peaks in Figure 5.2.

In all the experiments above, there seemed to be obvious changes to peak intensities at around -70°C , which is not far from the beta transition temperature of nifedipine (-66°C) and around 40°C , which is not far from the alpha transition temperature of felodipine (44°C). In order to check that the result from the peak fitting is reasonable, we plotted spectra either side of -66°C and 44°C in Figure 5.11 and Figure 5.12, respectively. The spectra at 5°C below and above the temperature of interest were plotted as a function of temperature. The subplots from the experiment 1, 2 and 3 were enumerated with (a), (b), and (c), respectively.

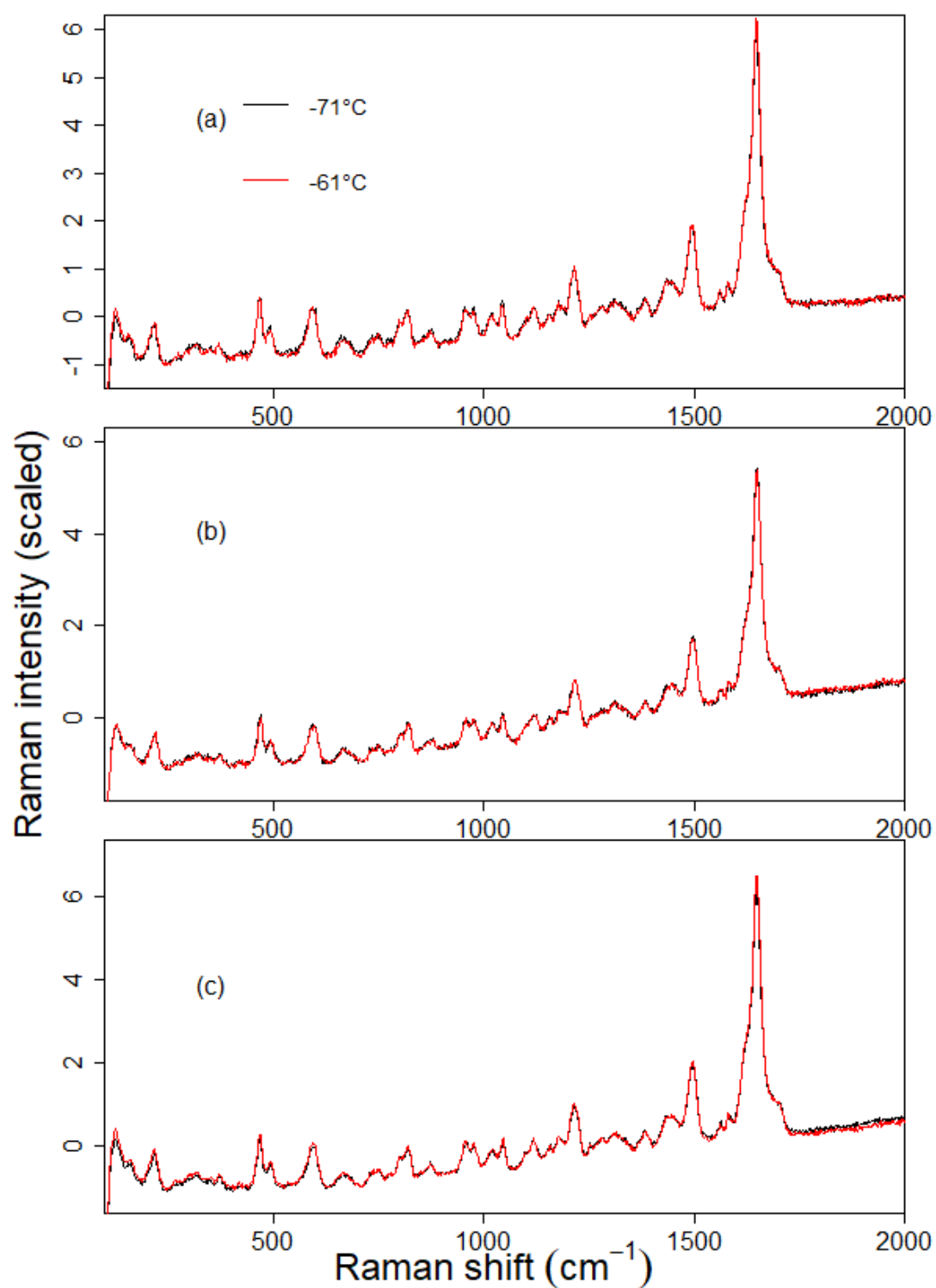


Figure 5.11. The Raman spectra of felodipine 5°C below and above -66°C, which is the reported beta transition temperature of nifedipine: -71°C (black line) and -61°C (red line), (a) experiment 1 (b) experiment 2 (c) experiment 3

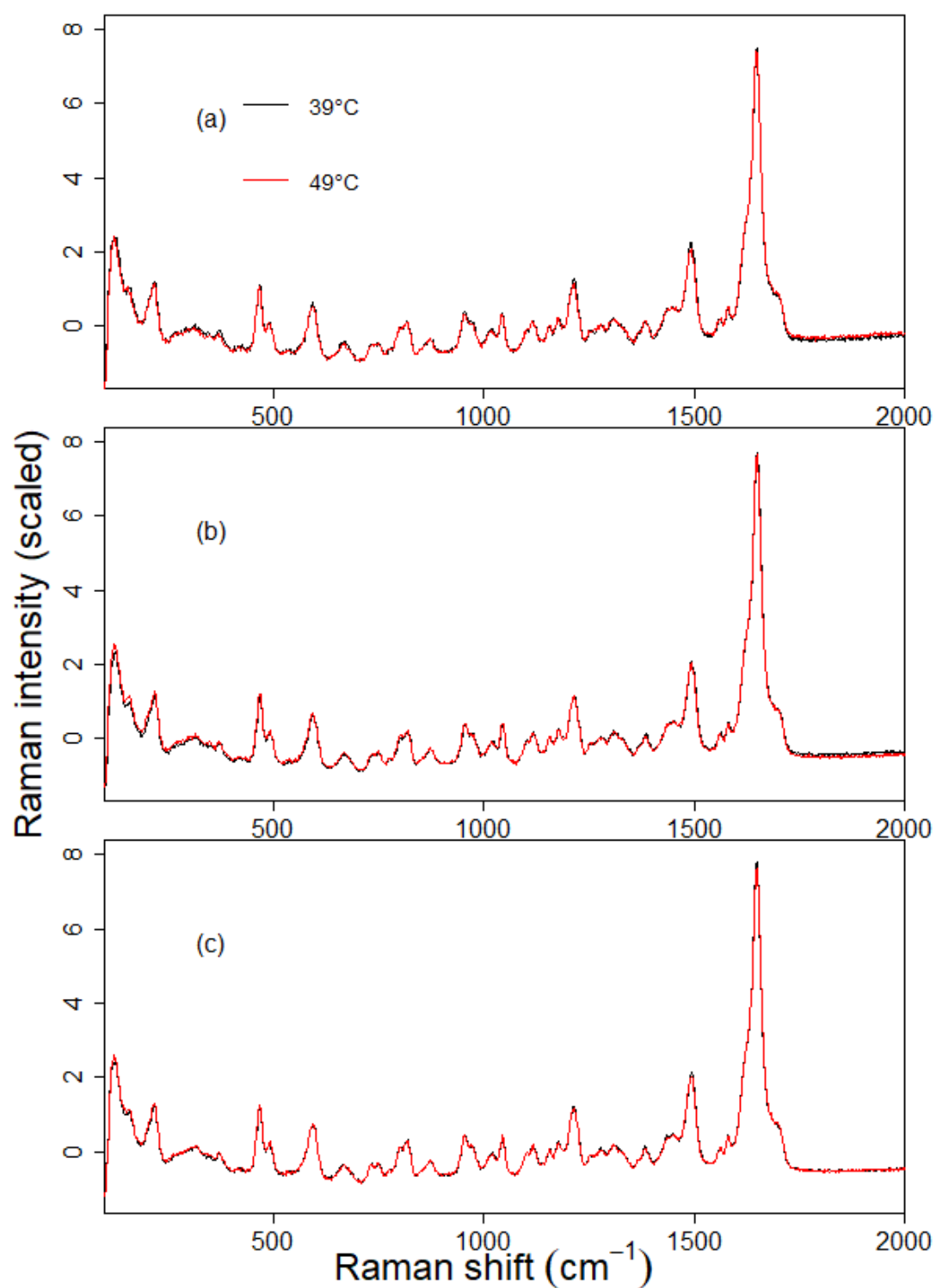


Figure 5.12. The Raman spectra of felodipine 5°C below and above alpha transition temperature of felodipine (44°C): 39°C (black line) and 49°C (red line), (a) experiment 1 (b) experiment 2 (c) experiment 3

All of the three felodipine experiments in Figure 5.11 show no change to Raman intensity at 5°C below and above -66°C. In Figure 5.12, all three experiments show no significant changes at 5°C below and above alpha transition temperature, 44°C. These plots do not support the result from the peak fitting of peak intensities in Figure 5.10. For felodipine based upon these studies, we do not have enough evidence to conclude that Raman intensity as a function of temperature could lead to detectable changes in Raman scattering of functional groups that are associated with alpha or beta relaxation.

5.4.3.2 Analysis of Gaussian fit curves of peak width

Figure 5.13 shows Gaussian fit curves of peak widths of felodipine plotted as a function of temperature at around 8 selected vibrational modes (Table 5.1). We intend to diagnose width of the peak as a function of temperature, which could give useful information on structural disorder and crystallinity, at the alpha transition temperature (44°C) of felodipine and the beta transition temperature (-66°C) of nifedipine.

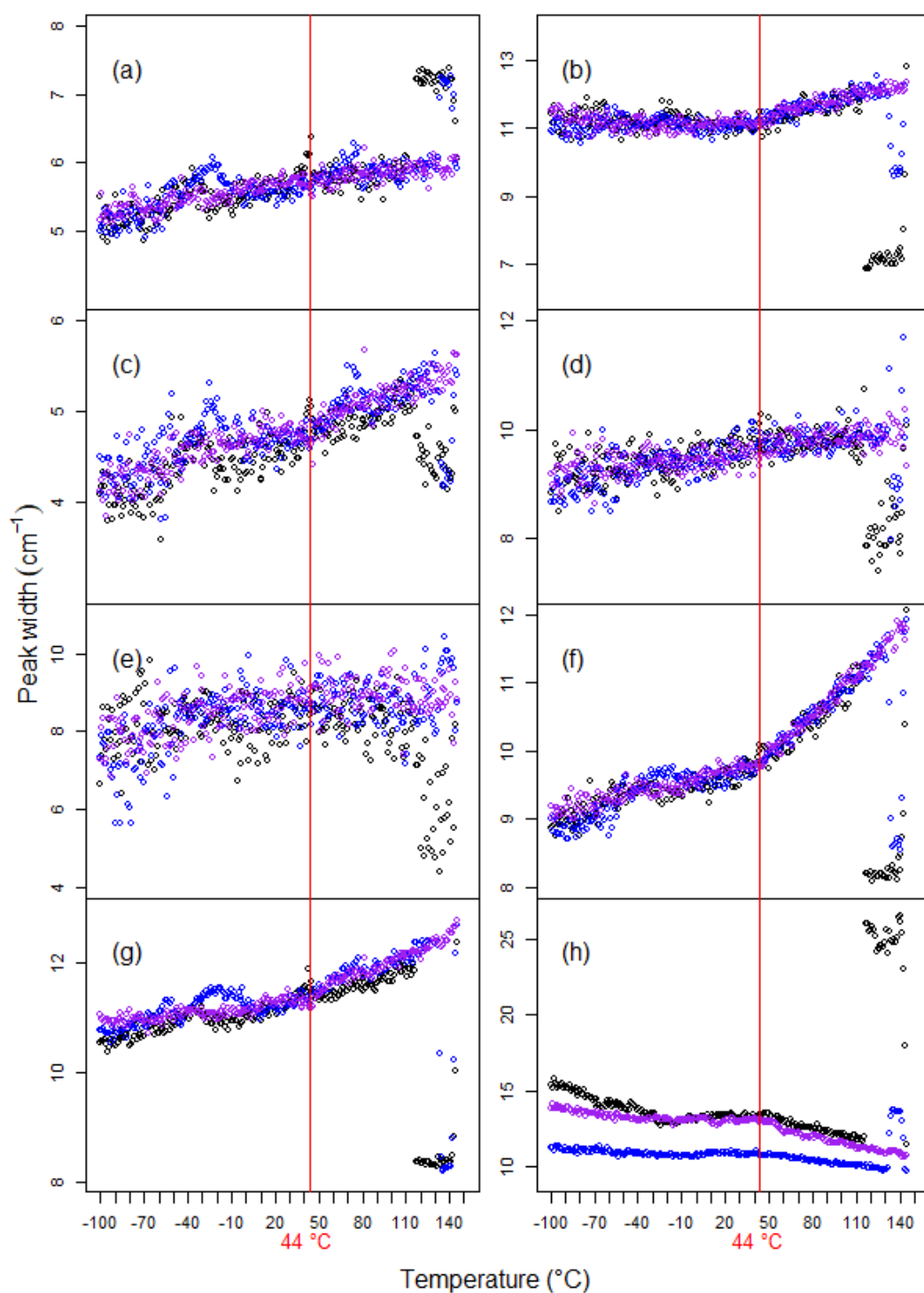


Figure 5.13. The peak fitting of width against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (44°C) transition temperature.

Experiment 1 (Figure 5.13, black dots) shows no obvious change to peak width in the non-crystalline region, but we see some subtle changes at around 44°C in peaks at 470 cm⁻¹ (C-O-C bend), 1049 cm⁻¹ (1,2 substituted ring) and 1648 cm⁻¹ (C=C stretching). We see obvious changes and discontinuities at around 120 and 140°C. In experiment 2 (Figure 5.13, blue dots), we observe some changes to peak width at around -30°C. Discontinuity is observed at around 140°C. Experiment 3 (Figure 5.13, purple dots) shows some changes to peak width at around 44°C in peaks at around 595 cm⁻¹ (in-plane ring deformation), 1049 cm⁻¹ (1,2 substituted ring), 1492 cm⁻¹ (aromatic C=C), 1648 cm⁻¹ (C=C stretching) and 1680 cm⁻¹ (C=O stretching). These changes in peak width are reasonable as we expect felodipine to go from amorphous to liquid at around 44°C, and liquid to crystal transition at around 140°C. Hence, for felodipine based upon these studies, we conclude that Raman band width as a function of temperature shows some detectable changes to disorder of structure that are associated with alpha relaxation.

5.4.3.3 Analysis of Gaussian fit curves of peak shift

Figure 5.14 present Gaussian fit curves of peak positions plotted as a function of temperature at around 8 selected vibrational modes (Table 5.1). We will proceed to review changes to peak position of felodipine sample captured across all temperatures to investigate potential structural changes caused by temperature change at around alpha, beta and other phase transition temperatures.

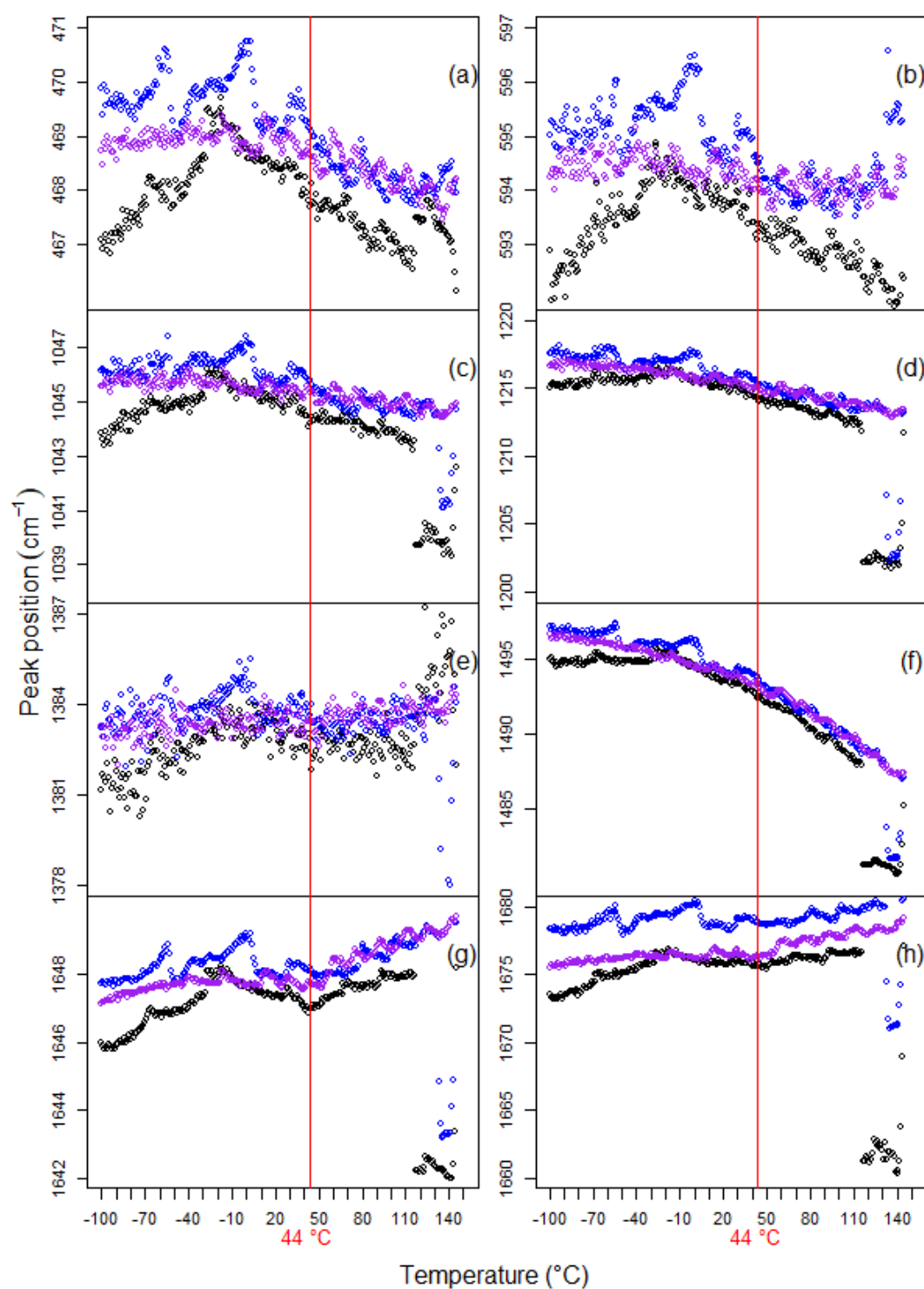


Figure 5.14. The peak fitting of position against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively. The red vertical lines pass through alpha (44°C) transition temperature.

In experiment 1 (Figure 5.14, black dots), we see changes to peak position at around -70°C which is not too far from the beta transition temperature of nifedipine (-66°C) and -30°C . We also see obvious changes and discontinuity at around 120 and 140°C . In experiment 2 (Figure 5.14, blue dots), we do see obvious changes to peak intensities in the non-crystalline region, at around 60°C , which is not too far from the beta transition temperature of nifedipine (-66°C). This change is not so prominent in peak at around 595 cm^{-1} , which corresponds to the in-plane ring deformation (Figure 5.14 (b)). We observe some changes and discontinuity in peak position at around 140°C . In experiment 3 (Figure 5.14, purple dots), the peaks generally show similar shift patterns from -100 to 140°C . The peaks fluctuate throughout the experiment. In all three experiments above, the most obvious changes in peak position are observed at around 70°C , at which peaks either show discontinuity or shift to different wavenumber. The findings on peak shifts were promising for the temperature at around the beta transition temperature but not the alpha transition temperature. In experiment 1 and 3, we observe changes around beta transition temperature of nifedipine. We conclude that for felodipine based upon the studies described above, the results do not support the reproducibility of the data in peak position of the Raman spectra in that they do not exhibit detectable changes around the alpha transition temperature but we do detect some changes around the beta transition temperature of nifedipine.

5.4.4 Low temperature repeat tests

From inspection of the contour maps and Gaussian peak fit curves from the previous section, some visual changes in peak intensity were observed for Raman spectra at around the non-crystalline region of felodipine. These results were not obviously reproducible in terms of the peak width and peak position. There seemed to be sample to sample variation in each of the experiments which was not expected given the samples were prepared in the same way, so the temperature cycling experiments of a single sample (three

repeats for each sample) were repeated at low temperature (-100 to 0°C) to test the reproducibility of the data around the beta transition temperature. Three sets of experiments were repeated, and each experiment consisted of three heat - cool - heat cycling experiments at low temperature.

5.4.4.1 Heat map – low temperature repeat tests

Figure 5.15 to Figure 5.22 are the key figures of the contour maps of the normalised Raman spectra collected for a quench-cooled felodipine in low temperature repeat experiments. The figures were plotted as a function of temperature similar to Section 5.4.2, but during heating of the sample from -100 to 0°C. The scaled intensities are plotted on the colour scale, similar to those in Section 3.4.2. Figure 5.16 and Figure 5.17 are the zoomed-in version of the low (0 to 400 cm^{-1}) and high (1200 to 1700 cm^{-1}) wavenumber region of the Figure 5.15. The high (1200 to 1700 cm^{-1}) region was selected due to presence of high intensity peaks. On the other hand, the low region (0 to 400 cm^{-1}) was zoomed-in in an effort to make comparison with other plots of similar wavenumber range in the previous chapter on nifedipine.

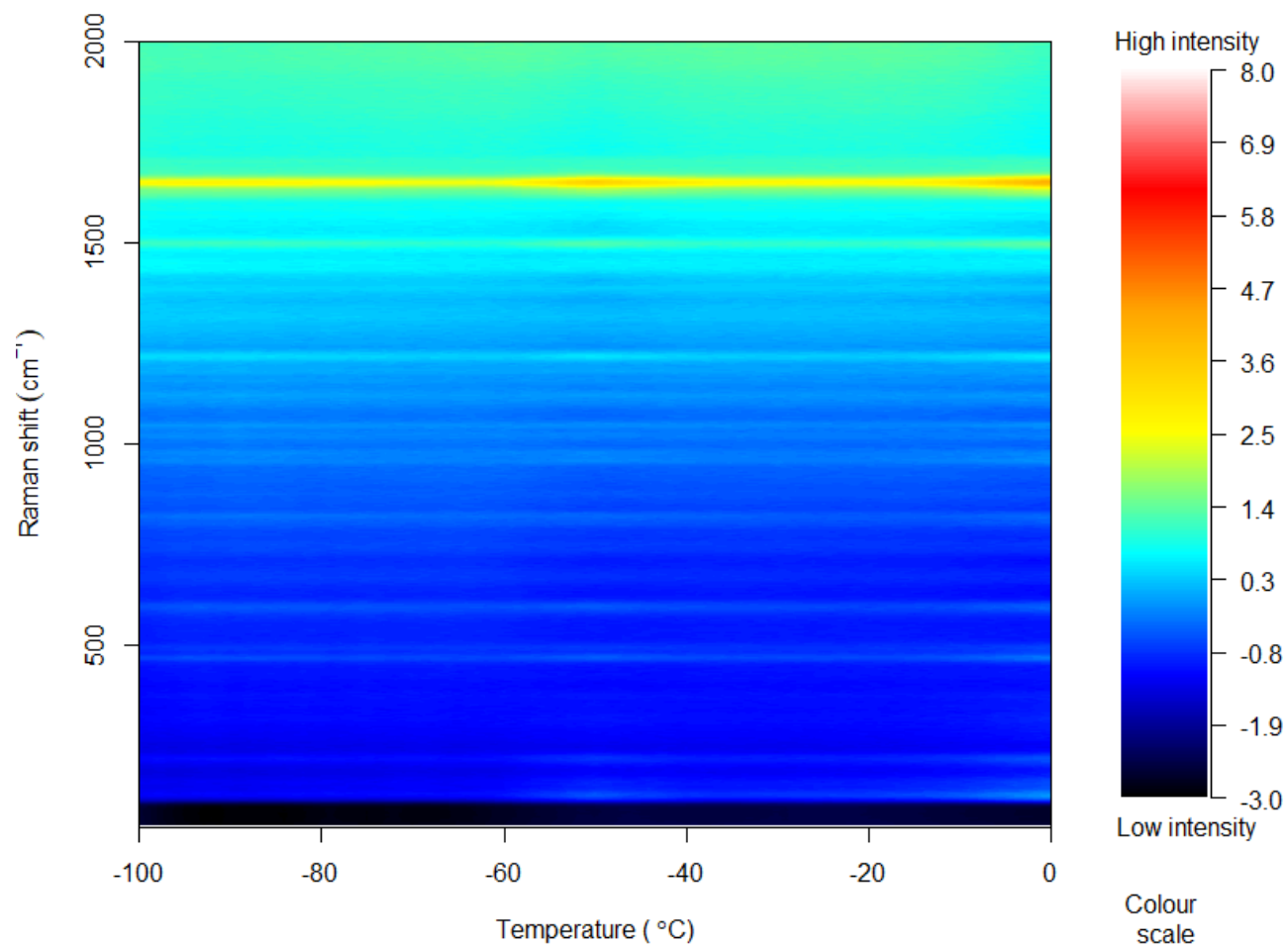


Figure 5.15. Low temperature felodipine repeat experiment 1 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

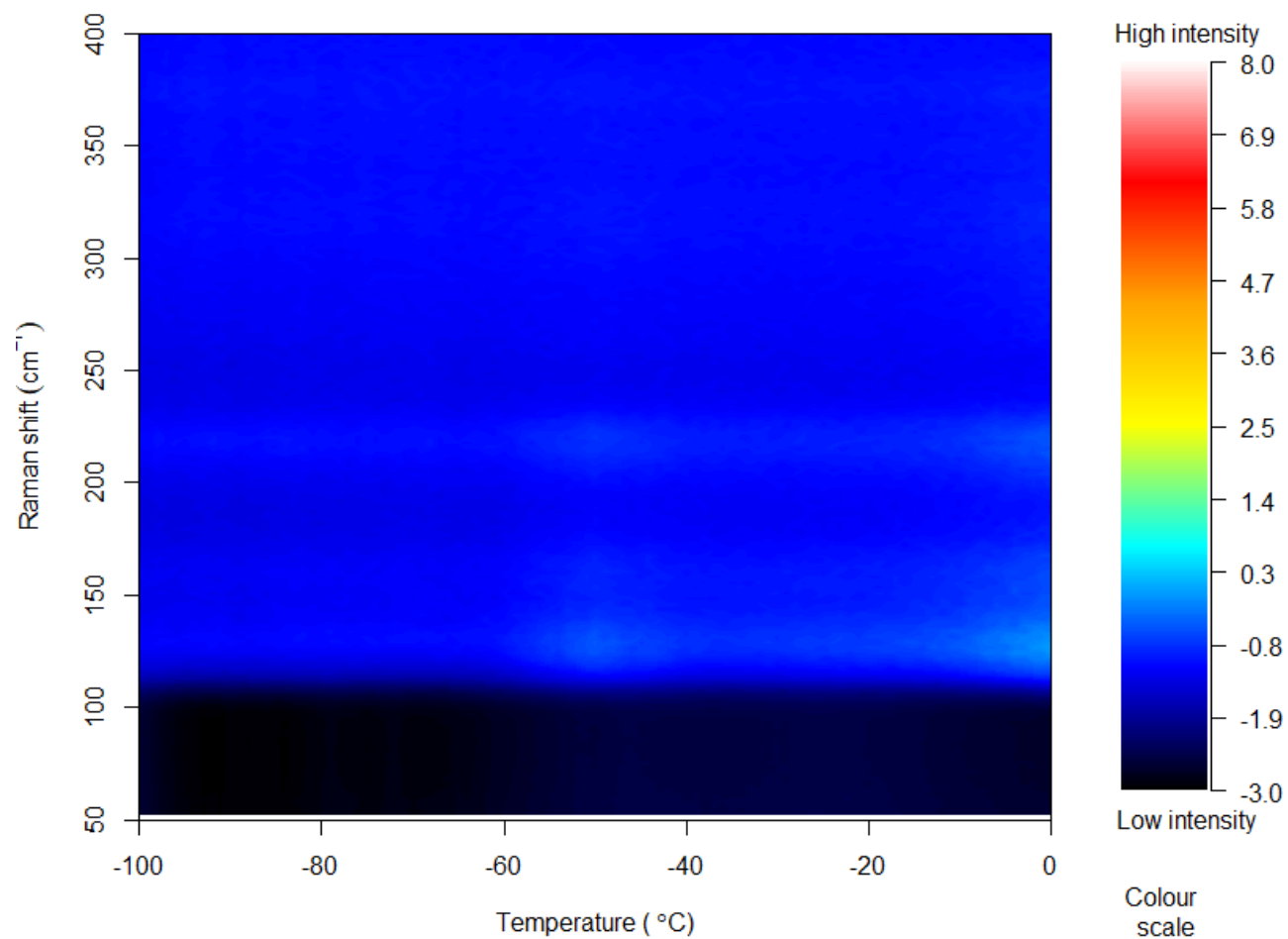


Figure 5.16. Low temperature felodipine repeat experiment 1 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

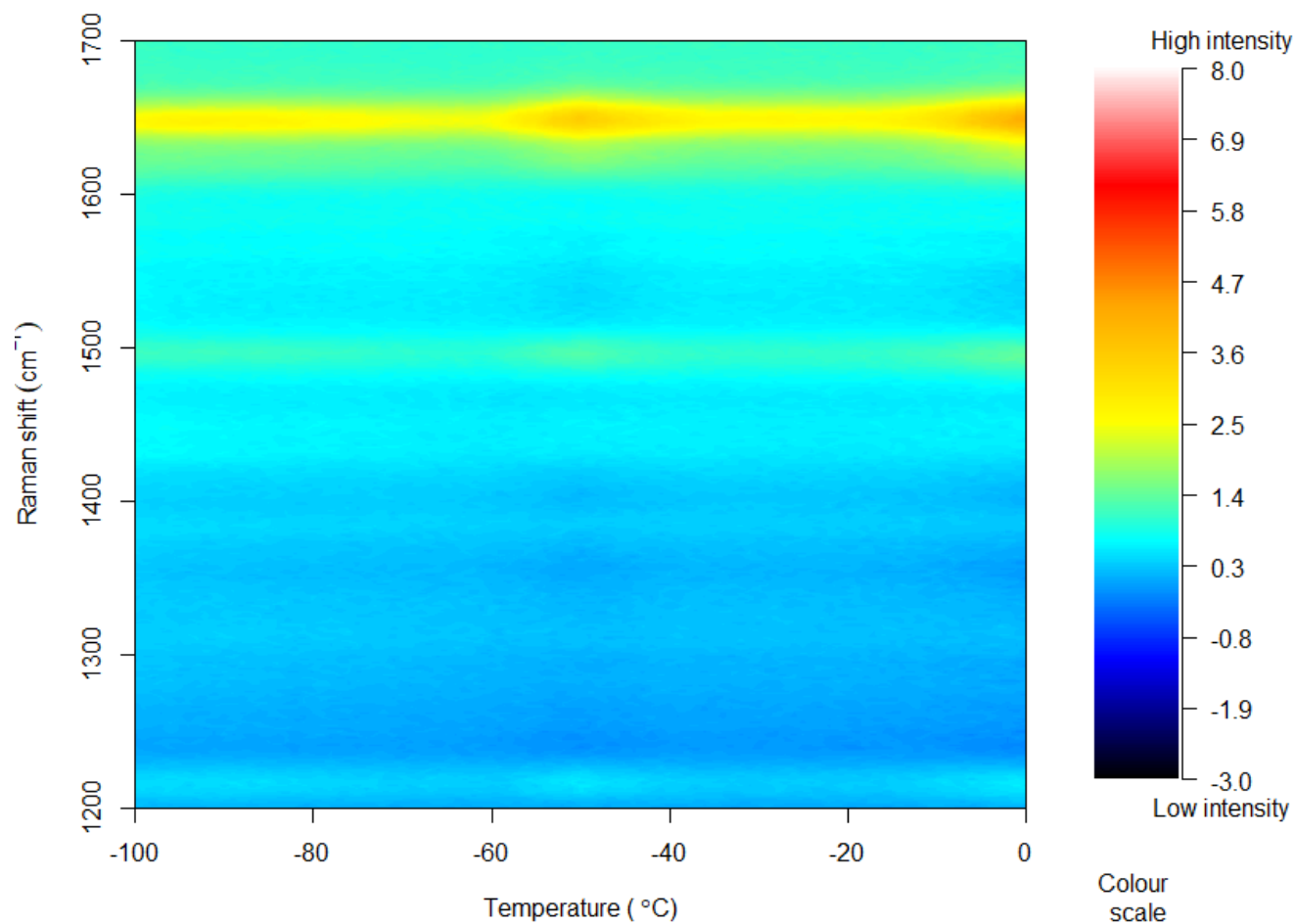


Figure 5.17. Low temperature felodipine repeat experiment 1 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

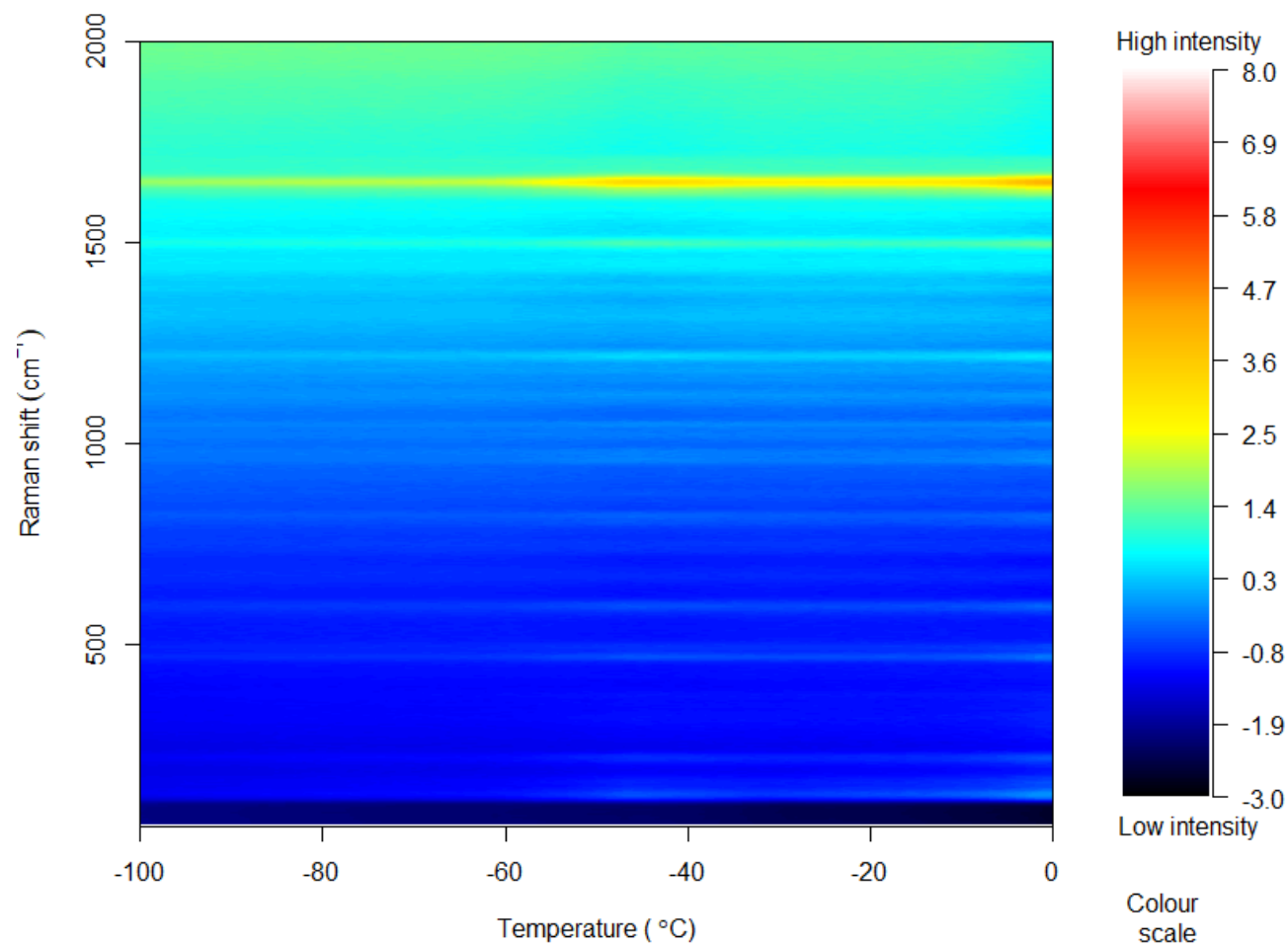


Figure 5.18. Low temperature felodipine repeat experiment 1 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

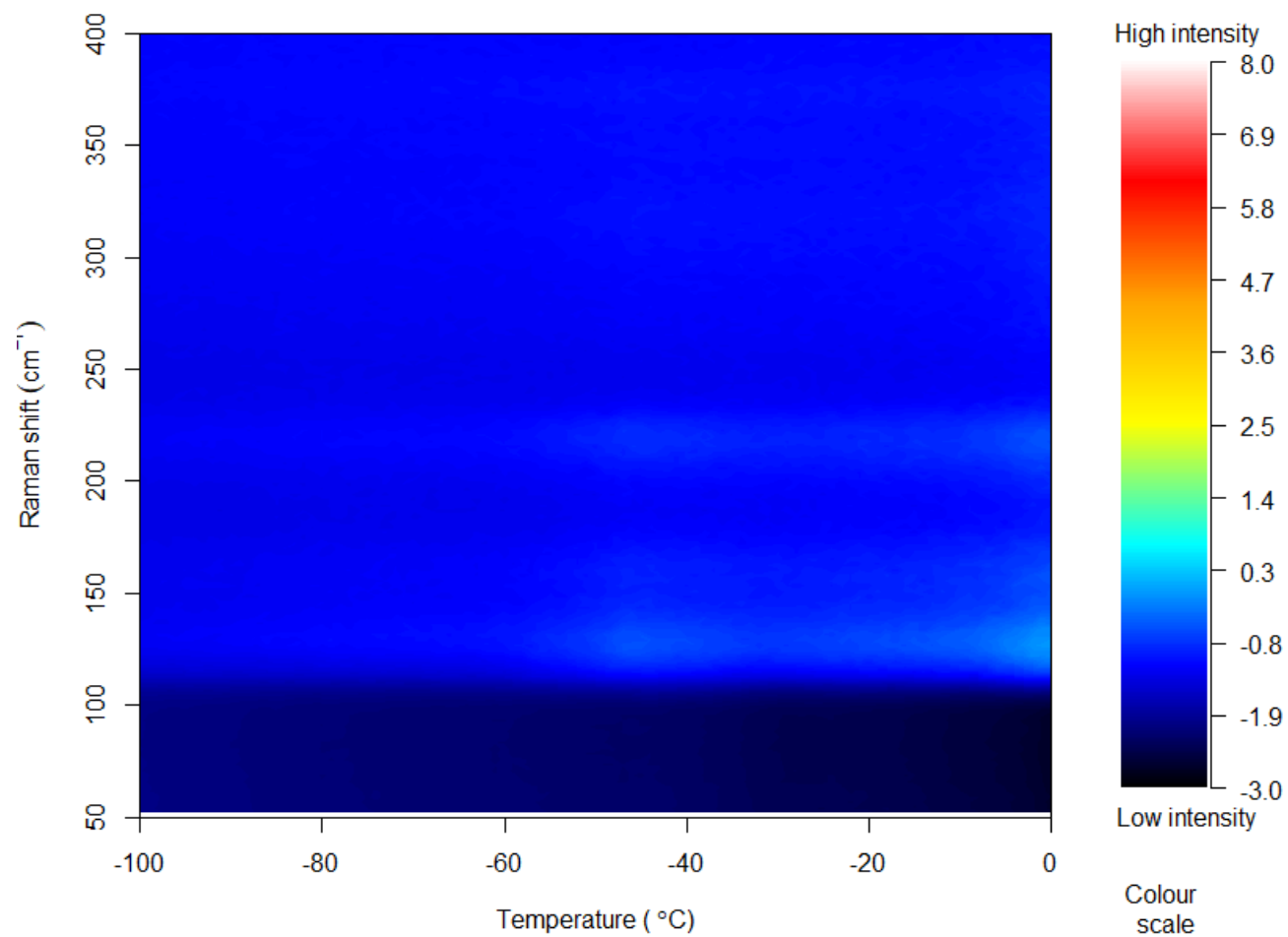


Figure 5.19. Low temperature felodipine repeat experiment 1 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

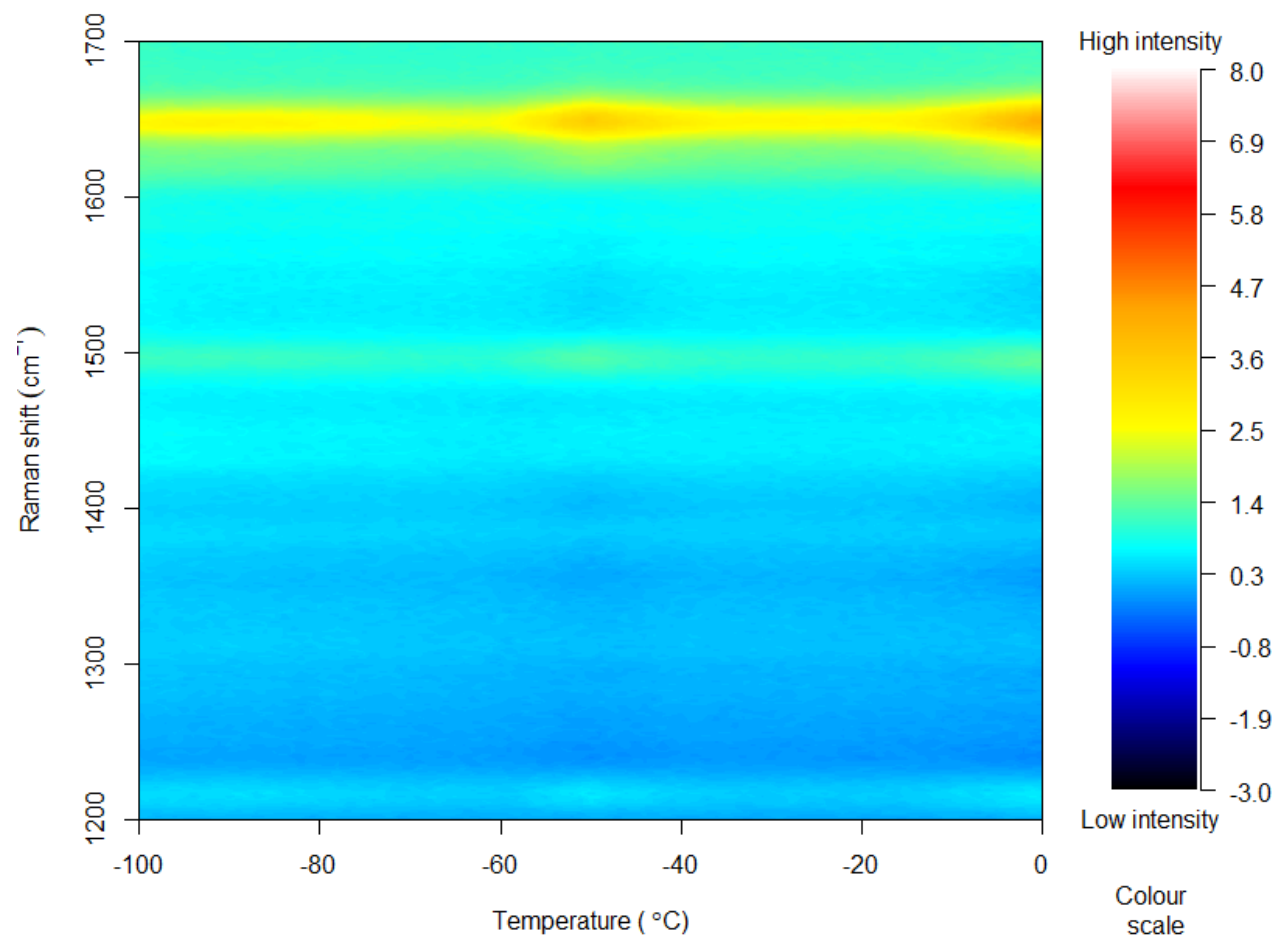


Figure 5.20. Low temperature felodipine repeat experiment 1 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

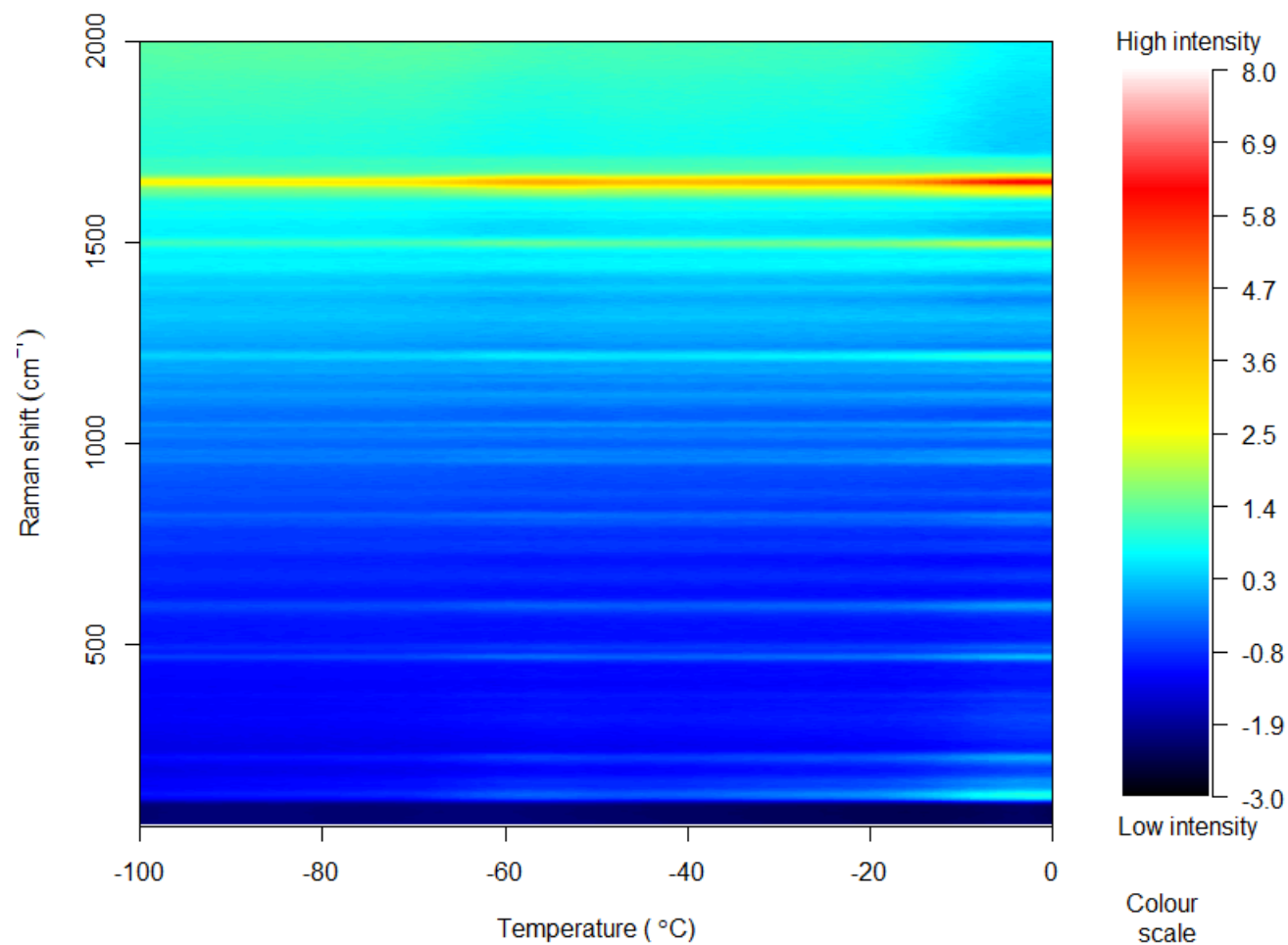


Figure 5.21. Low temperature felodipine repeat experiment 1 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

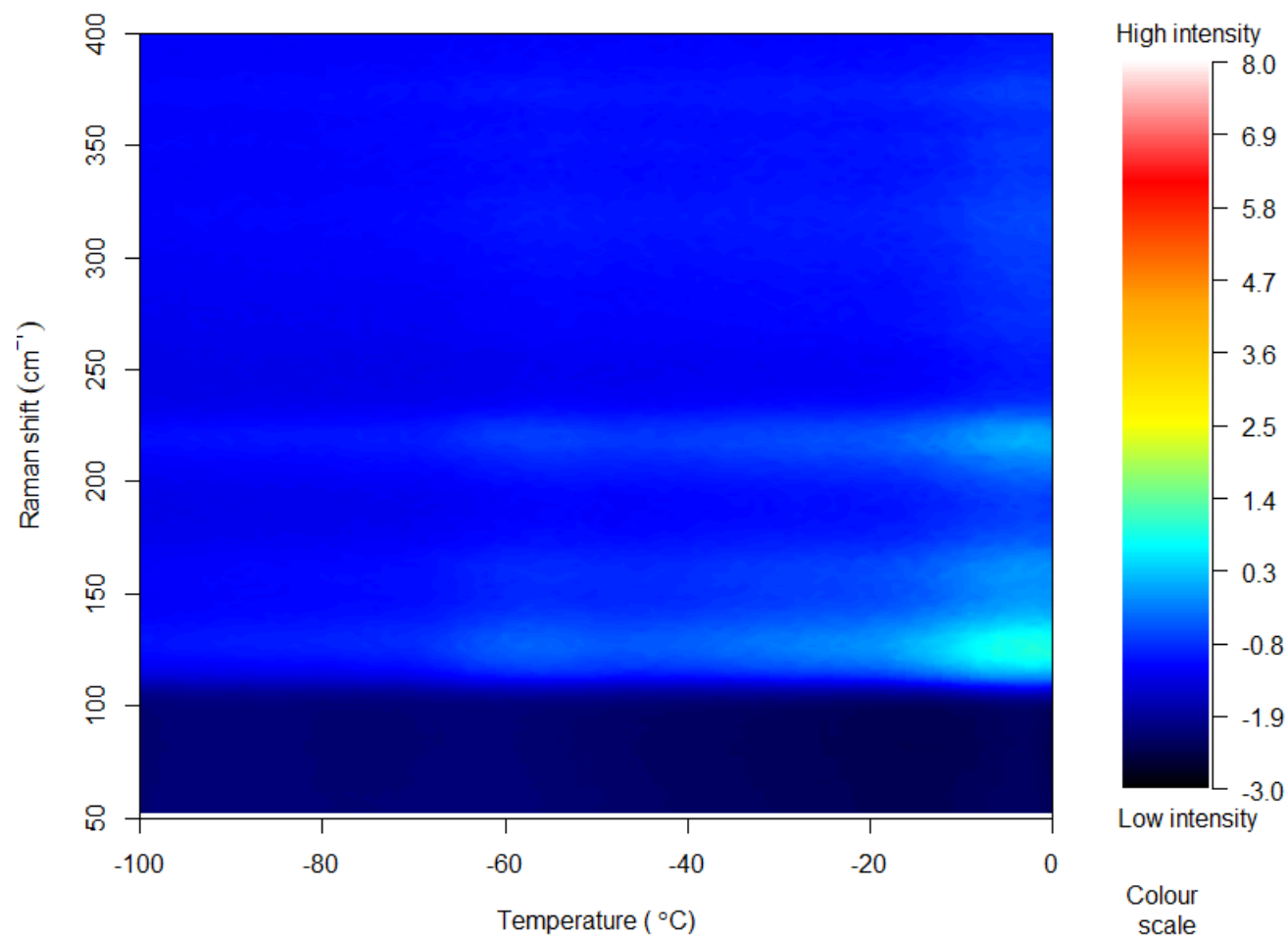


Figure 5.22. Low temperature felodipine repeat experiment 1 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

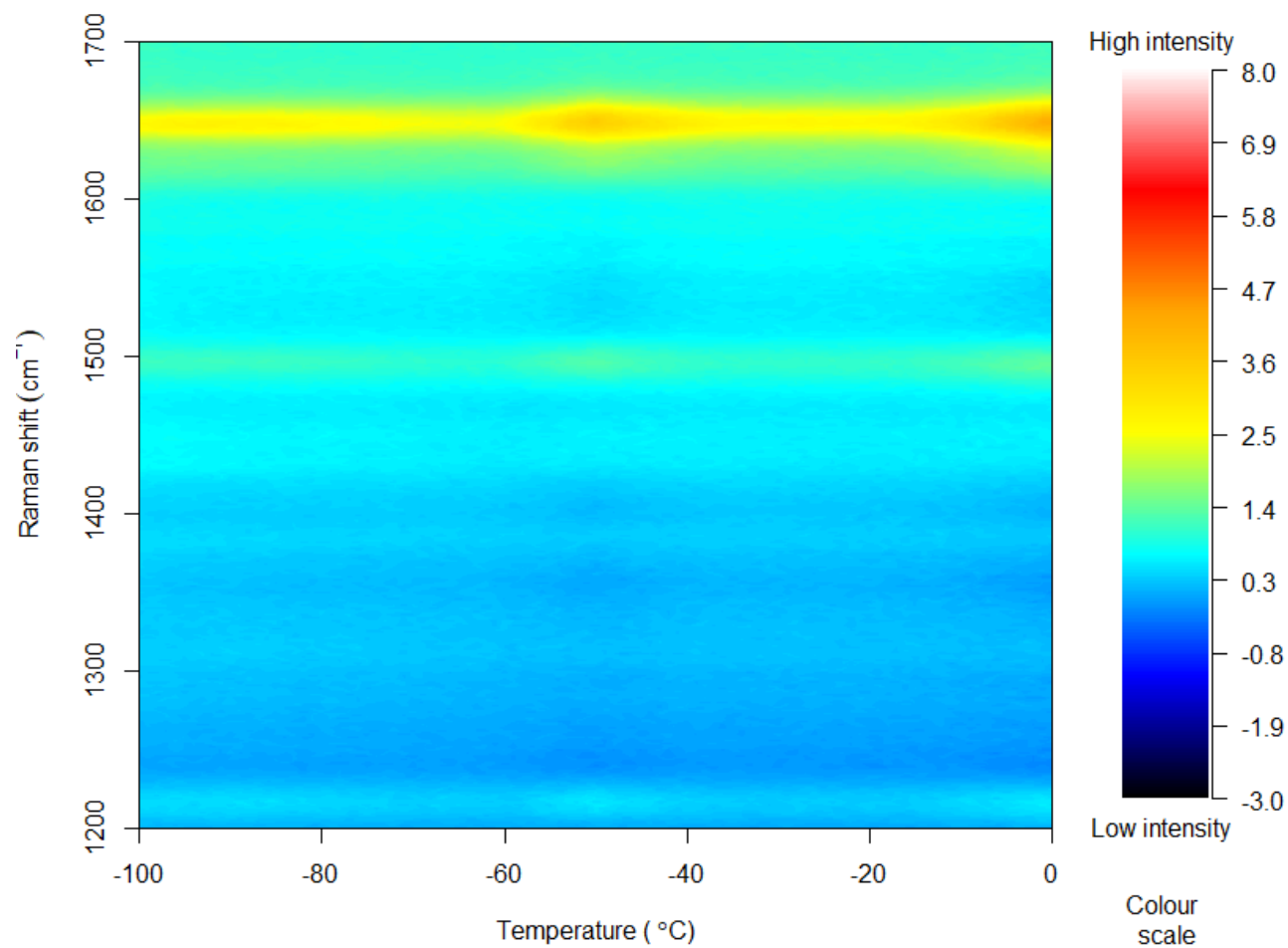


Figure 5.23. Low temperature felodipine repeat experiment 1 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

In cycle 1 of experiment 1 (Figure 5.15, Figure 5.16 and Figure 5.17), there is no obvious change observed around the beta (-66°C) transition temperature, therefore repeated the experiment of the same quench-cooled nifedipine sample by re-freezing and reheating, for the second (**Error! Reference source not found., Error! Reference source not found. and Error! Reference source not found.**) and the third (**Error! Reference source not found., Error! Reference source not found. and Error! Reference source not found.**) time. No obvious changes were observed in the second and third repeat cycle. We repeated two more sets of the experiments but did not see obvious changes around the beta transition temperature. The plots of the experiment 2 (Figure D 1 to Figure D 9 of Appendix D) and experiment 3 (Figure D 10 to Figure D 18 of Appendix D) are found in the appendix. Hence, we cannot fully conclude that for felodipine based upon these studies, temperature could lead to detectable changes in Raman scattering of functional groups that are associated with the beta transition temperature.

5.4.4.2 Analysis of Gaussian fit curves of peak intensity in low temperature repeat tests

We have seen the contour maps of the low temperature repeat experiments and we proceed to test the reproducibility of the data in the low temperature region of felodipine. The spectral peaks from the low temperature repeat experiments were analysed to see different Raman features in the low temperature region of felodipine in the 8 selected intramolecular vibrational modes (Table 5.1). Figure 5.24, Figure 5.25 and Figure 5.26 show Gaussian fit curves of peak intensities plotted as a function of temperature of cycle 1, 2 and 3, respectively. Each dot on the scatterplot represents peak intensity of selected peaks in the range of -100 to 0°C. The black, blue and purple dots represent low temperature experiment 1, 2 and 3, respectively.

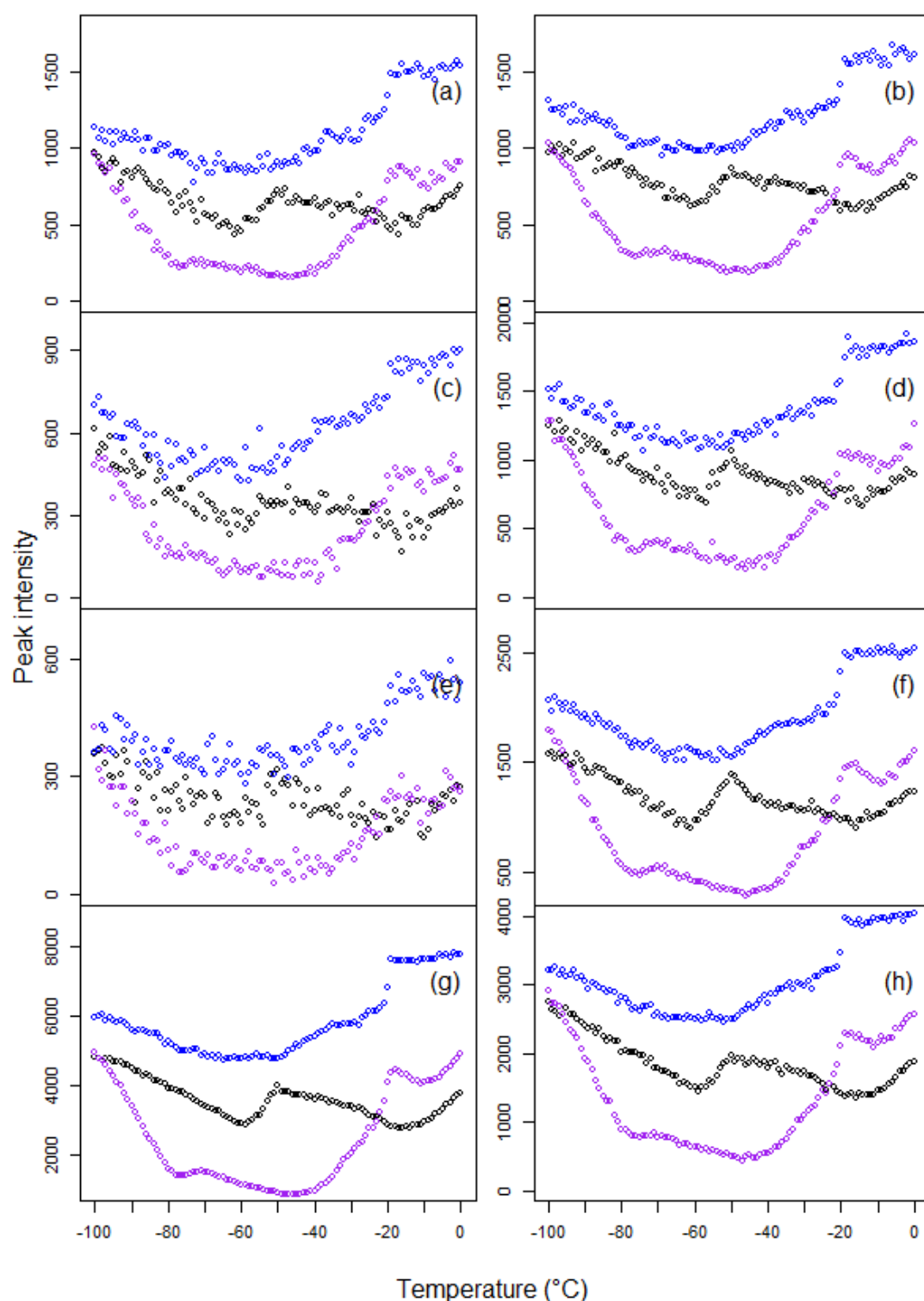


Figure 5.24. Low temperature experiment (cycle 1) - The peak fitting of intensity against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

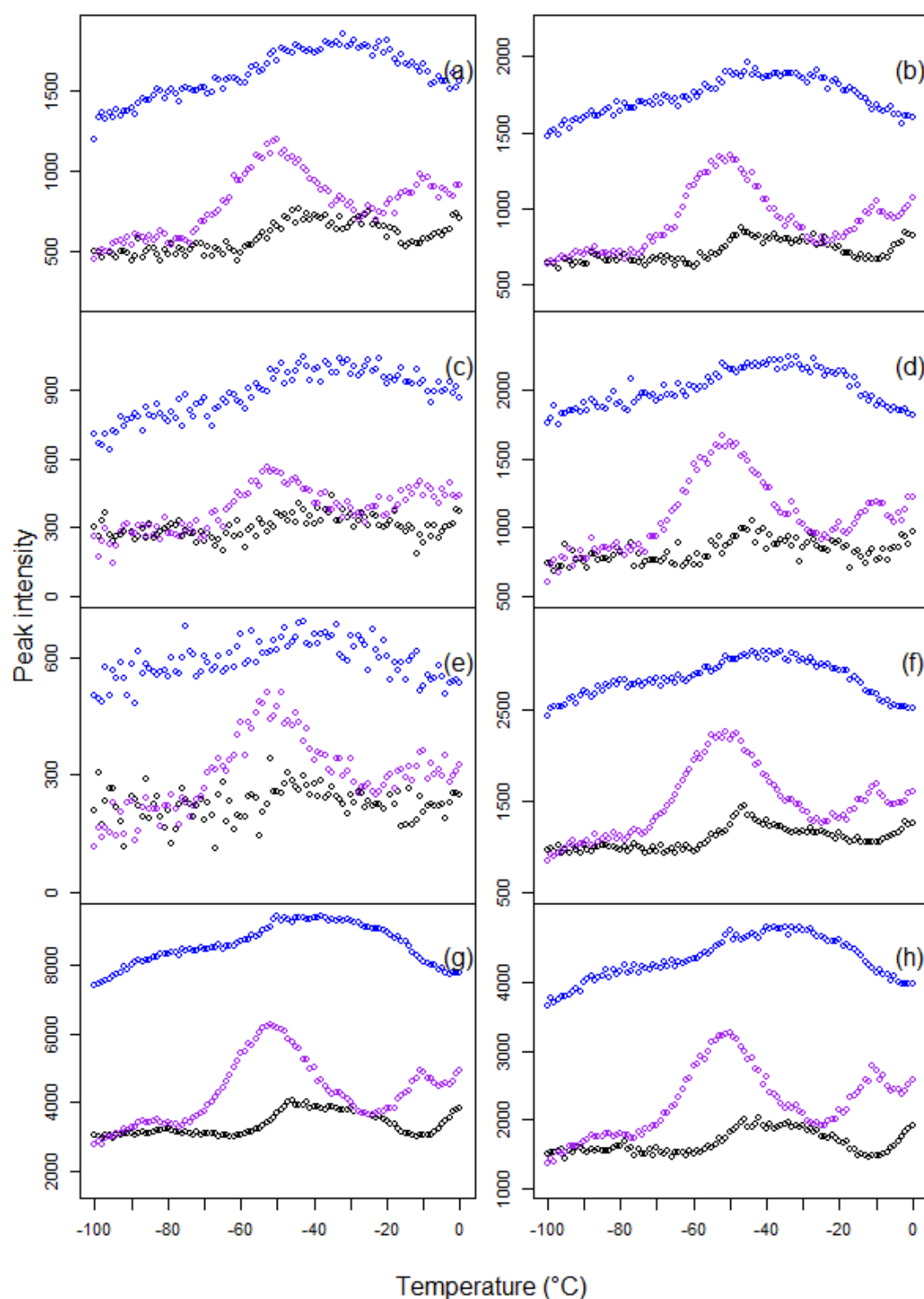


Figure 5.25. Low temperature experiment (cycle 2) - The peak fitting of intensity against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

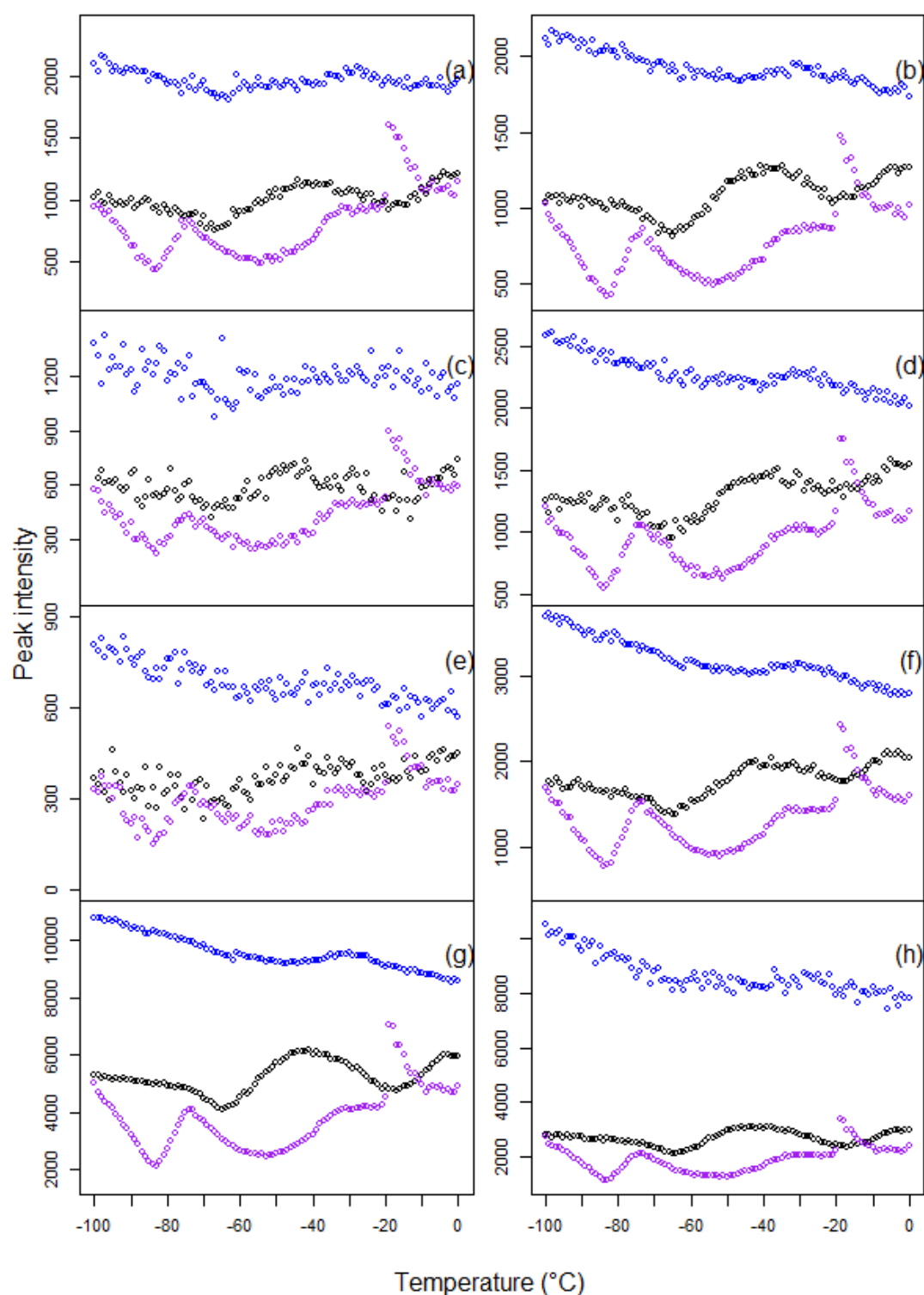


Figure 5.26. Low temperature experiment (cycle 3) - The peak fitting of intensity against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

Cycle 1 (Figure 5.24) shows U-shaped curve with an obvious change at around -20°C. In cycle 2, only experiment 3 shows obvious change to peak intensity around -50°C (Figure 5.25, purple dots). Cycle 3 of experiment 1 (Figure 5.26, black dots) shows obvious change at around -85, -75 and 20°C, whereas in experiment 3 (Figure 5.26, purple dots), we observe some changes at around -65°C. We could observe changes in peak intensities at around -80 and -20°C in most peaks but the Gaussian fit curves of peak curves do not fully support reproducibility of the data. Given the figures are not reproducible, we conclude that for felodipine based upon these studies, the beta temperature does not appear to cause detectable changes in Raman scattering of functional groups.

5.4.4.3 Analysis of Gaussian fit curves of peak width in low temperature repeat tests

The spectral peaks from the low temperature repeat experiments were analysed to see potential changes to Raman bandwidth caused by beta transition temperature at -66°C in the 8 selected intramolecular vibrational modes (Table 5.1). Figure 5.27, Figure 5.28 and Figure 5.29 show Gaussian fit curves of peak width plotted as a function of temperature of cycle 1, 2 and 3, respectively.

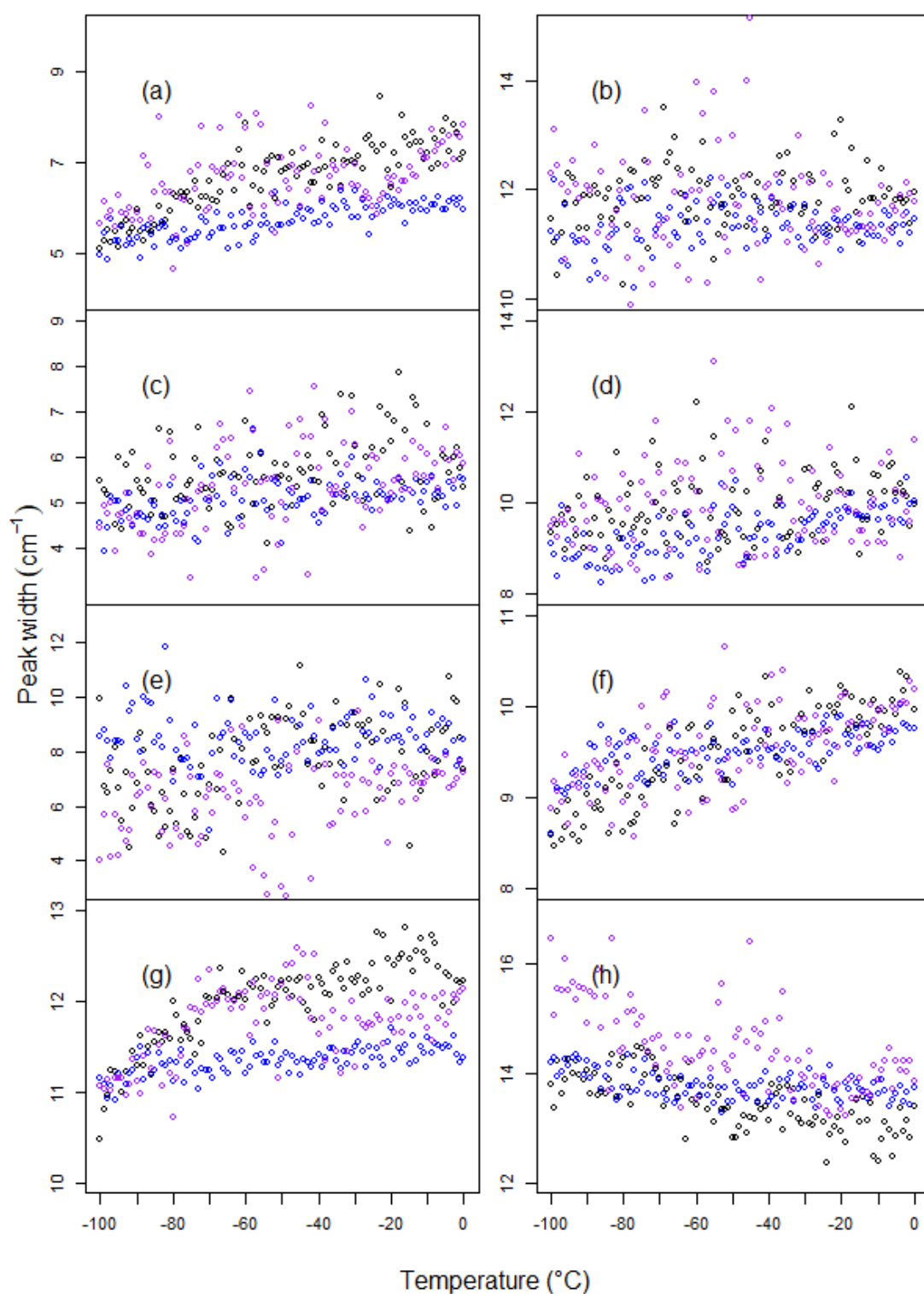


Figure 5.27. Low temperature experiment (cycle 1) - The peak fitting of width against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

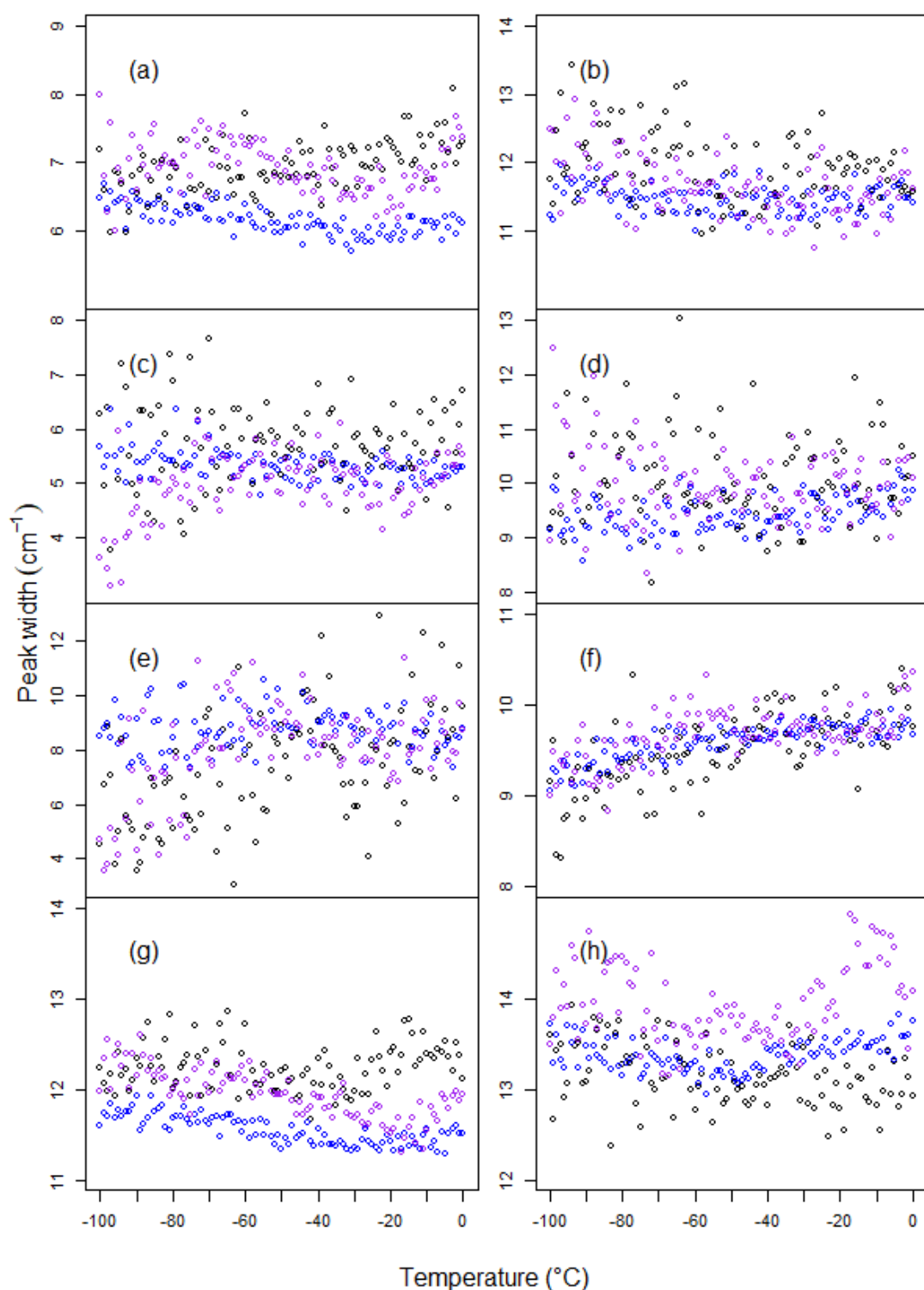


Figure 5.28. Low temperature experiment (cycle 2) - The peak fitting of width against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

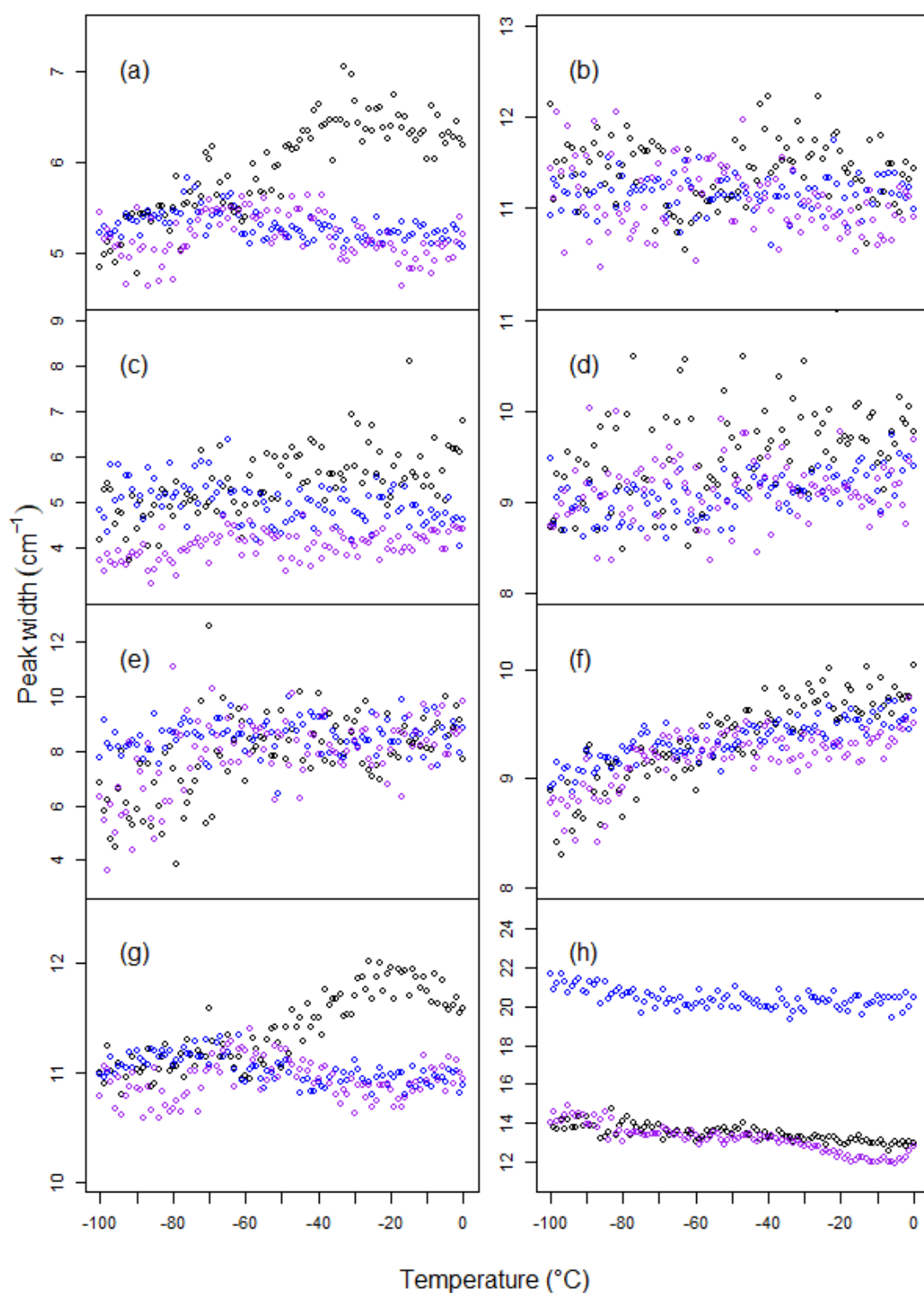


Figure 5.29. Low temperature experiment (cycle 3) - The peak fitting of width against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

In Figure 5.27, Figure 5.28 and Figure 5.29, we observe no obvious changes in peak width. This is reasonable as we see no change to the non-crystalline region in the previous experiments (Figure 5.13). For felodipine based upon these studies, we conclude that disorder of the system may not be affected by beta transition temperature at -66°C because the widths of the Raman peaks do not seem to show detectable changes in that temperature range.

5.4.4.4 Analysis of Gaussian fit curves of peak shift in low temperature repeat tests

The Gaussian fit curves of peak position of felodipine were plotted to investigate different Raman features in the 8 selected intramolecular vibrational modes (Table 5.1). Figure 5.30, Figure 5.31 and Figure 5.32 show Gaussian fit curves of peak position plotted as a function of temperature of cycle 1, 2 and 3, respectively.

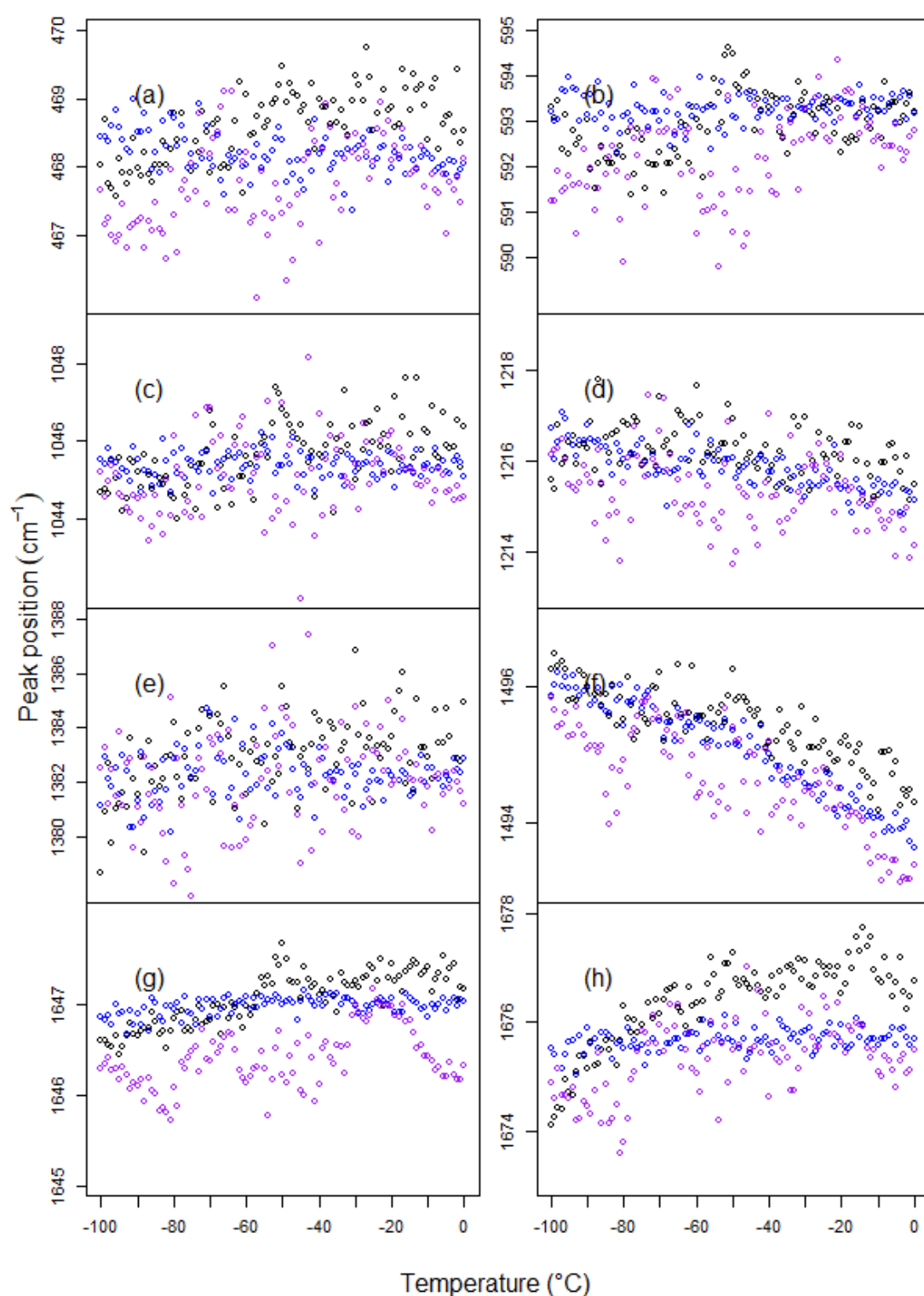


Figure 5.30. Low temperature experiment (cycle 1) - The peak fitting of position against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

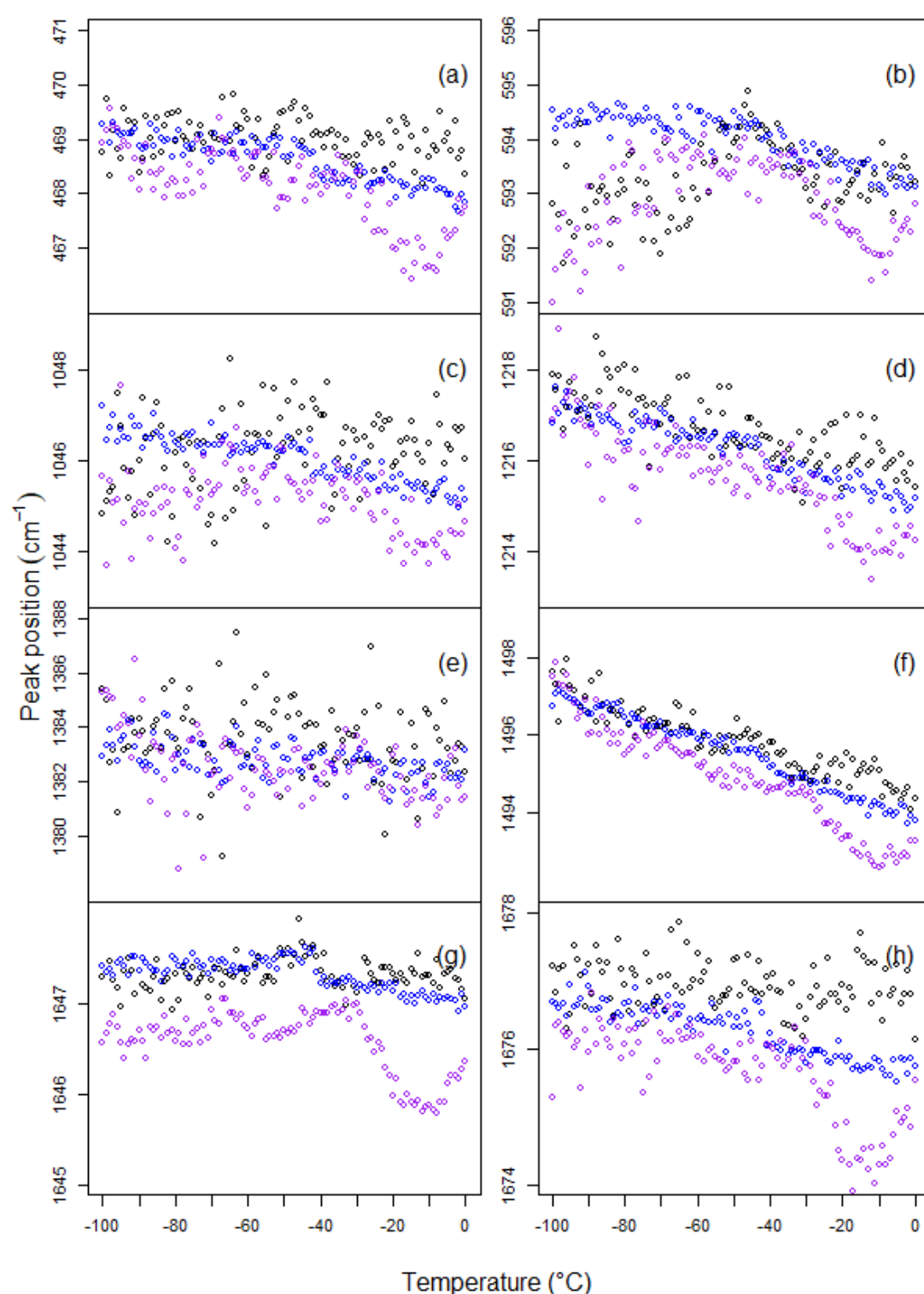


Figure 5.31. Low temperature experiment (cycle 2) - The peak fitting of position against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

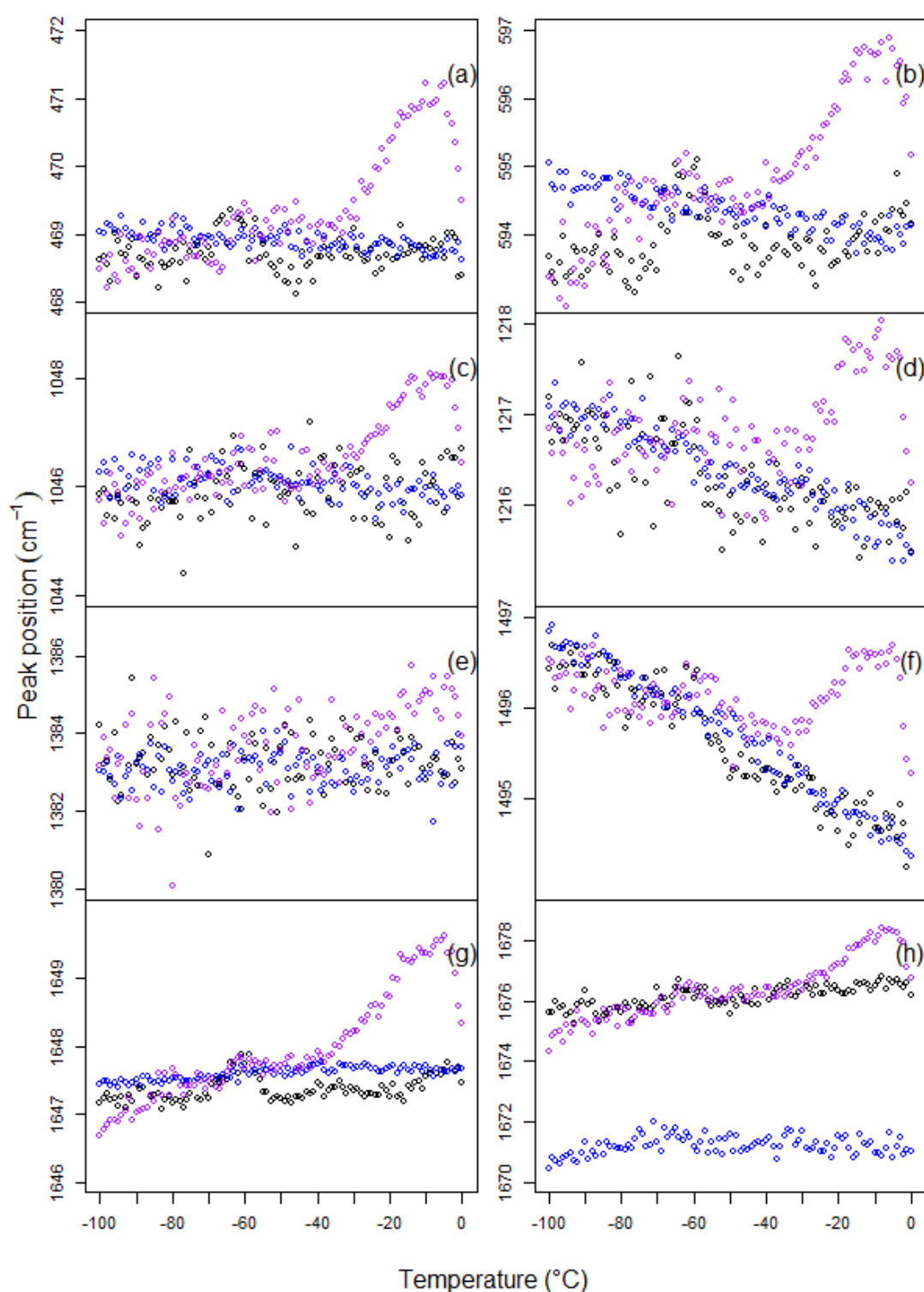


Figure 5.32. Low temperature experiment (cycle 3) - The peak fitting of position against temperature for the 10 selected peaks of felodipine: 470 cm^{-1} , 595 cm^{-1} , 1049 cm^{-1} , 1224 cm^{-1} , 1380 cm^{-1} , 1492 cm^{-1} , 1648 cm^{-1} , and 1680 cm^{-1} . The peak positions are approximate. The black, blue and purple dots represent experiment 1, 2 and 3, respectively.

Cycle 1 (Figure 5.30) shows peaks that are unevenly distributed. Cycle 2 (Figure 5.31, purple dots) and cycle 3 (Figure 5.32, purple dots) of experiment 3 show obvious changes at around -20°C . This is in agreement with some of the changes in peak intensities that were observed at around -20°C in the previous figures (Figure 5.14). The peak shift figures of the low temperature experiments do not show any obvious change around -60°C . Hence, we conclude that for felodipine based upon the studies described above, structural changes do not seem to occur at the beta transition temperature around -66°C .

5.5 Conclusion

This chapter showed detailed study to see any changes around alpha (44°C) and beta (-66°C) relaxation of felodipine in heating the amorphous state from Raman spectroscopy. We summarised the findings from Gaussian fit curves of peak intensity, width and position of nifedipine in Table 5.2.

While we have performed detailed studies to see any detectable changes around alpha (44°C) and beta (-66°C) transition temperatures of felodipine in heating the amorphous state from Raman spectroscopy, we do see some changes in peak intensity that are reproducible at around the alpha and beta transition temperature. When we did single variable temperature Raman experiment, some changes in peaks intensities were observed around the beta transition temperature and having conducted three experiments on different samples, the results were still reproducible. When we did the low temperature cycling experiment, we did not see reproducibility in the low temperature region.

We do not see any changes to peak width around the beta transition temperature but saw some subtle changes in peak width around the alpha transition temperature in two of the three experiments (Experiment 1 and 3). The findings were not particularly reproducible in that we only see detectable changes in two of the three repeated experiments. This is surprising in light of

the JG model which predicts inhomogeneous chemical environment, but we expected to see some changes during solid to liquid transition (at around the alpha transition temperature). Furthermore, we do see some changes at around the beta transition temperature in experiment 1 and 3, but the changes were not reproducible when we did the low temperature repeat experiments.

For felodipine based upon the studies described above, we cannot fully conclude that there is no solid evidence for detectable change to the Raman spectra around the alpha and beta transition temperature. Given the previous chapter showed no obvious around the alpha transition in paracetamol or nifedipine, we did not expect to observe changes at around those temperature. We have looked at nifedipine and felodipine, which make a good model system, but lack of reproducibility of the data made it difficult to further study.

Table 5.2. Summary of the results from variable temperature set up Raman experiments of paracetamol (The symbol “o” indicates presence of visual changes; “x” indicates absence of visual change)

Experiment No.		Change around beta transition temperature			Change around alpha transition temperature		
		Peak intensity	Peak width	Peak position	Peak intensity	Peak width	Peak position
Experiment 1		o	x	o	o	o	x
Experiment 2		o	x	o	o	x	x
Experiment 3		o	x	x	o	o	x
Low temperature experiment 1	Cycle 1	o	x	x			
	Cycle 2	x	x	x			
	Cycle 3	o	x	x			
Low temperature experiment 2	Cycle 1	x	x	x			
	Cycle 2	x	x	x			
	Cycle 3	x	x	x			
Low temperature experiment 3	Cycle 1	x	x	x			
	Cycle 2	x	x	x			
	Cycle 3	x	x	x			

Chapter 6 Conclusions and outlook

6.1 Overall conclusions

We have performed the most detailed study to date using vibrational spectroscopy of the alpha and beta relaxation. While we have performed detailed studies to see any changes around alpha and beta transition temperature of paracetamol, nifedipine and felodipine, we do not see any changes that are obviously reproducible around the temperature of these transitions.

We have looked at a selection of peaks from a variety of chemical functionalities and no obvious change to peak intensity, width and position is seen in the non-crystalline region as a function of temperature. While some changes were observed in some individual experiments around the alpha and beta transition temperatures, they were not particularly reproducible when we did the low temperature repeat tests. In the variable temperature Raman experiments, some changes in peaks are observed around the reported beta transition temperature but having conducted three experiments on different samples, and cycling experiment on the same sample, we can conclude that there is no obvious and reproducible evidence of any spectroscopic changes associated with the beta or alpha relaxation temperatures.

Changes in peak intensity are not particularly reproducible between experiments in the present study and it may be that the intensities are more subject to external fluctuations, e.g., room temperature or icing on Linkam stage. The changes are not related to order or disorder of the system because the peak width did not seem to change. The changes in peaks are not obviously related to any structural changes occurring because the peaks shift data were not reproducible. Therefore, we could conclude that for paracetamol, nifedipine and felodipine based upon the studies described above, there is no strong evidence for change in peak intensity, width and position to the Raman spectra around the alpha and beta transition temperature.

For alpha transition, where there are massive changes in the physical properties from a solid that does not flow to a liquid that does flow, it is surprising to see no changes as we would expect at the alpha transition to see changes in the peak widths. Even the crystal transitions among the polymorphs do not exhibit properties that change as much - they stay solid. From the results and analysis of our findings, we found no reproducible evidence of obvious spectroscopic changes in paracetamol, nifedipine and felodipine around the alpha transition temperatures, but we did see clear and obvious and reproducible changes when the transitions involve crystalline forms, whether it is non-crystalline to crystalline form or between the different crystalline forms in accord with the literature.

It is notable to observe that changes have been spotted in peak positions around beta transition temperature but not around at alpha transition temperatures given glass is similar to liquid (**Error! Reference source not found.**, **Error! Reference source not found.** and **Error! Reference source not found.**). We observed some detectable changes to peak width at around the alpha transition temperature, only in nifedipine and felodipine, but not in paracetamol (**Error! Reference source not found.** and **Error! Reference source not found.**). These findings were not particularly reproducible in that we did not see detectable changes in all of the repeated experiments. Alpha transition temperature is typically determined by thermal analysis techniques, such as differential thermal analysis (DTA) differential scanning calorimeter (DSC), thermomechanical analysis (TMA) and dynamic mechanical analysis (DMA) (Materials Characterization by Thermomechanical Analysis, 1991). During alpha relaxation, if heat flows in, there is more freedom of motion into which the heat can be distributed. Hence, there is an increase in heat capacity, which is detected in thermal analysis. On the other hand, in our study, structural changes at the alpha relaxation are undetectable, which presumably indicates that the structure of the amorphous glass and the viscous liquid are extremely similar.

Table 6.1. Previous studies on detection of alpha or beta transition temperatures using vibrational spectroscopy

Sample	Method	Alpha transition temperature	Beta transition temperature	Citation
poly(ethylene terephthalate)	FTIR	○	×	(Zhang et al, 2004)
poly(p-phenylene sulfide)	FTIR	○	×	(D'Ilario & Martinelli, 2006)
poly(L-lactide)	FTIR	○	×	(Pan et al, 2008)
polystyrene (PS)	FTIR	○	×	(Liang & Huang, 2012)
polystyrene (PS) film	Raman spectroscopy	○	×	(Liem, 2004)
poly(ethylene terephthalate)	Raman spectroscopy	×	×	(Hafsia et al, 2016)
nylon 6,6	Raman spectroscopy	○	○	(Bertoldo Menezes et al, 2016)

Our study differs significantly from previous literatures where the alpha and/or beta relaxations have been detected via vibrational spectroscopy (Table 6.1), possibly due to:

- We have looked at a different sample (i.e., small molecules instead of polymers).
- The experiments presented in this thesis are repeats. We have seen some effects that we could probably claim if we had one experiment related to beta transition, but they are not reproducible.
- The samples studied in the present work were prepared and analysed in a short period of time (a few tens of minutes) whereas the literature on polymers has invariably used pre-prepared samples which had been stored in the amorphous state for months or longer.

The obvious difference is that we are looking at small molecules, instead of polymers. The change of heat capacity or signal on heat flow at T_g is usually prominent in polymers (Liu et al, 2009). Polymers in amorphous forms, exhibit a distinct glass transition behaviour due to the presence of disordered, random arrangement of polymer chains.

All the experiments in this thesis were done in triplicates to ensure the validity and accuracy of the data. Experiments without reproducibility cannot be reliably replicated or confirmed by other researchers using the same methods, materials, and conditions. Such experiments could be considered unreliable and raise concerns about the validity and credibility of the reported

findings. Our results show discrepancy because we had to reproduce them three times but report limitations of the study and potentially provide valuable insights for future research.

The amorphous polymers possibly had the opportunity to undergo relaxation during their storage period and which our samples did not because our samples were only available in crystalline forms and therefore we had to make them amorphous in every experiments with no extended relaxation period. Longer annealing times can lead to more significant changes in the structure. Extended annealing periods have been reported to allow for more complete relaxation of the polymer chains, resulting in a more prominent and well-defined T_g and enthalpic recovery. The relevant figure is shown in Figure 6.1(Menczel, 2009).

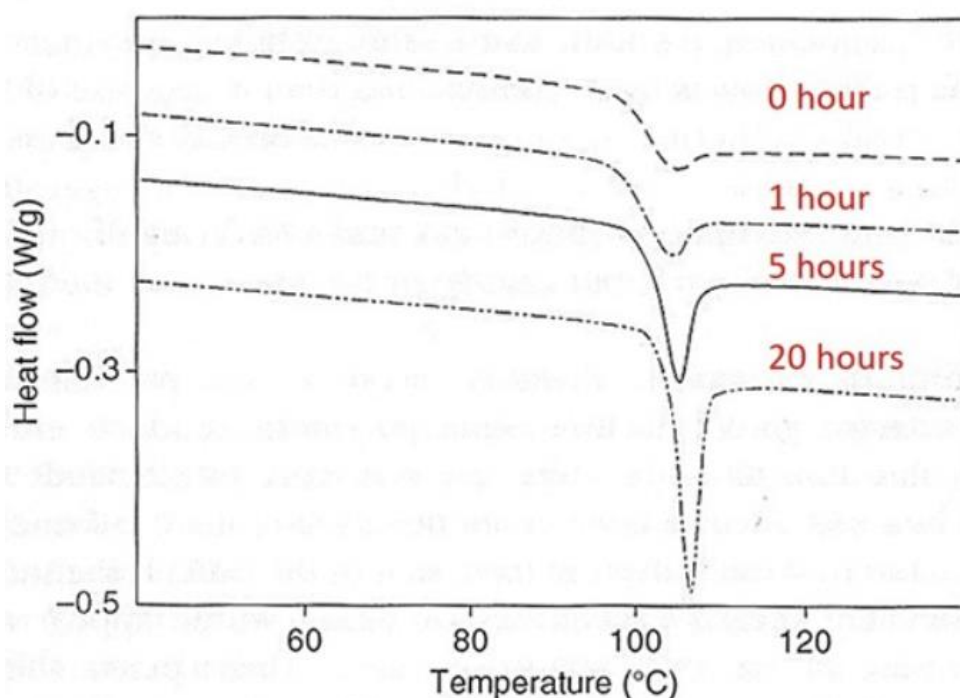


Figure 6.1. DSC curves showing the development and growth of an enthalpy relaxation peak immediately following the glass transition of polystyrene, after aging below $T_g \sim 100^\circ\text{C}$. Annealing times at 80°C from top to bottom: 0 hr, 1 hr, 5 hr, 20 hr; Used with permission of John Wiley & Sons - Books, from (Menczel & Prime, 2009); permission conveyed through Copyright Clearance Center, Inc.

6.2 Future outlook

We know from previous study using DSC that annealing small molecules such as nifedipine and felodipine allows the sample to relax through alpha relaxation (Marsac et al, 2006). Figure 6.2 shows change in enthalpy recovery as a function of time for nifedipine and felodipine at 23.5°C. Instead of doing heat-cool-heat experiment on a sample (as we have done with our present study), we could try heat-cool-anneal-heat experiment to investigate the enthalpic relaxation of the sample and its effect in alpha and beta transition temperatures using Raman spectroscopy.

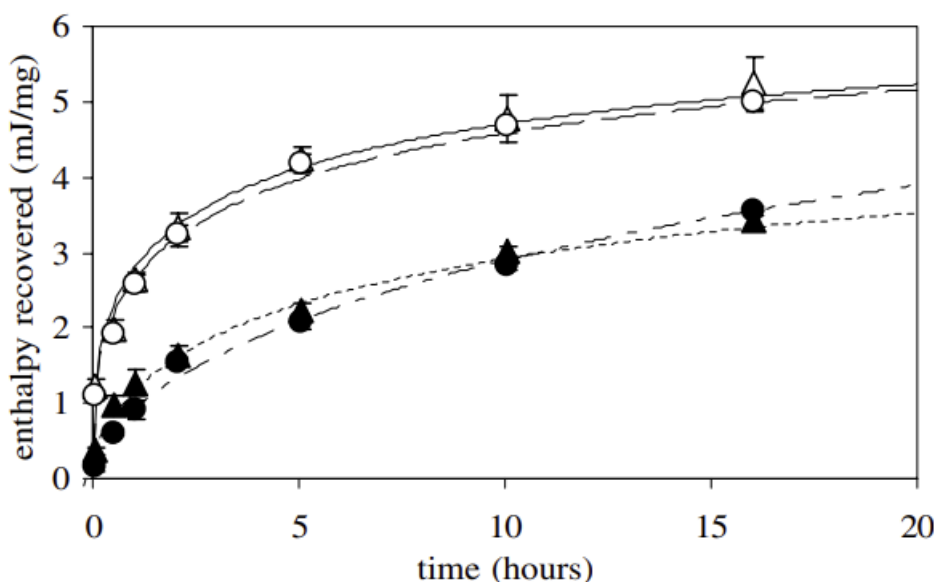


Figure 6.2. Enthalpy recovered (mJ/mg) as a function of storage time at 23.5-°C for nifedipine (○), felodipine (Δ), and amorphous molecular level solid dispersions of nifedipine with 10 wt.%PVP (●) and felodipine with 10 wt.%PVP (▲). Error bars represent one standard deviation; Used with permission of Springer Nature BV, from (Marsac et al, 2006); permission conveyed through Copyright Clearance Center, Inc.

Solid-state NMR can be performed at variable temperatures to study how the amorphous structure evolves with changes at around alpha and beta temperatures. By acquiring NMR spectra at different temperatures, subtle

changes in chemical shifts, linewidths, and peak intensities can be analyzed, providing insights into the amorphous phase transitions and molecular mobility. Furthermore, variable temperature solid-state NMR can be applied to annealed amorphous samples to reveal information about molecular dynamics and mobility.

Raman spectroscopy alone may not provide complete insight into all factors influencing the stability of amorphous materials. However, it is easily accessible complementary analytical technique to more commonly used thermal and spectroscopic methods, such as DSC and dielectric spectroscopy.

Appendix A

Supplementary information part I

Gaussian peak fitting script (Initial expected differences in which the intensities were judged by visual inspection on a sample basis):

```
library(fields)
myfiles <- list.files(pattern=".txt")
number_of_files <- length(myfiles)
temperature_sequence <- matrix(NA, ncol=1, nrow=number_of_files)
for (myfile in myfiles)
{
  myindex <- which(myfiles == myfile)
  temperature <- as.numeric(unlist(strsplit(myfile, "_"))[7])
  print(temperature)
  temperature_sequence[myindex,1] <- temperature
}
temperature_sequence <- sort(temperature_sequence)

offset <- 0.2
ymin <- 0
ymax <- offset*number_of_files+5

# Separate plots
xmin <- 30
xmax <- 450

# Initial parameters
background_estimate <- 2000
background_gradient_estimate <- 0
peak_intensity_estimate_1 <- 3000
peak_width_estimate_1 <- 10
peak_position_estimate_1 <- 388

fit_parameters <- matrix(NA, ncol=5, nrow=261)
for (temperature in seq(from=-100, to=160, by=1))
{
  myindex <- which(temperature_sequence == temperature)
  search_term <- paste("_", temperature, "_deg", sep="")
  relevant_file <- myfiles[grep(search_term, myfiles)]
  mydata <- read.csv(file=relevant_file, header=FALSE, sep="\t")
  mydata <- subset(mydata, mydata[,1] >= xmin & mydata[,1] <= xmax)
  names(mydata) <- c("Wavenumber", "Intensity")
  plot(mydata, main=relevant_file)

  fit <- nls(
    Intensity ~
    peak_intensity_1*exp(-1/2*(Wavenumber-peak_position_1)^2/peak_width_1^2) +
    background + background_gradient*Wavenumber,
```



```

    start=c(peak_position_1=peak_position_estimate_1,
peak_width_1=peak_width_estimate_1, peak_intensity_1=peak_intensity_estimate_1,
background=background_estimate, background_gradient=background_gradient_estimate),
data = mydata, control = nls.control(maxiter=10000, minFactor=0.01/1024, warnOnly =
TRUE)
)
lines(mydata$Wavenumber, predict(fit), col="red")
xline(as.numeric(fit$m$getPars())[4]), col="red")

# Update fit estimates
background_estimate <- 2000
background_gradient_estimate <- 0
peak_intensity_estimate_1 <- as.numeric(fit$m$getPars())[3])
peak_width_estimate_1 <- as.numeric(fit$m$getPars())[2])
peak_position_estimate_1 <- as.numeric(fit$m$getPars())[1])

fit_parameters[myindex,] <- as.numeric(fit$m$getPars())

}

```

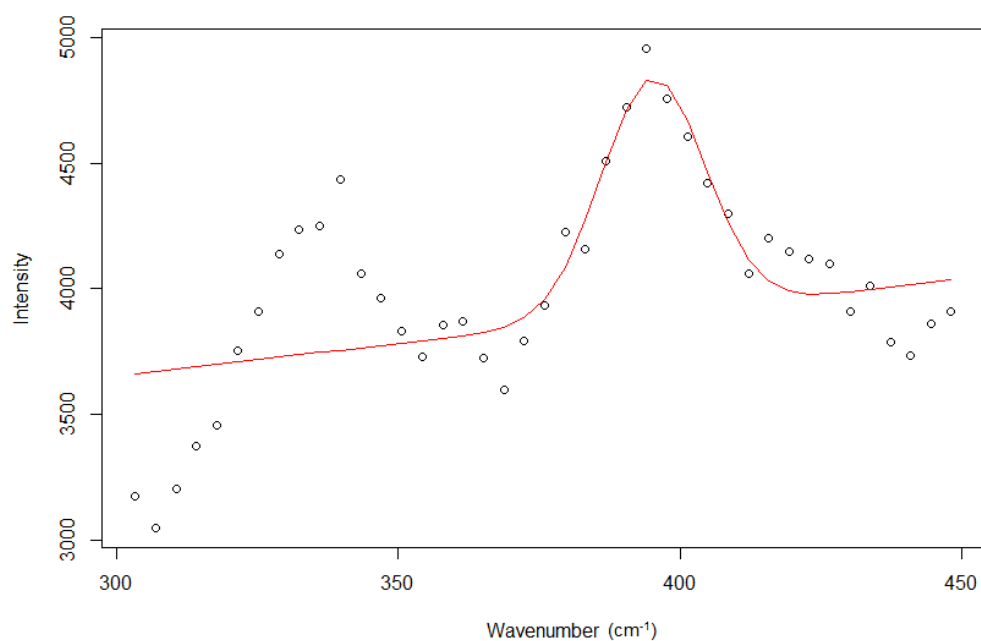


Figure A 1. Raman spectra of paracetamol at -100°C (The model fitted to the data - Gaussian peak fitted images of Raman spectra)

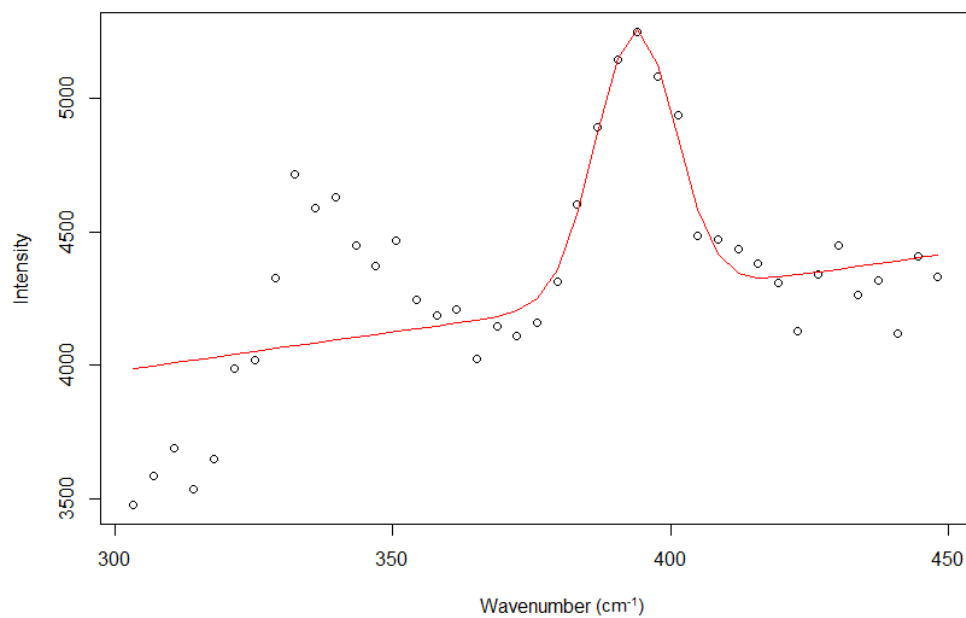


Figure A 2. Raman spectra of paracetamol at -99°C

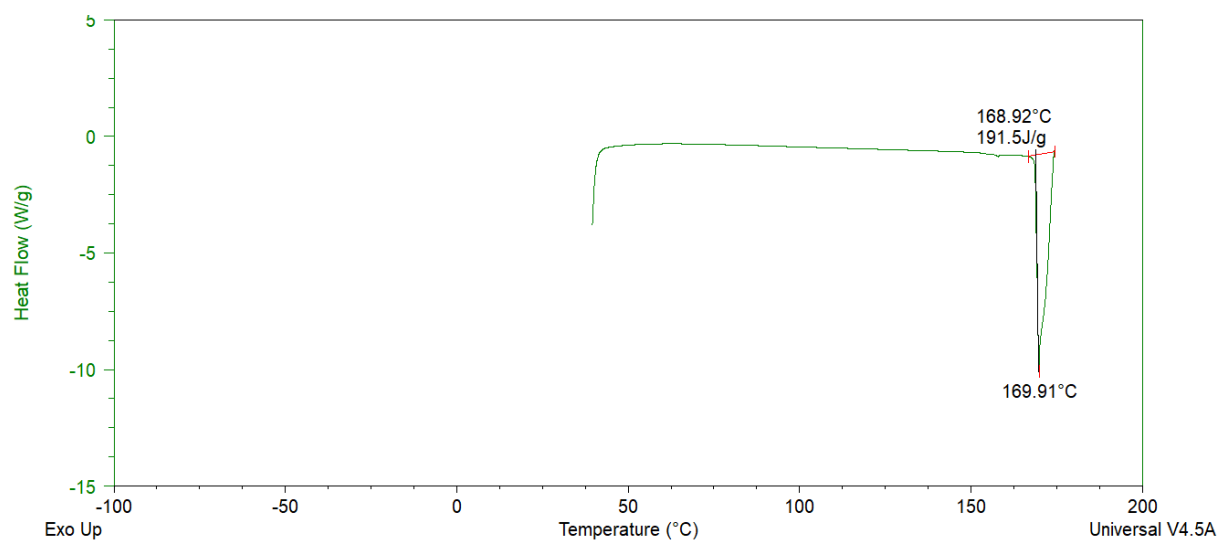


Figure A 3. DSC thermogram of paracetamol sample

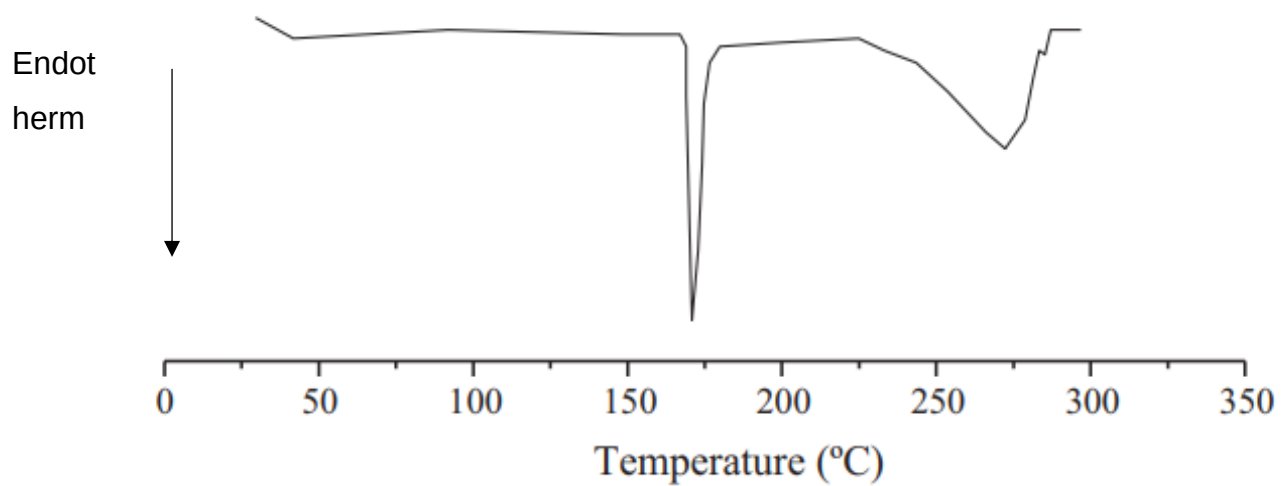


Figure A 4. Reference DSC thermogram of pure paracetamol. Used with permission of Elsevier Science & Technology Journals from (de Oliveira et al, 2017); permission conveyed through Copyright Clearance Center, Inc.

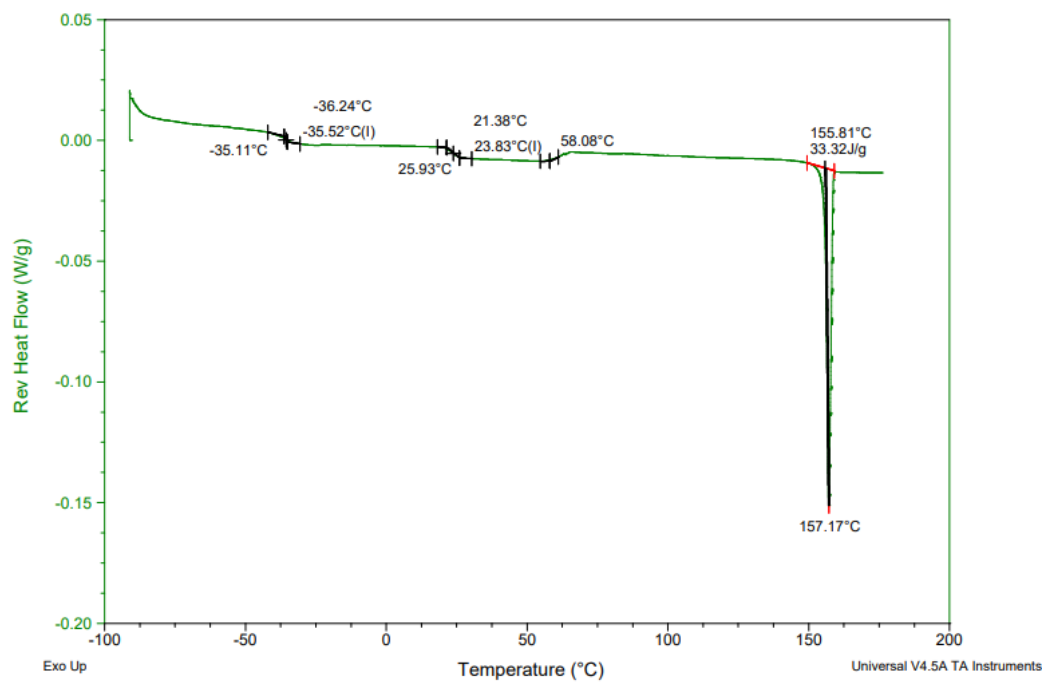


Figure A 5. Modulated DSC thermogram of paracetamol sample

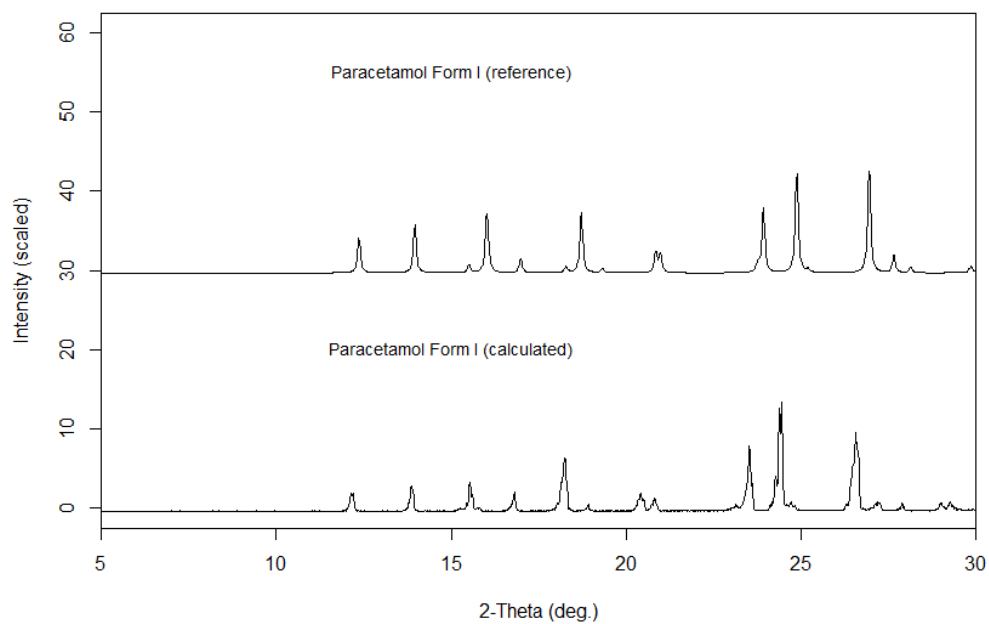


Figure A 6. XRD diagram of paracetamol

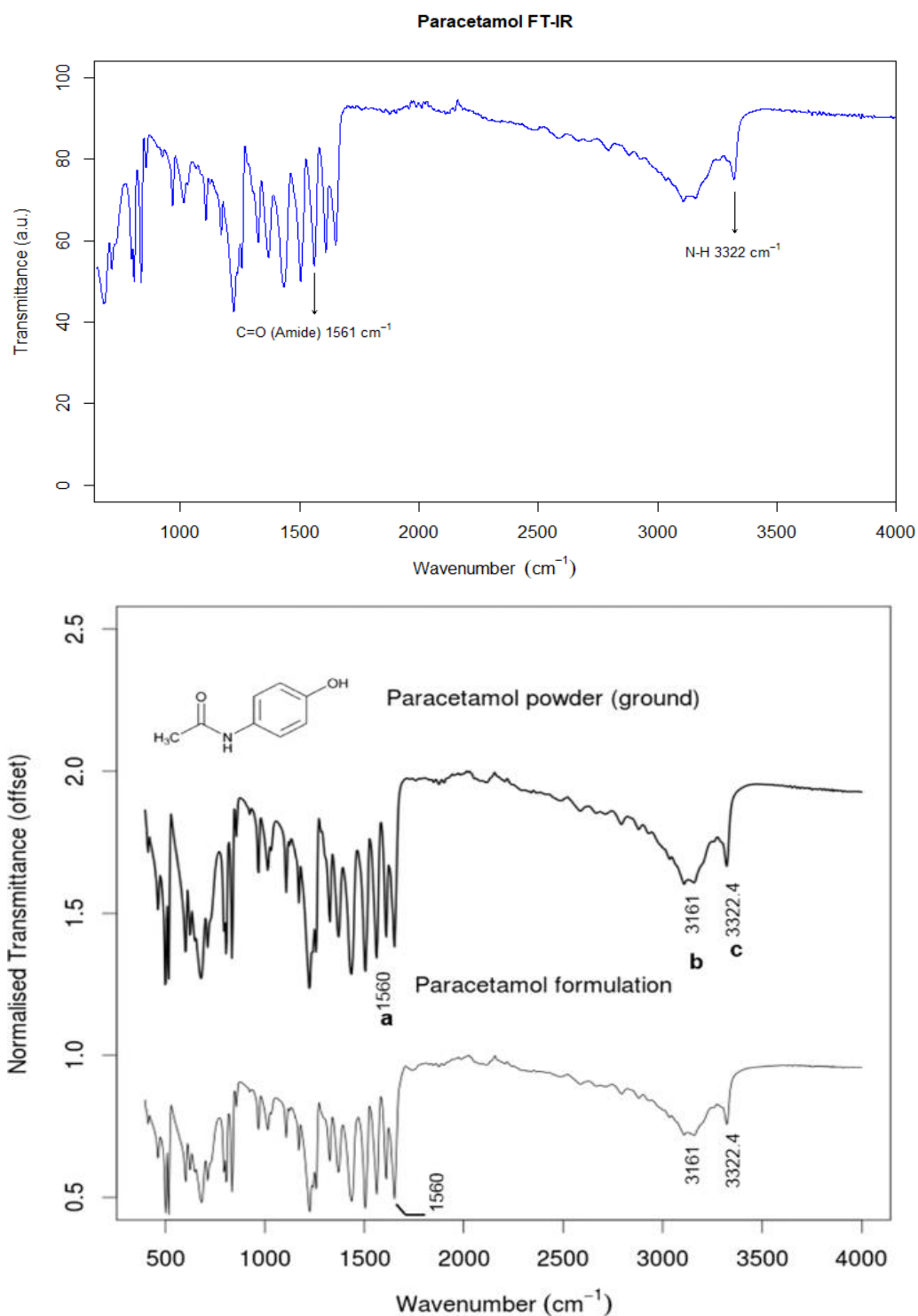


Figure A 7. FTIR diagram of paracetamol (top) and reference FTIR diagram of paracetamol (bottom). Used with permission of Elsevier Science & Technology Journals, from (Khaled et al, 2018) ; permission conveyed through Copyright Clearance Center, Inc.

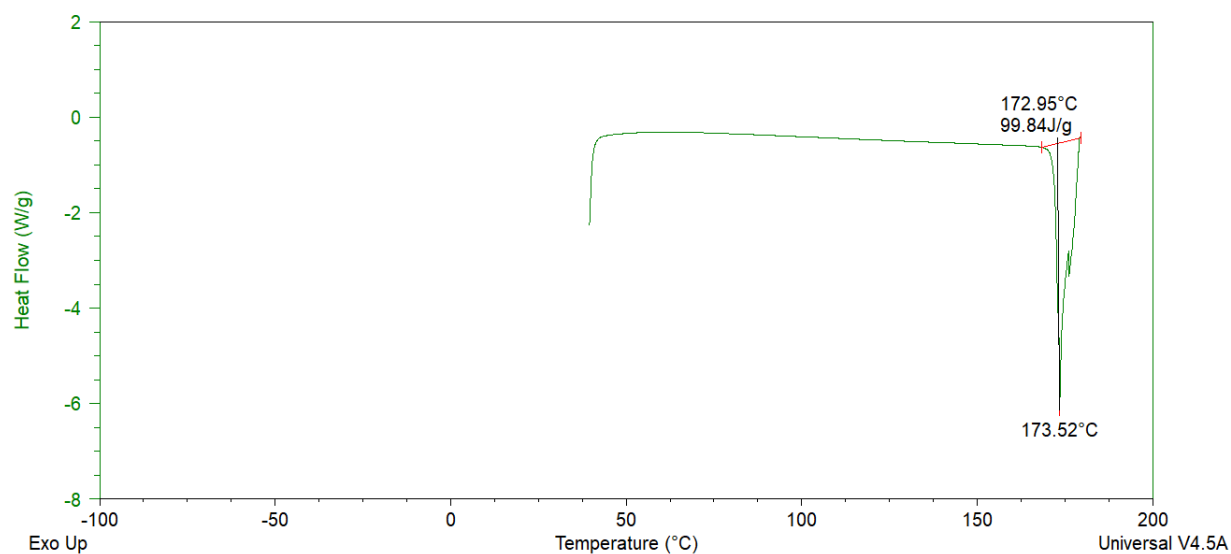


Figure A 8. DSC thermogram of nifedipine sample

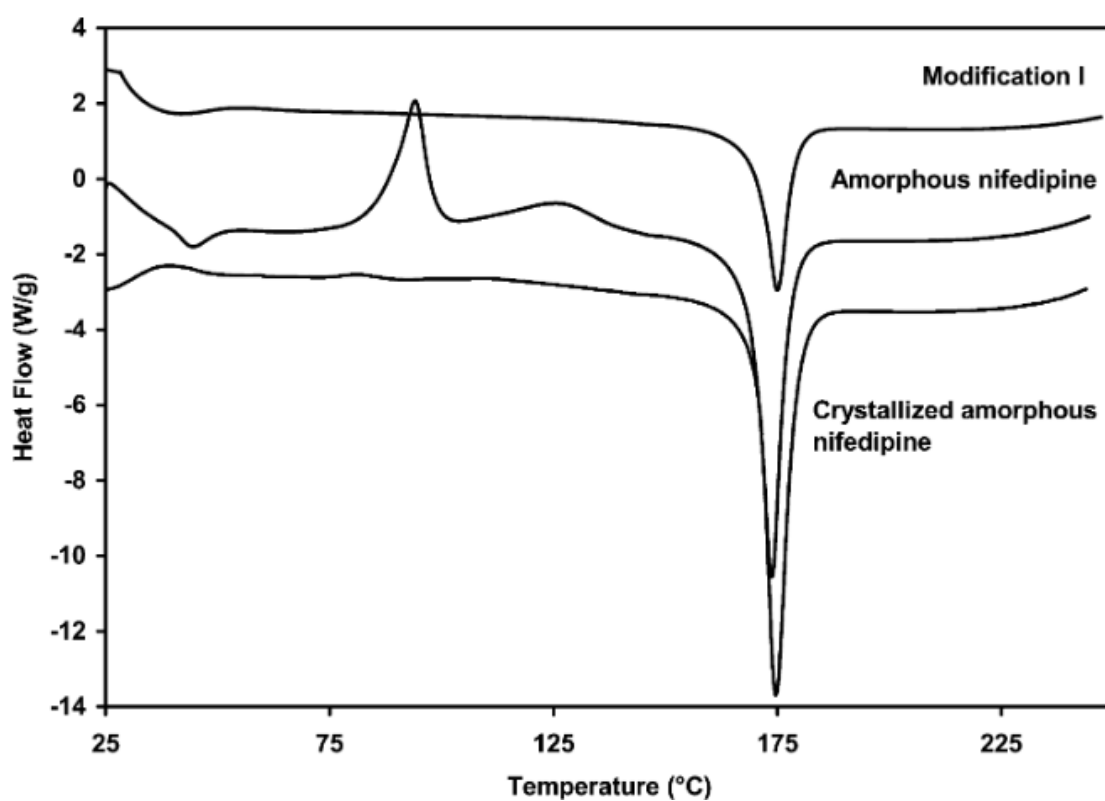


Figure A 9. Reference DSC thermogram of nifedipine Used with permission of Taylor & Francis Informa UK Ltd - Journals, from (Song et al, 2005); permission conveyed through Copyright Clearance Center, Inc.

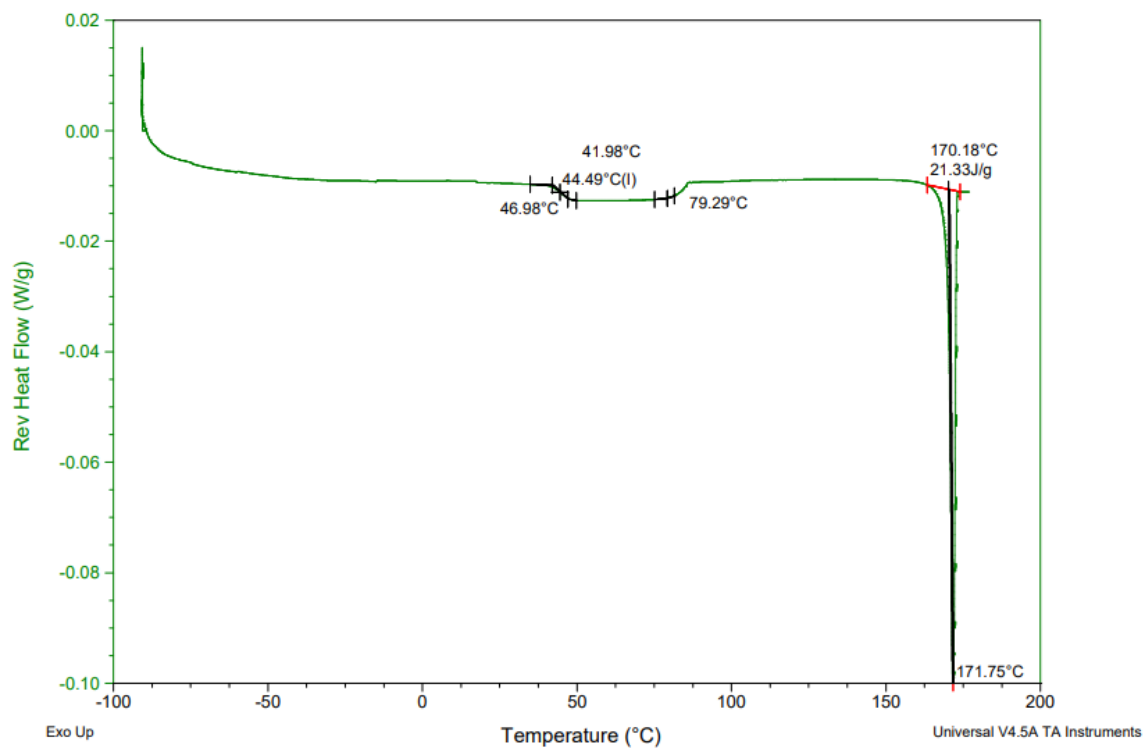


Figure A 10. Modulated DSC thermogram of nifedipine sample

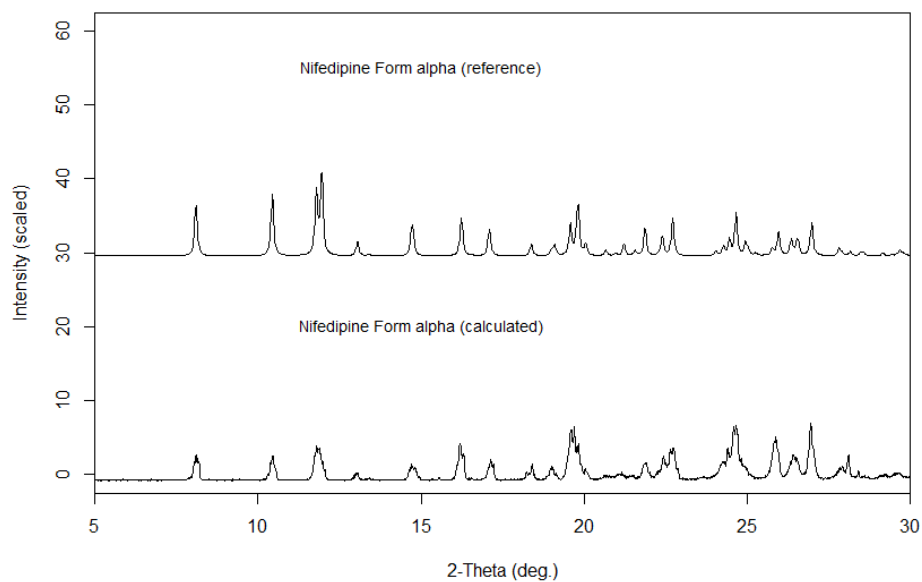


Figure A 11. XRD diagram of nifedipine

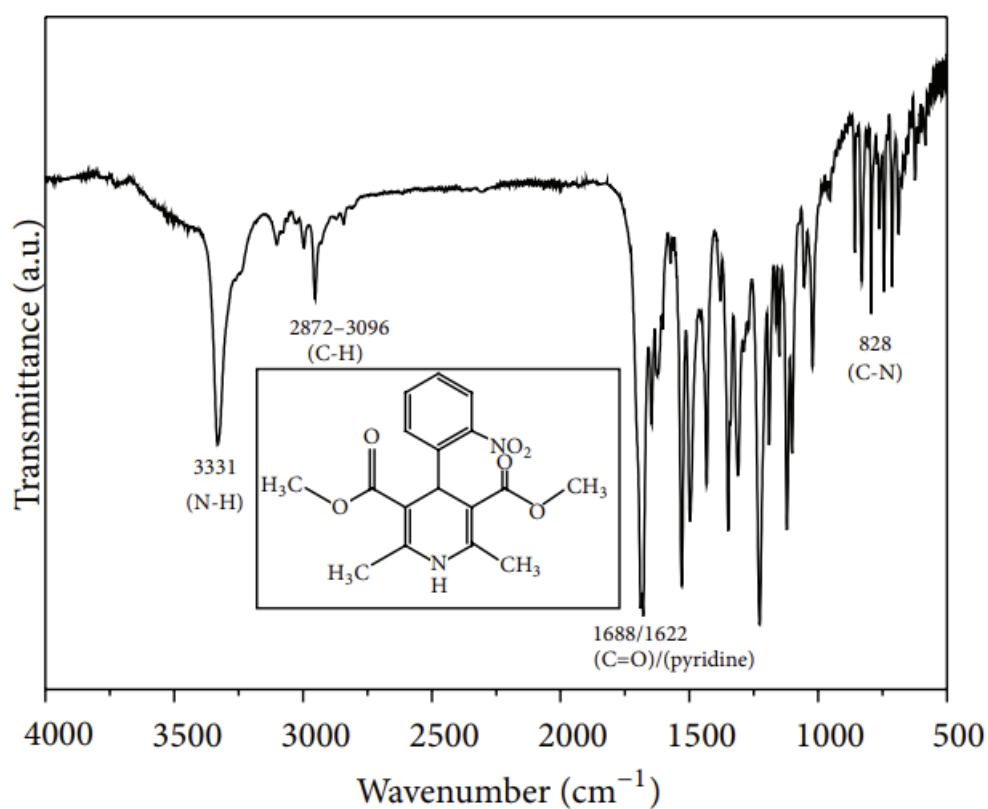
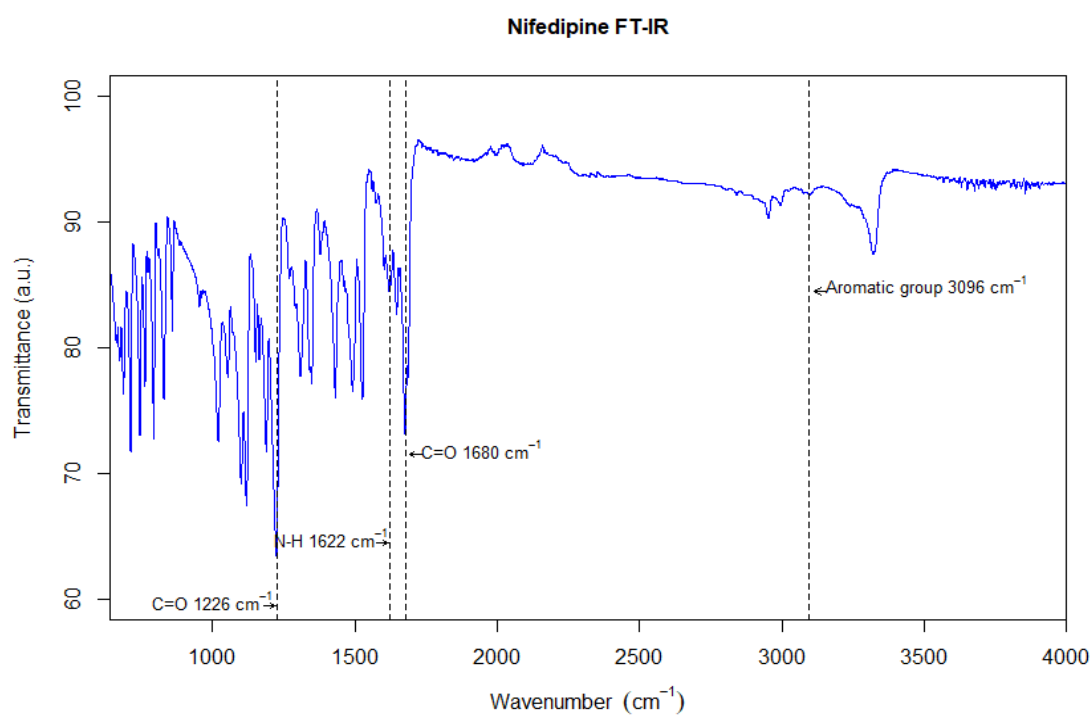


Figure A 12. FTIR diagram of nifedipine (top) and reference FTIR diagram of pure nifedipine (da Costa et al, 2015)

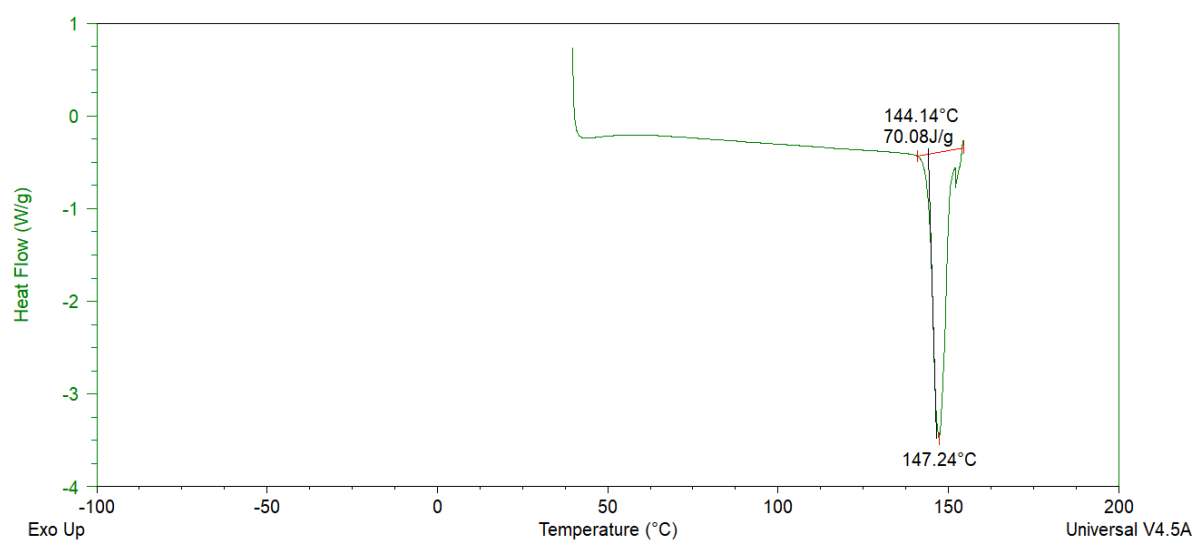


Figure A 13. DSC thermogram of felodipine sample

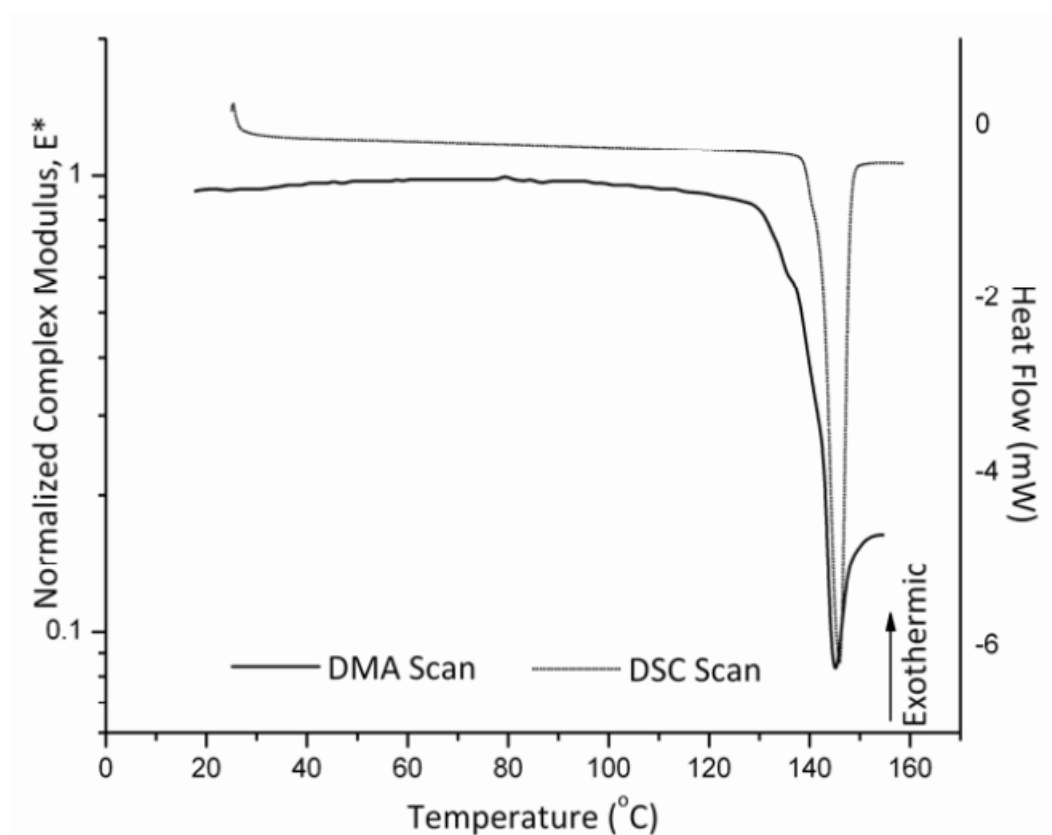


Figure A 14. Reference DSC thermogram of felodipine (Abiad et al, 2010)

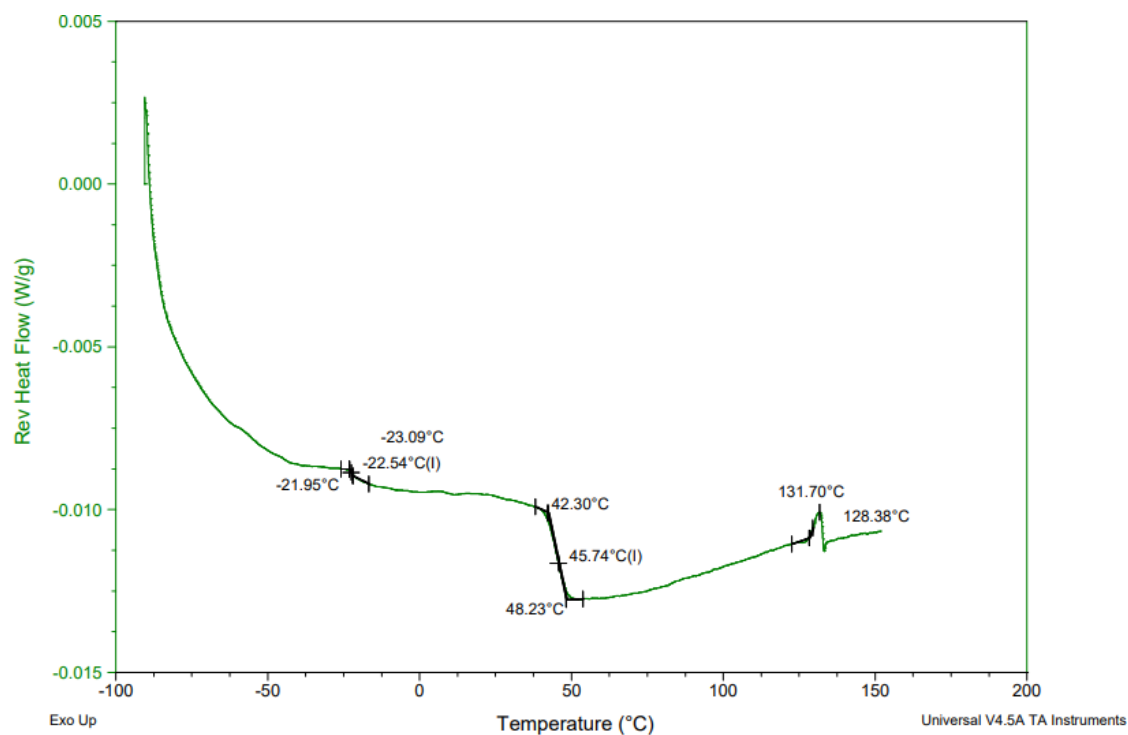


Figure A 15. Modulated DSC thermogram of felodipine sample

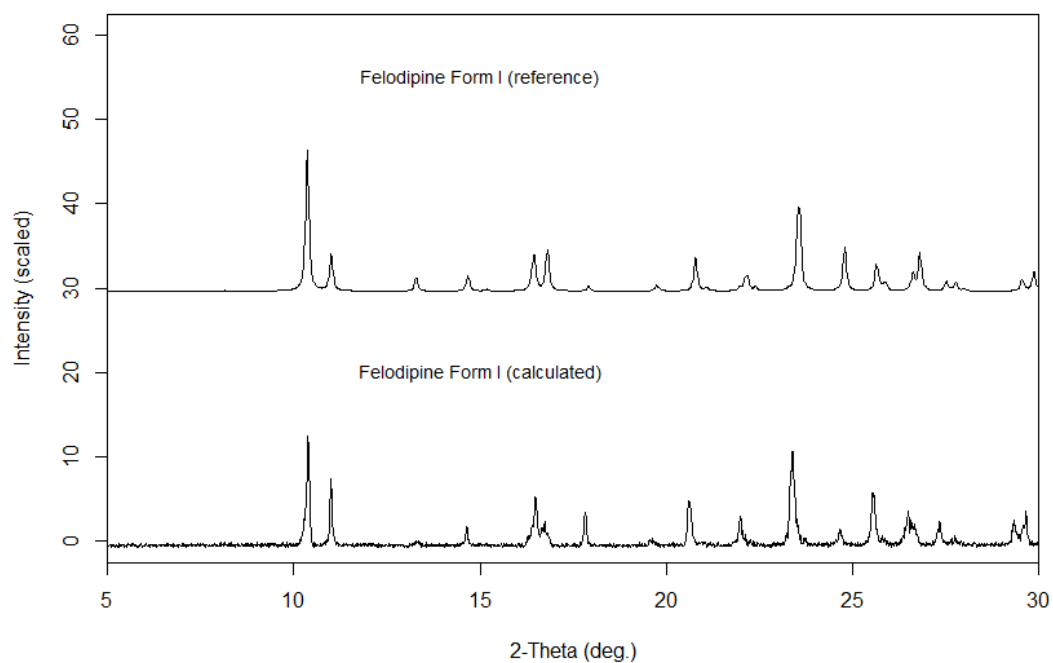


Figure A 16. XRD diagram of felodipine

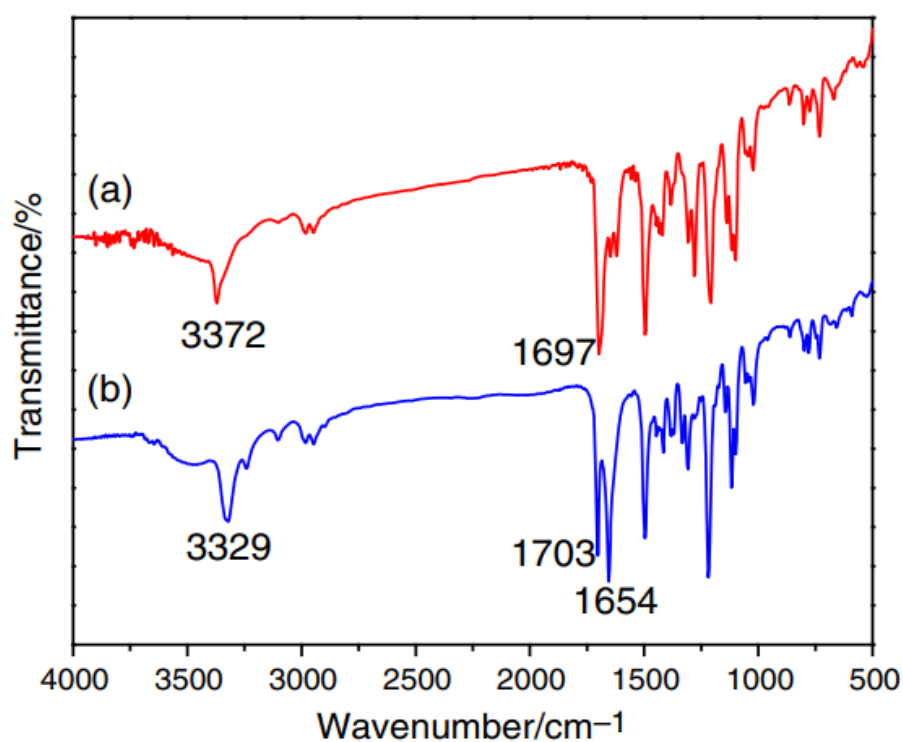
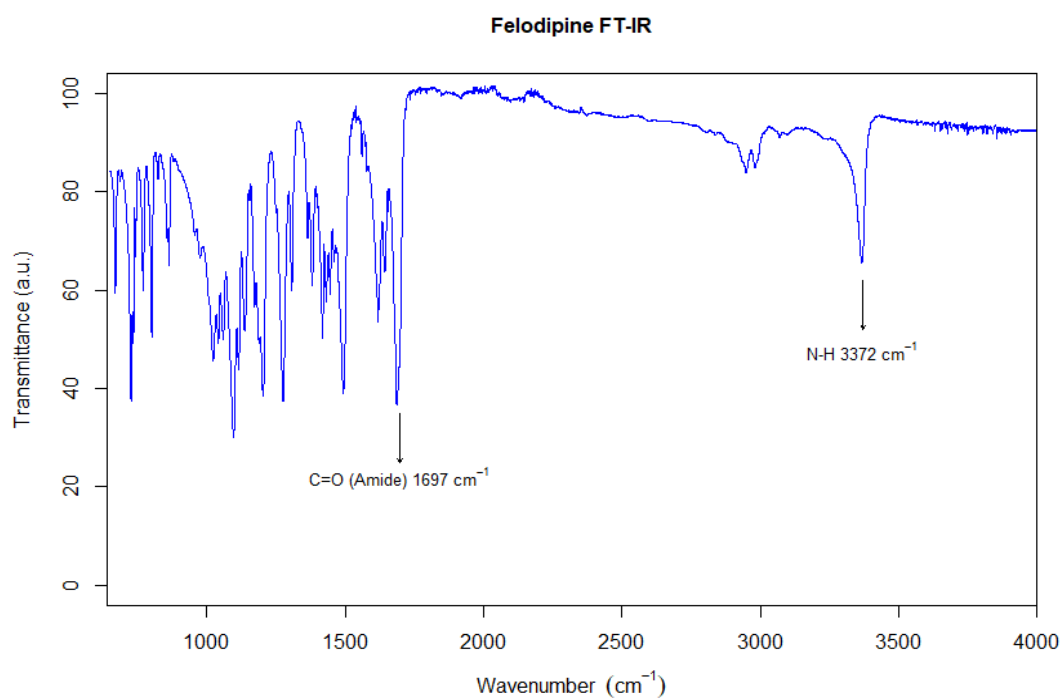


Figure A 17. FTIR diagram of felodipine (top) and reference FTIR diagram of felodipine form I (a) and form IV (b) (bottom). Used with permission of Springer Nature BV, from (Wang et al, 2015); permission conveyed through Copyright Clearance Center, Inc.

Appendix B

Supplementary information part II

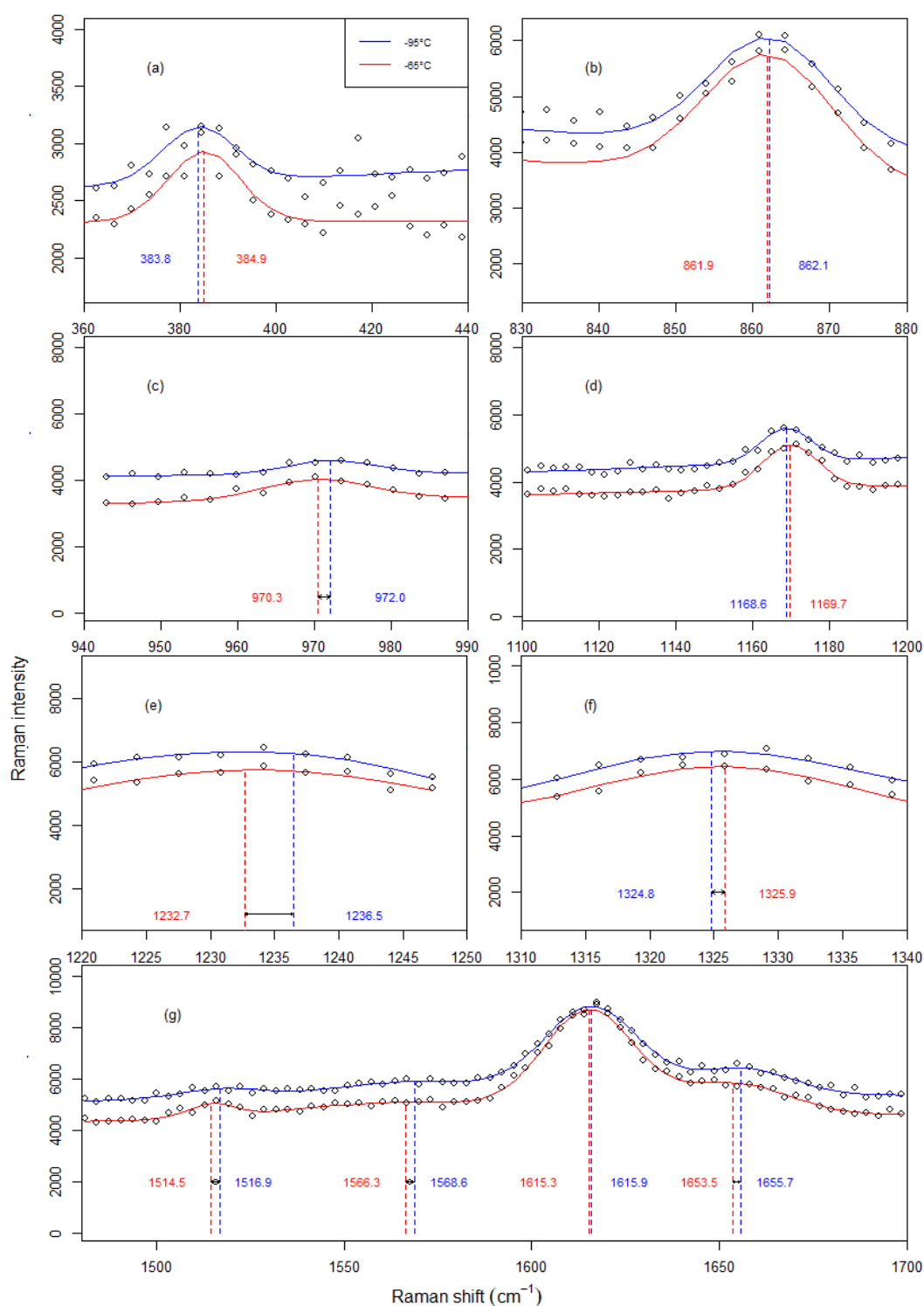


Figure B 1. Experiment 2 Raman peaks at -95 and -65°C to illustrate peak position shift around beta transition temperature (-79.15°C): (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

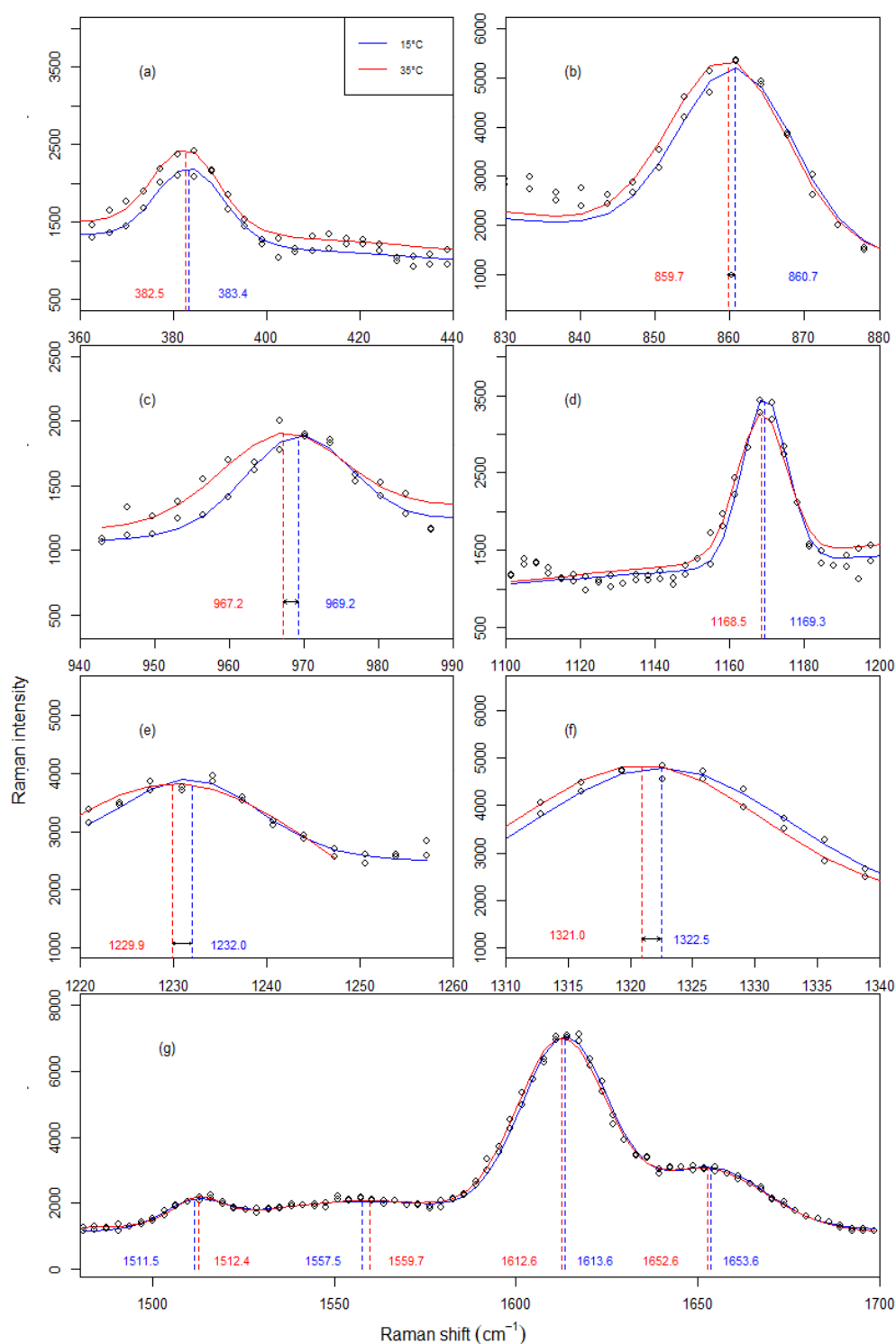


Figure B 2. Experiment 2 Raman peaks at 15 and 35°C to illustrate peak position shift around alpha transition temperature (23.85°C): (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

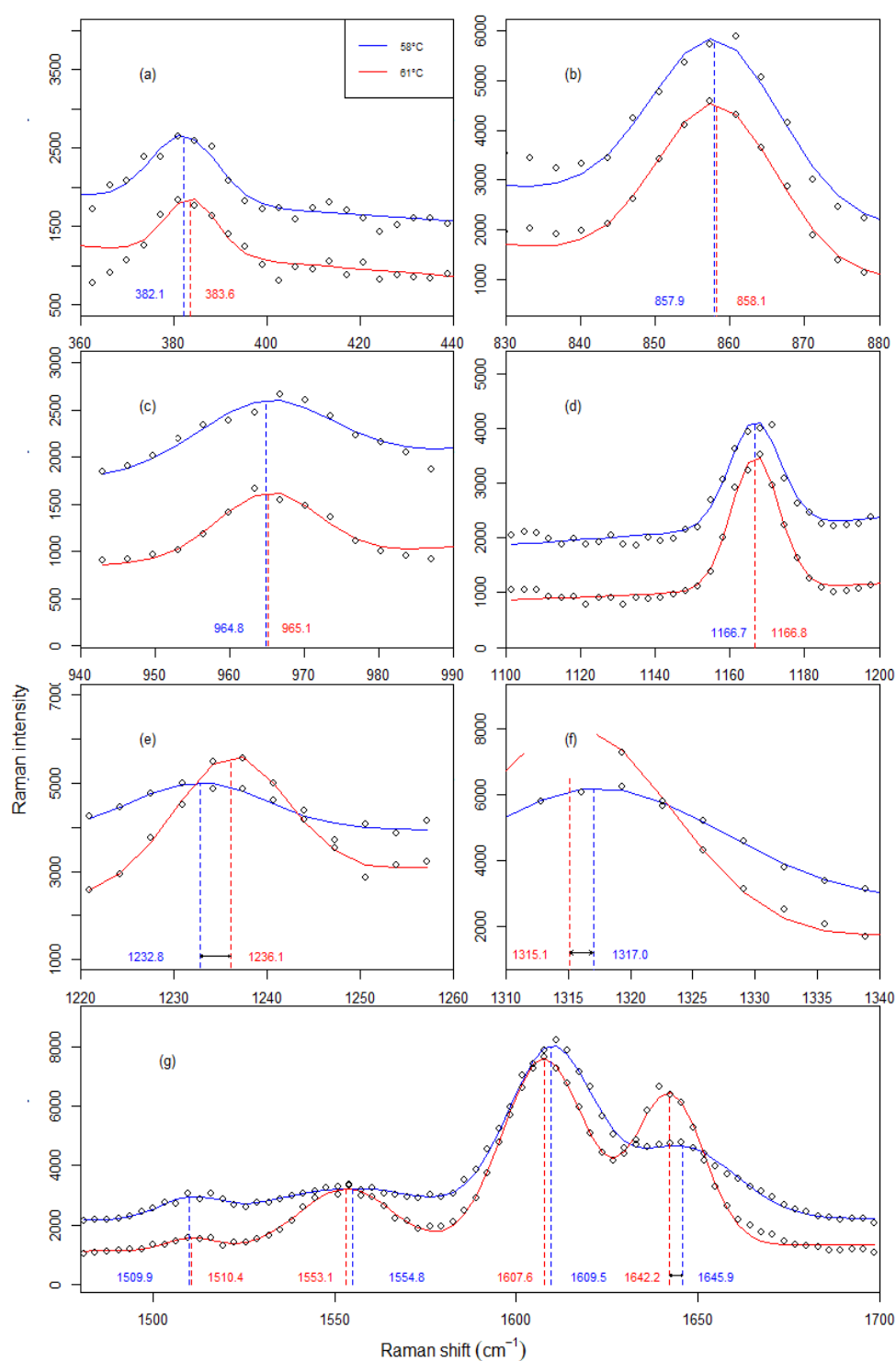


Figure B 3. Experiment 2 Raman peaks at 58 and 61°C to illustrate peak position shift around form III phase transition temperature: (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

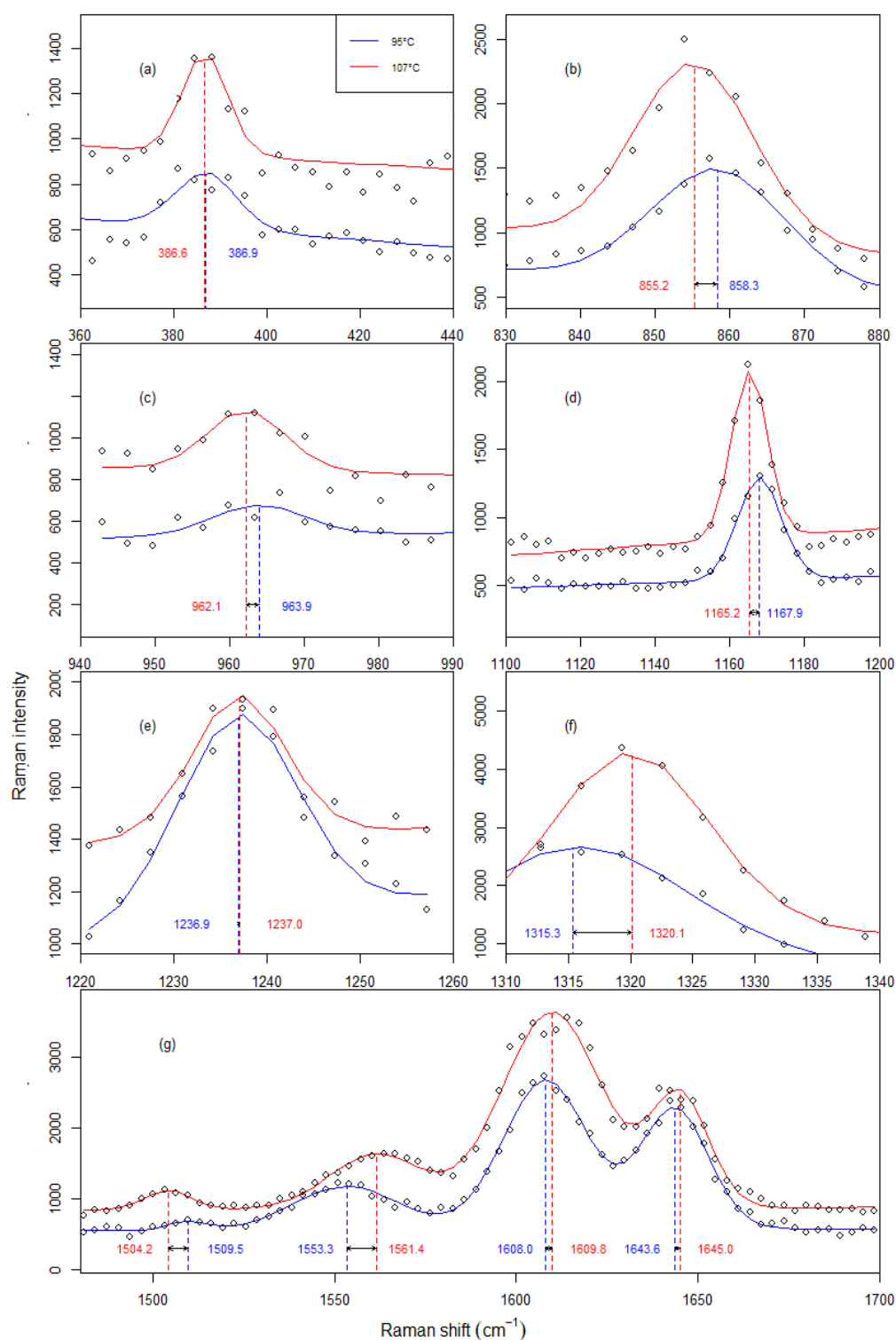


Figure B 4. Experiment 2 Raman peaks at 95 and 107°C to illustrate peak shift around form II phase transition temperature: (a) 390 cm^{-1} ; (b) 865 cm^{-1} ; (c) 975 cm^{-1} ; (d) 1175 cm^{-1} ; (e) 1230 cm^{-1} ; (f) 1330 cm^{-1} ; (g) 1520 cm^{-1} , 1560 cm^{-1} , 1620 cm^{-1} , and 1660 cm^{-1} . These peak positions are approximate.

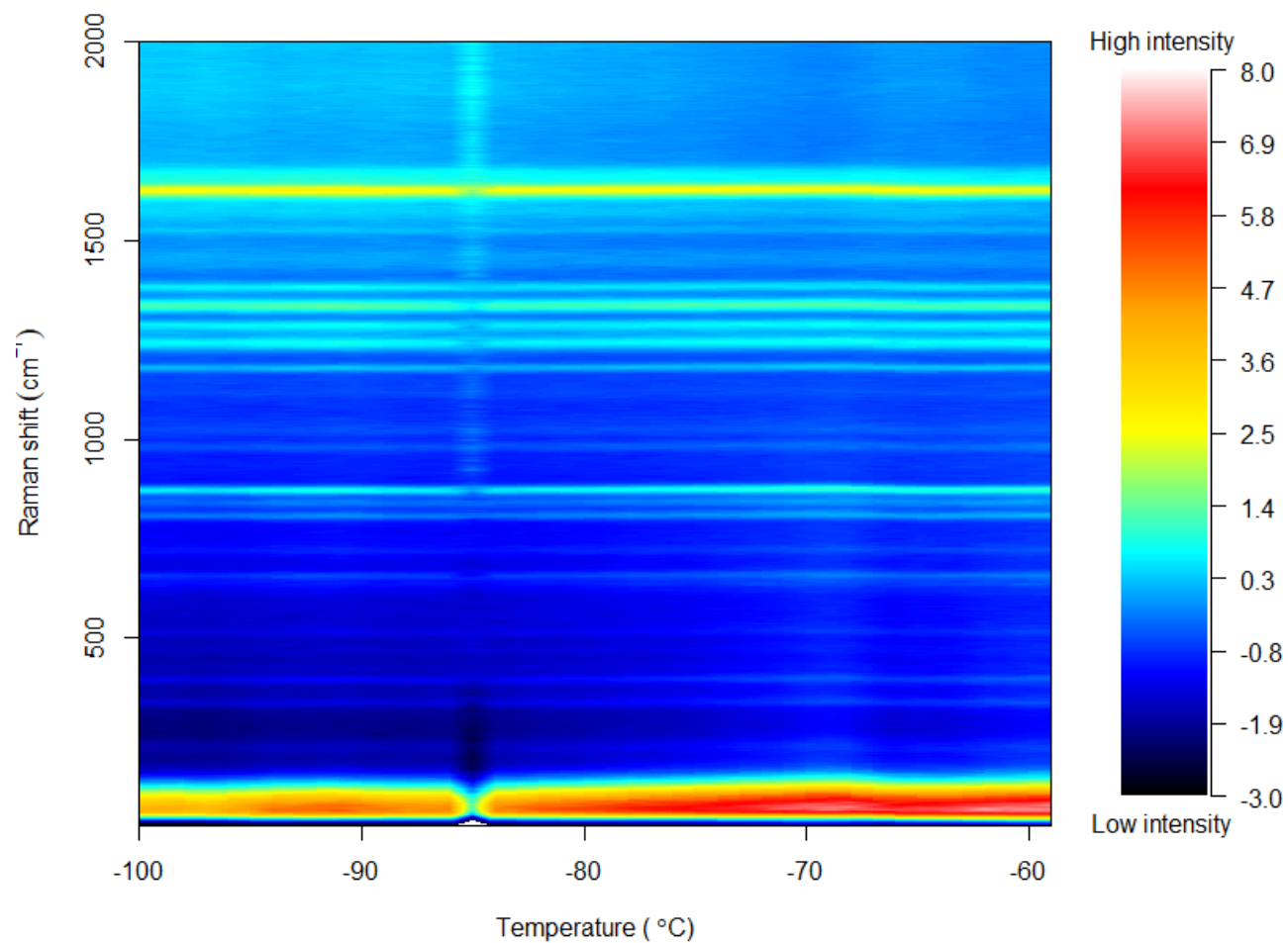


Figure B 5. Low temperature paracetamol repeat experiment 2 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

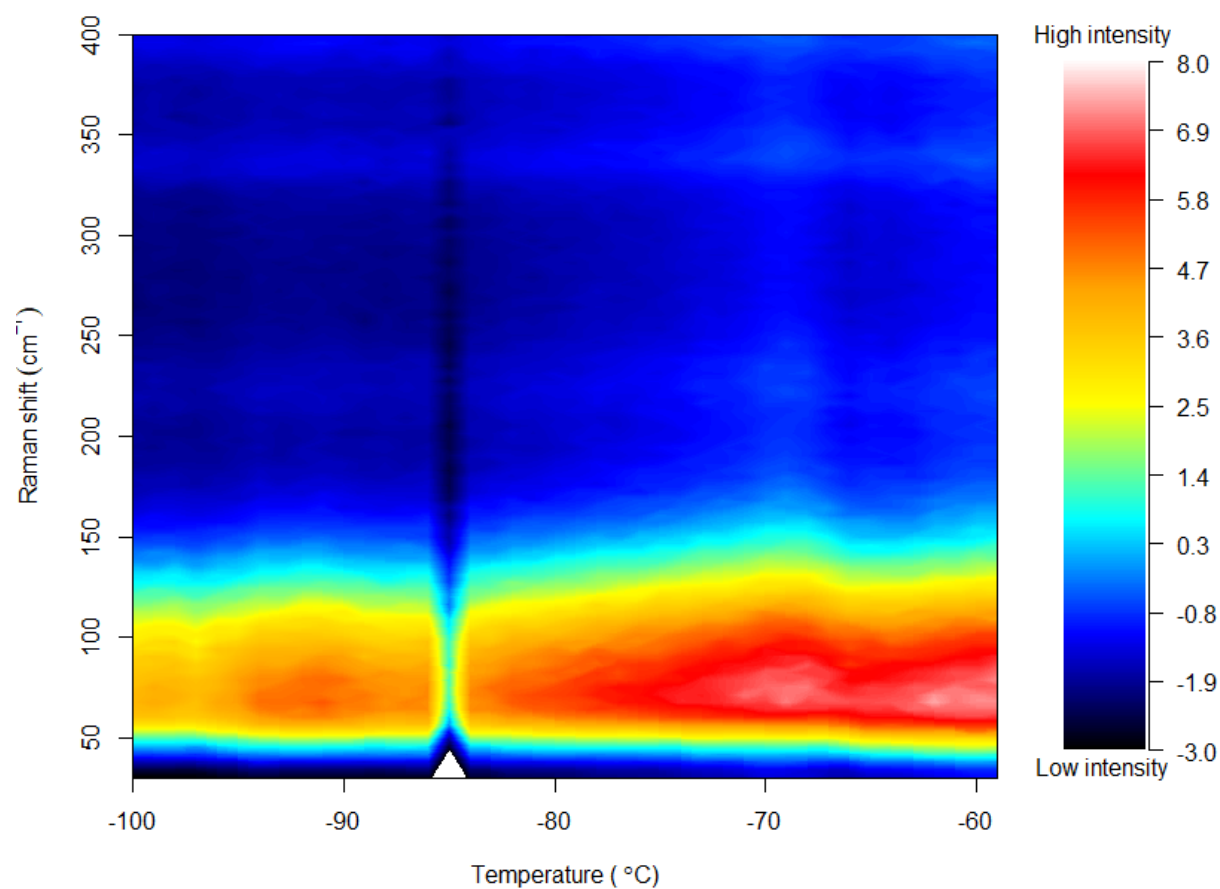


Figure B 6. Low temperature paracetamol repeat experiment 2 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

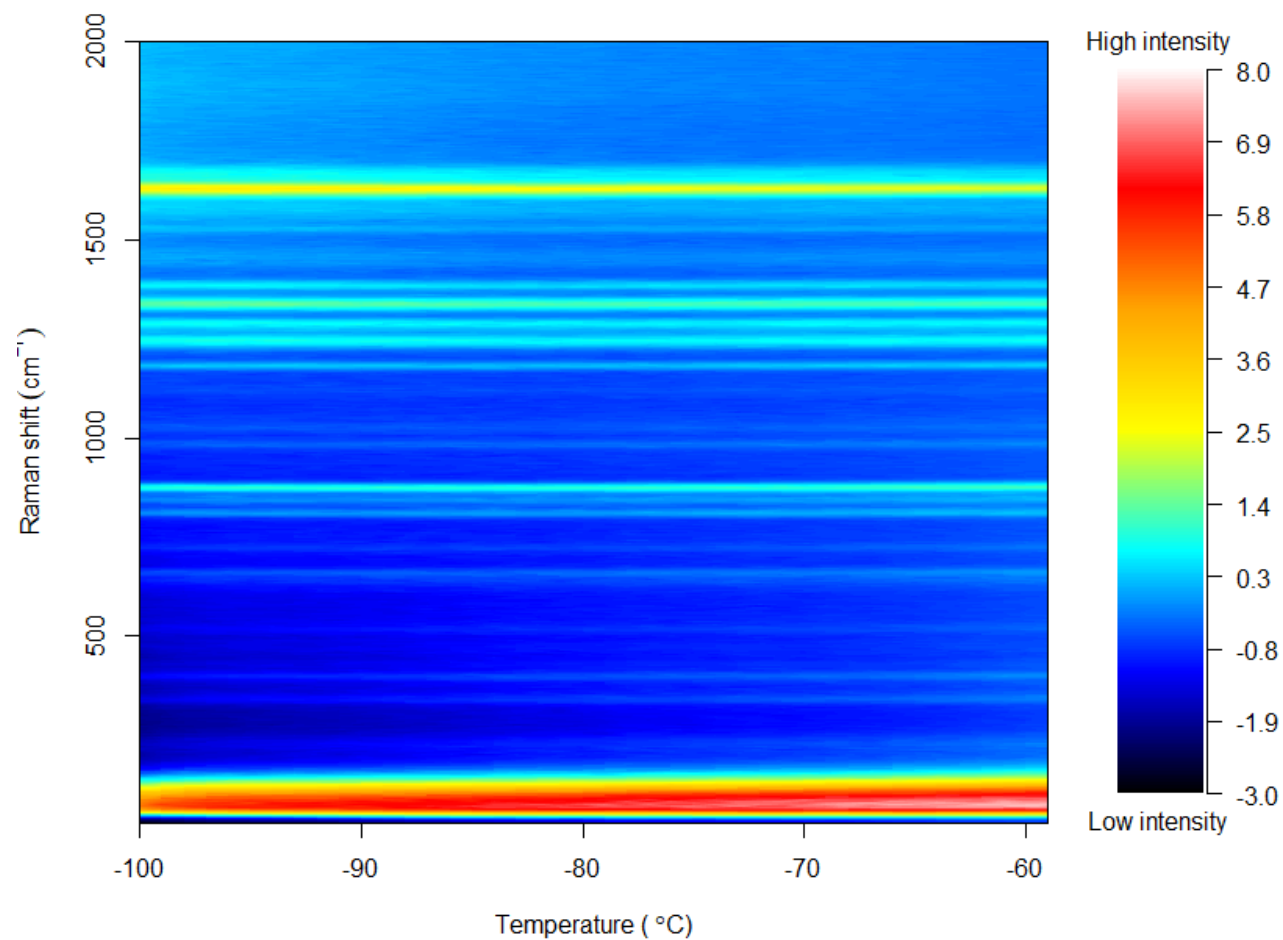


Figure B 7. Low temperature paracetamol repeat experiment 2 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

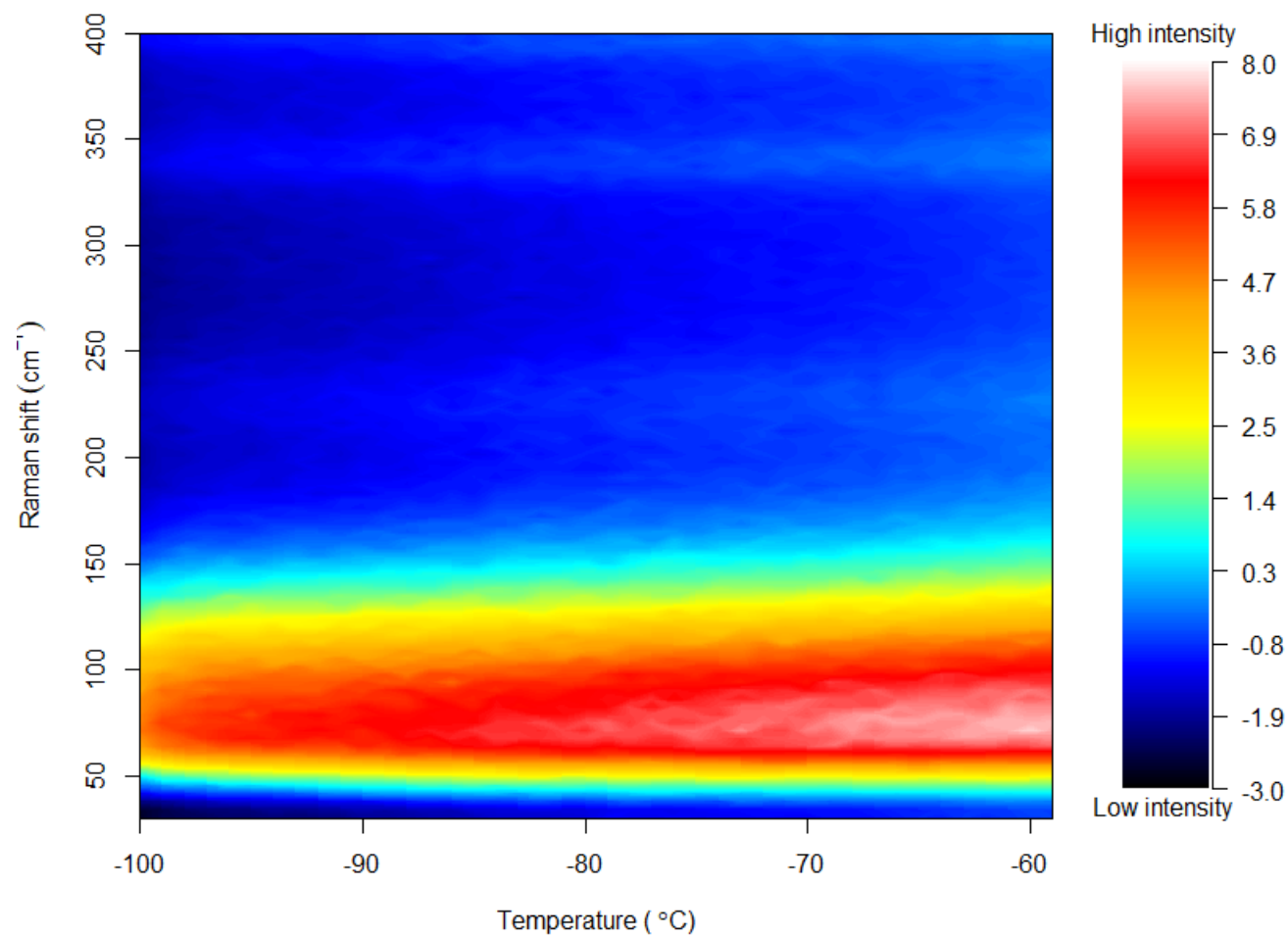


Figure B 8. Low temperature paracetamol repeat experiment 2 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

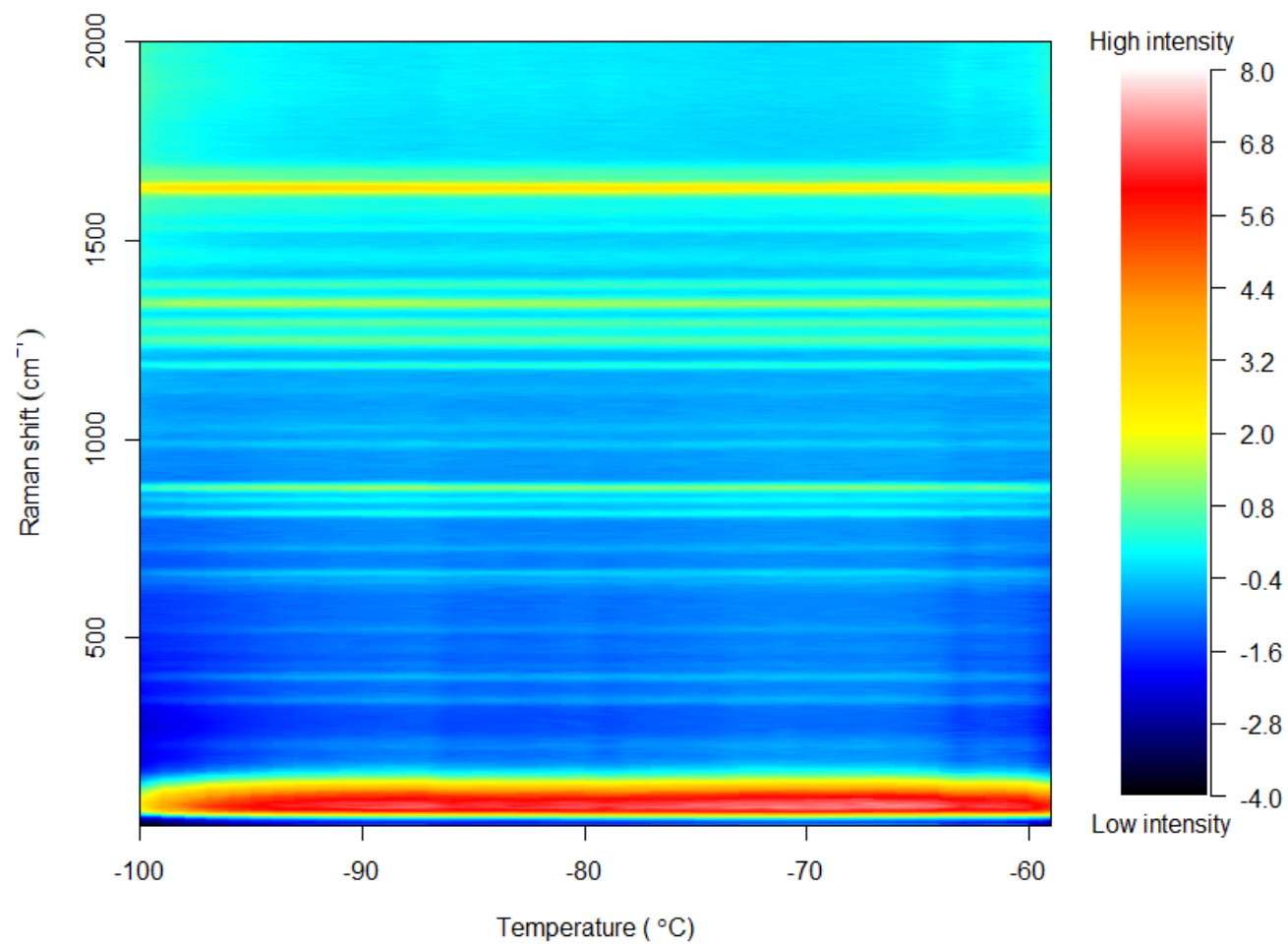


Figure B 9. Low temperature paracetamol repeat experiment 2 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

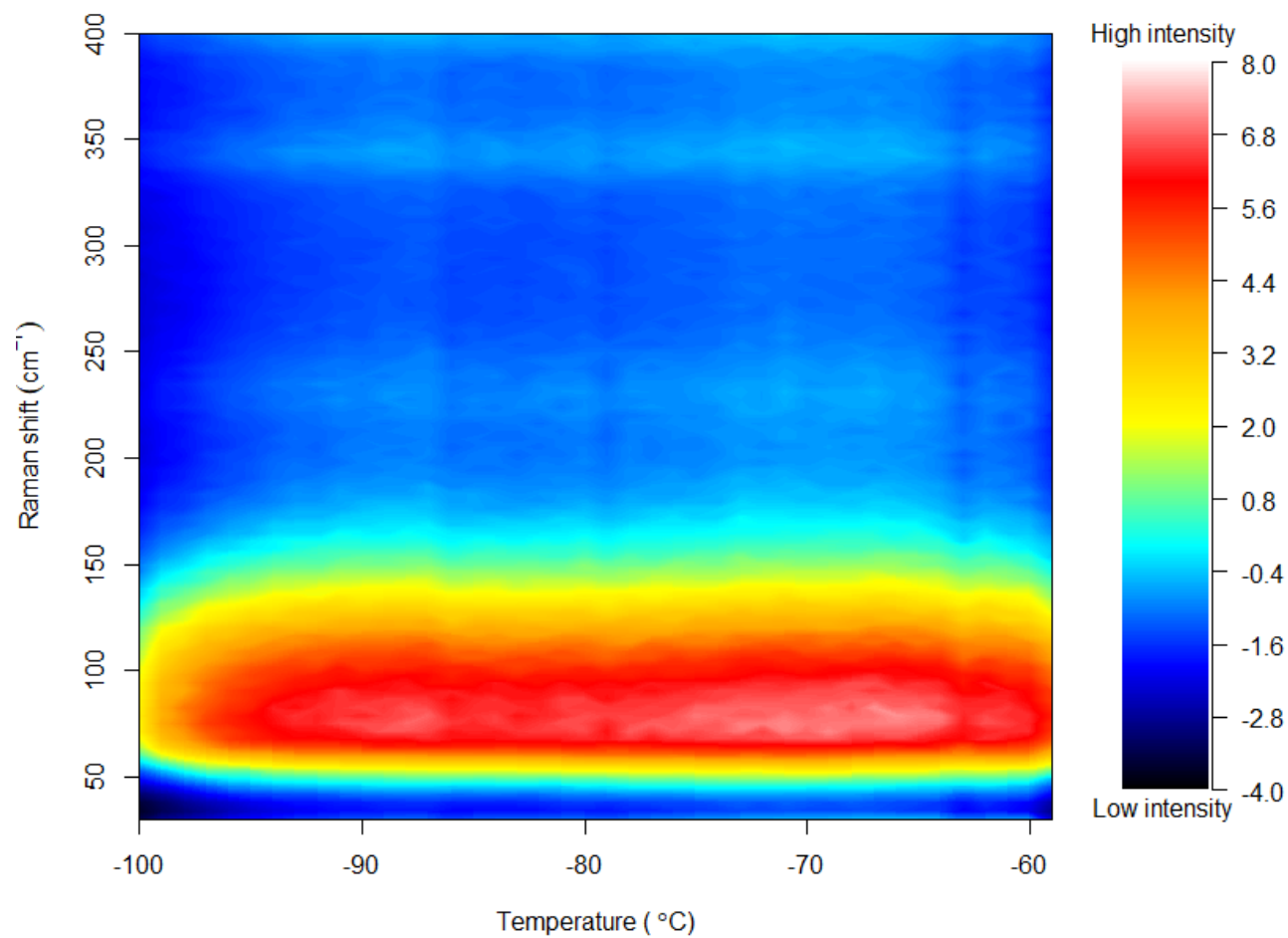


Figure B 10. Low temperature paracetamol repeat experiment 2 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

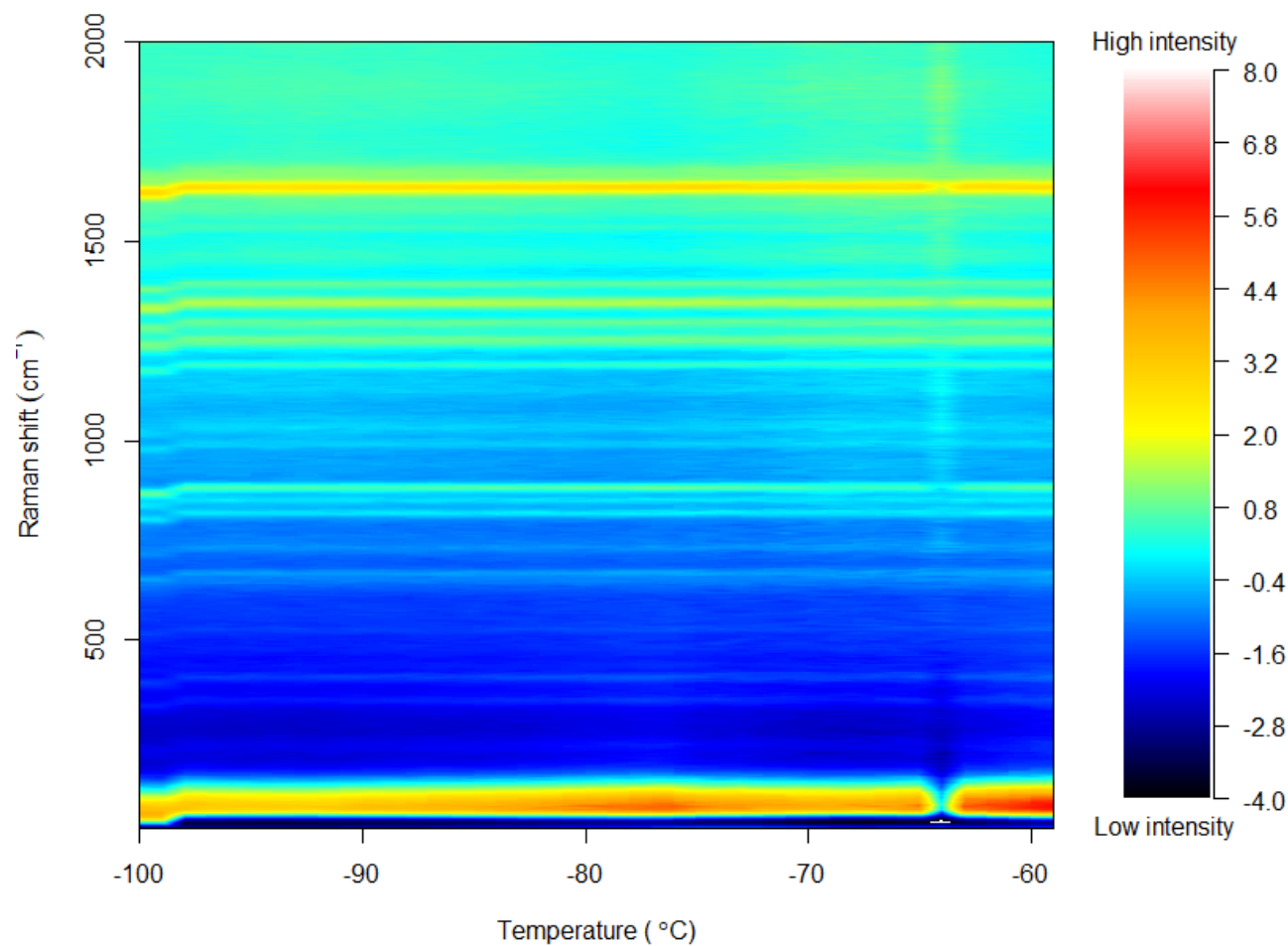


Figure B 11. Low temperature paracetamol repeat experiment 3 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm^{-1} heated from -100 to -59 $^{\circ}\text{C}$. As noted in the text, the sample is heated to 180 $^{\circ}\text{C}$ and cooled to 30 $^{\circ}\text{C}/\text{min}$. It is thought to be amorphous at low temperatures.

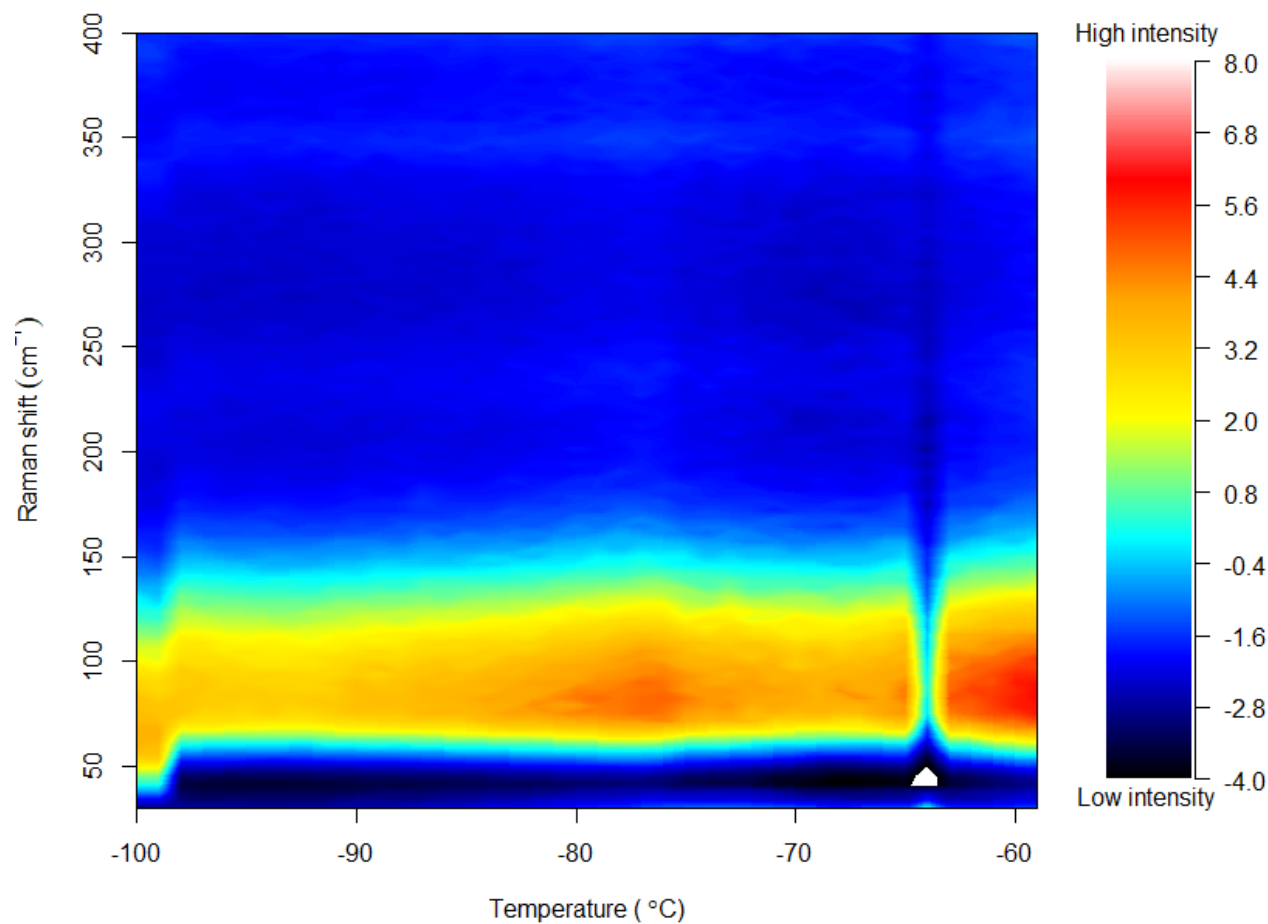


Figure B 12. Low temperature paracetamol repeat experiment 3 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

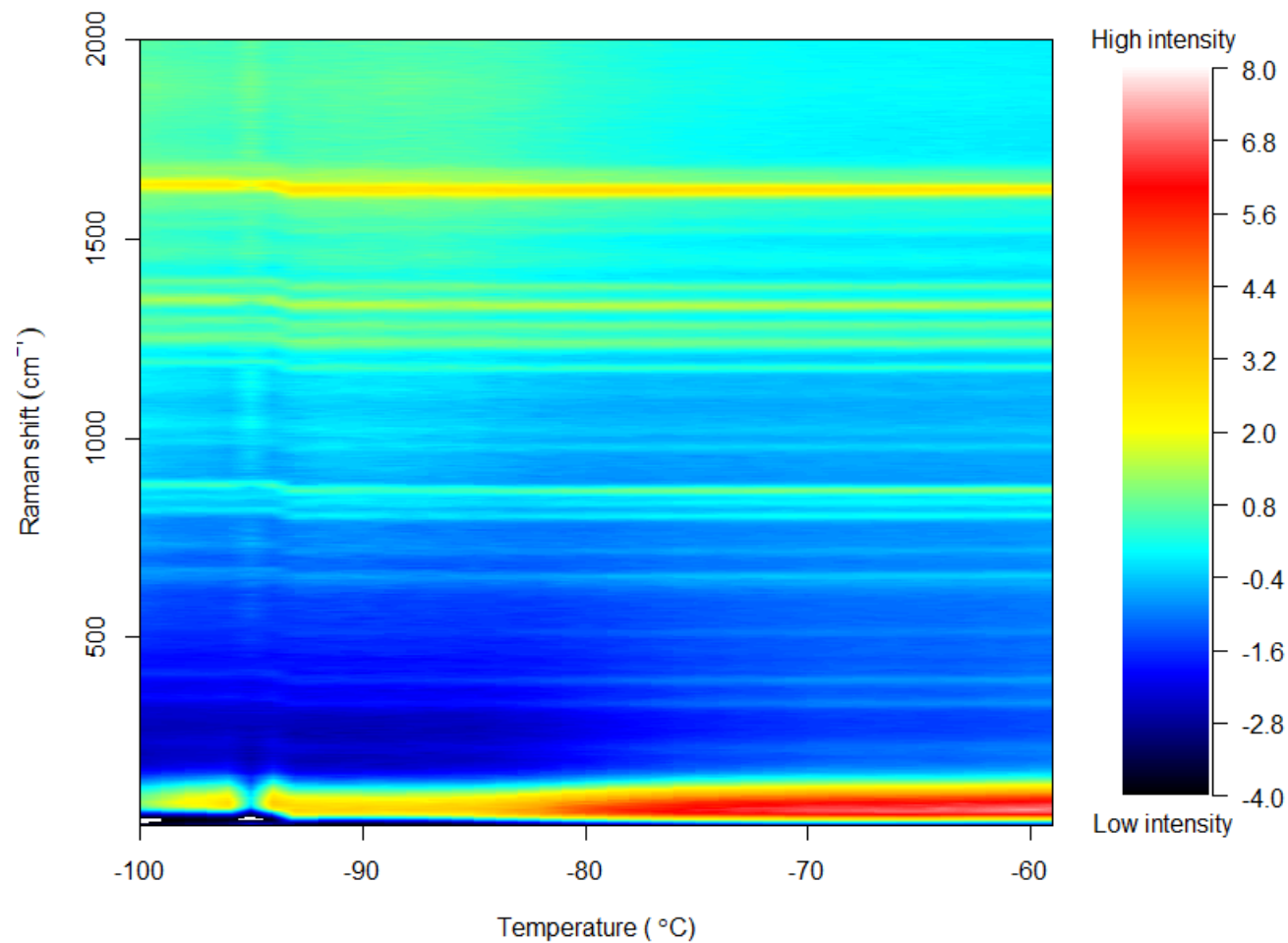


Figure B 13. Low temperature paracetamol repeat experiment 3 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

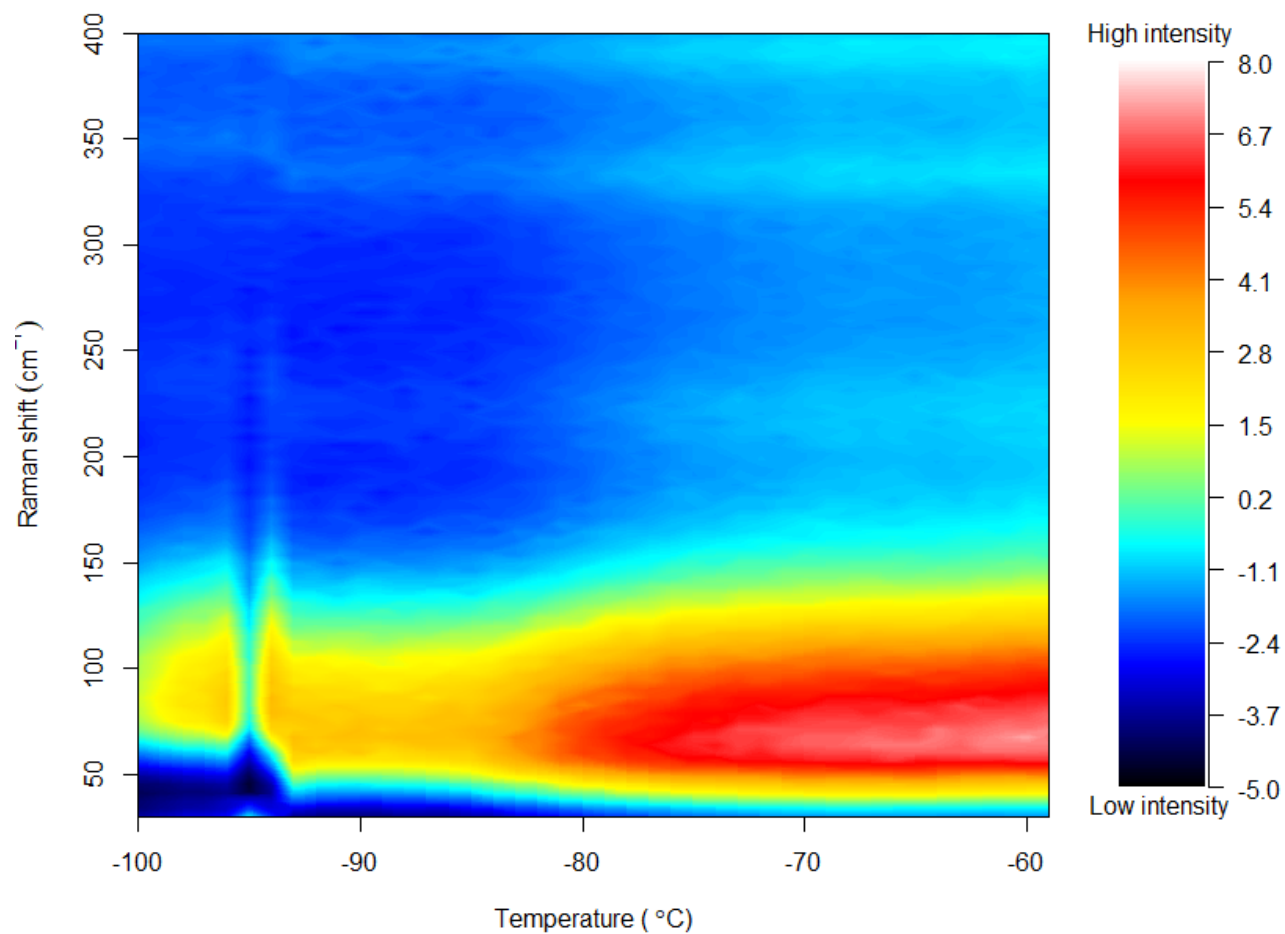


Figure B 14. Low temperature paracetamol repeat experiment 3 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm^{-1} heated from -100 to -59°C . As noted in the text, the sample is heated to 180°C and cooled to $30^{\circ}\text{C}/\text{min}$. It is thought to be amorphous at low temperatures.

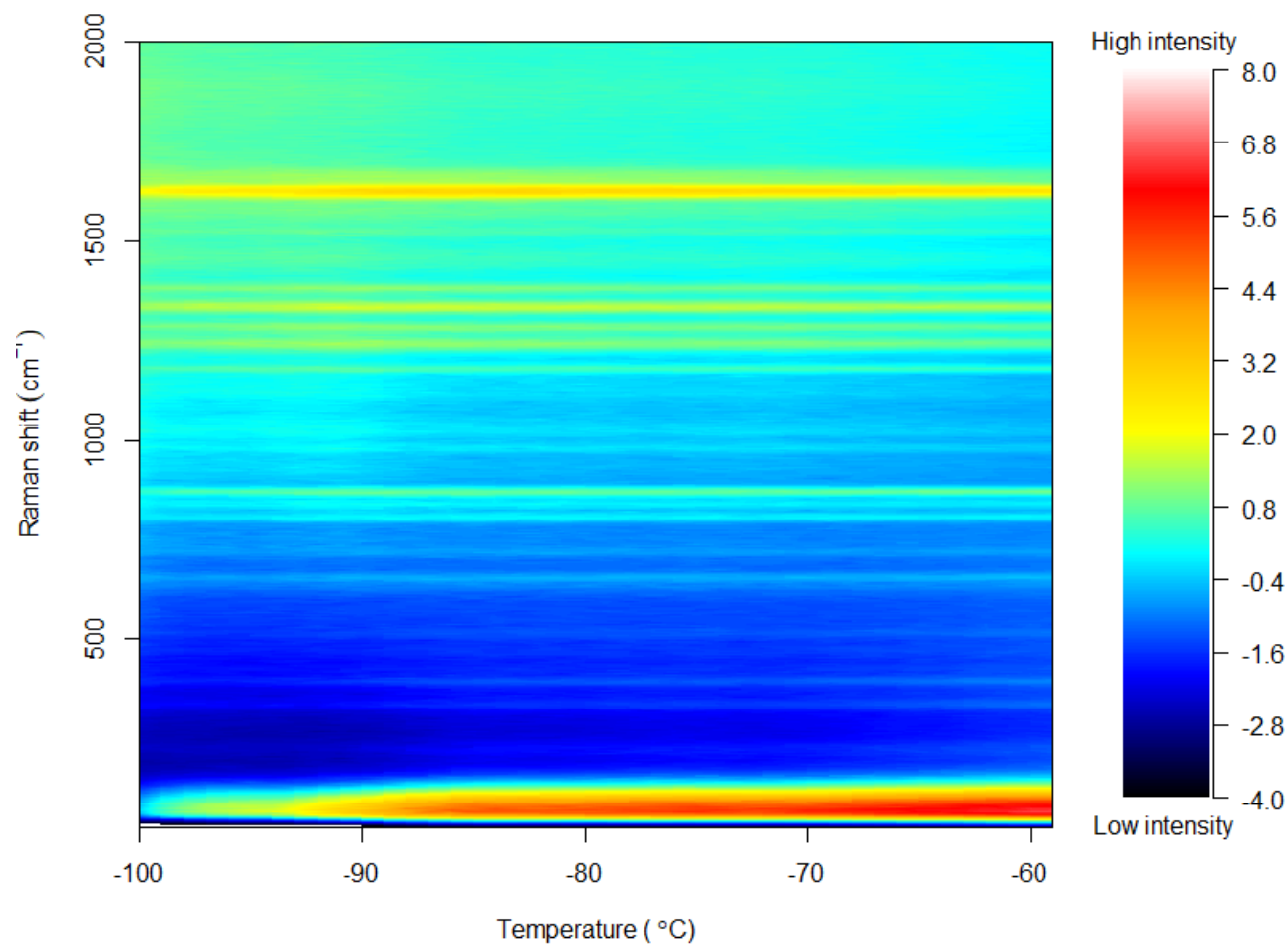


Figure B 15. Low temperature paracetamol repeat experiment 3 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

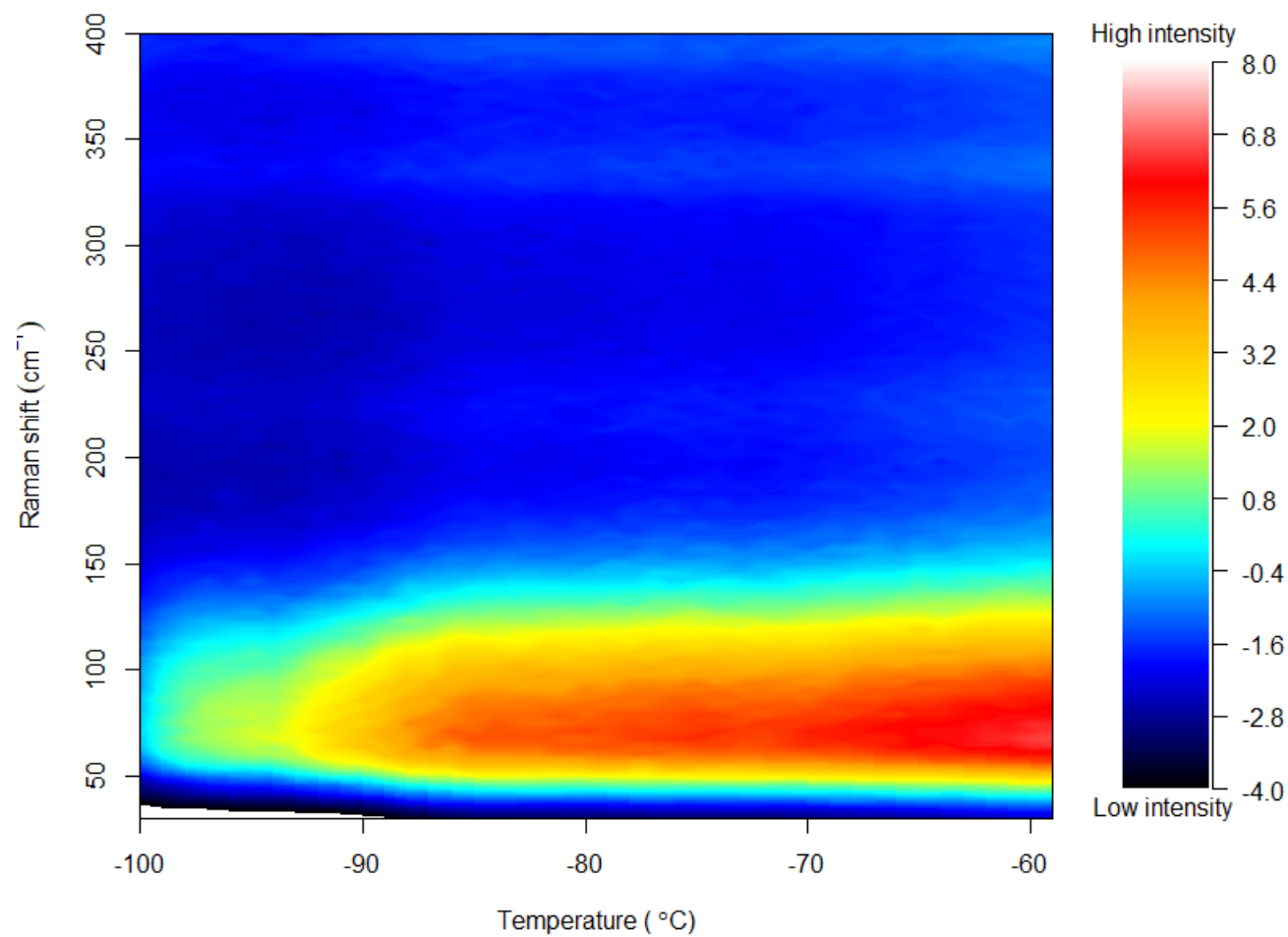


Figure B 16. Low temperature paracetamol repeat experiment 3 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -59°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

Appendix C

Supplementary information part III

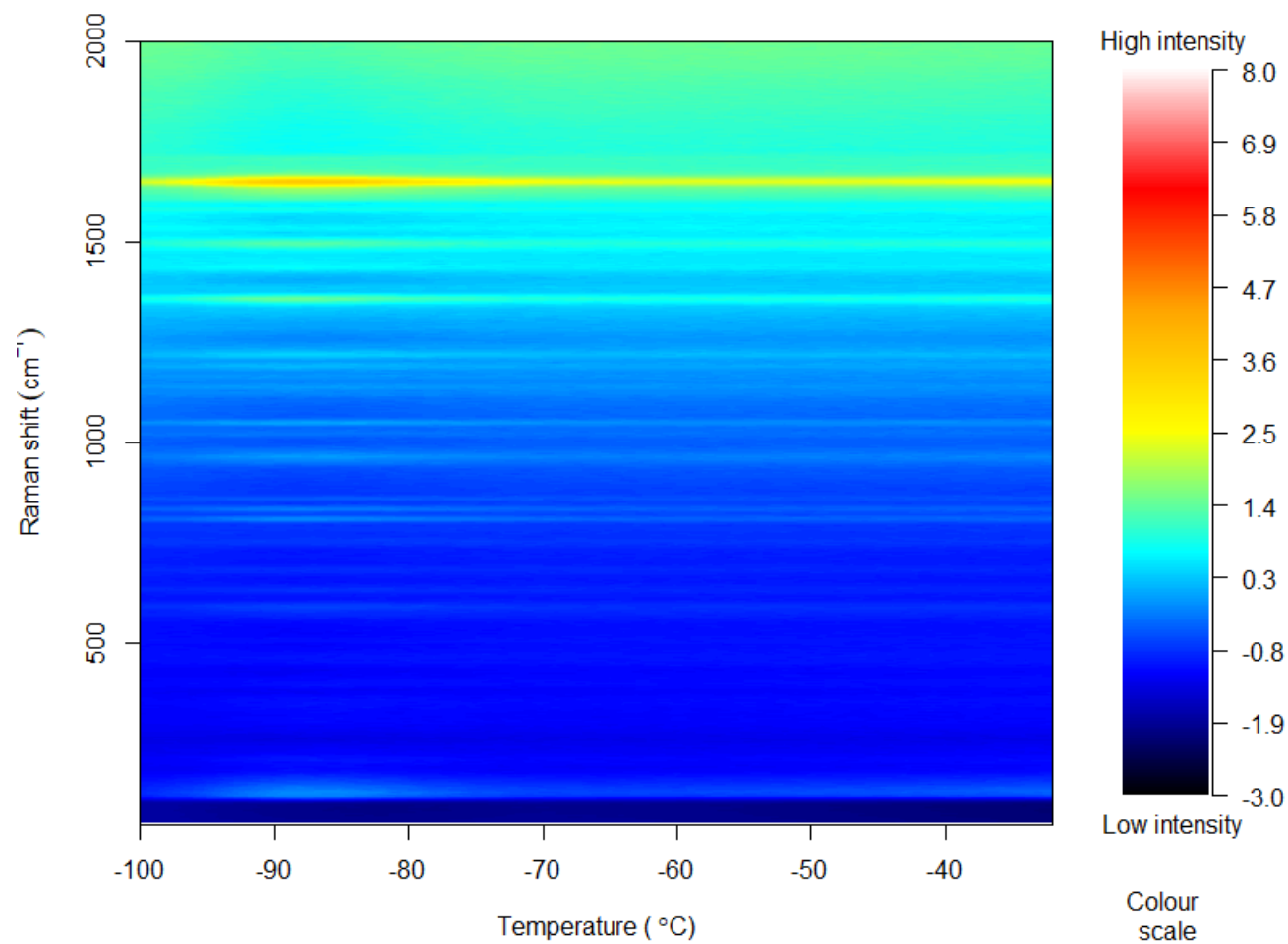


Figure C 1. Low temperature nifedipine repeat experiment 2 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

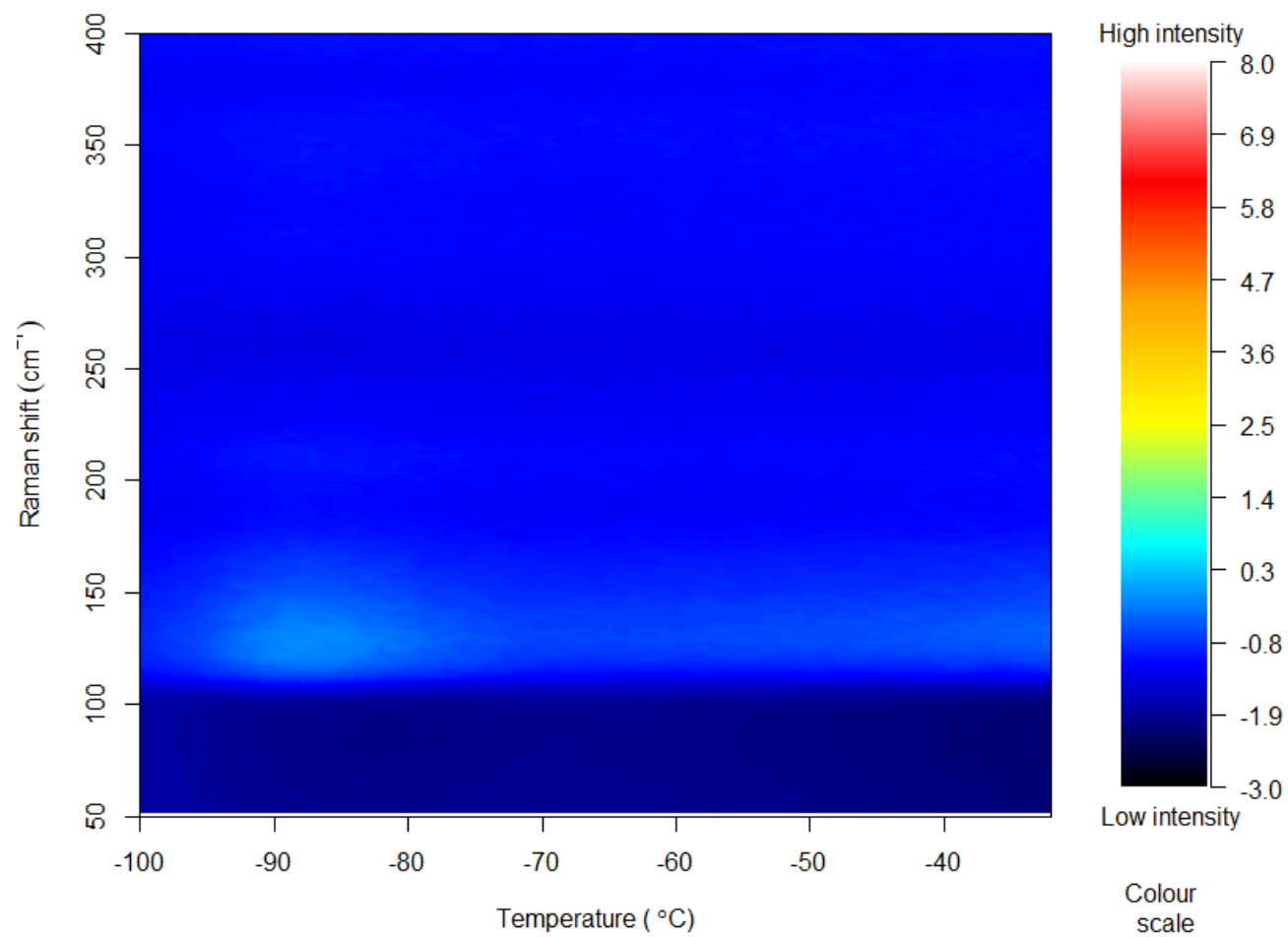


Figure C 2. Low temperature nifedipine repeat experiment 2 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

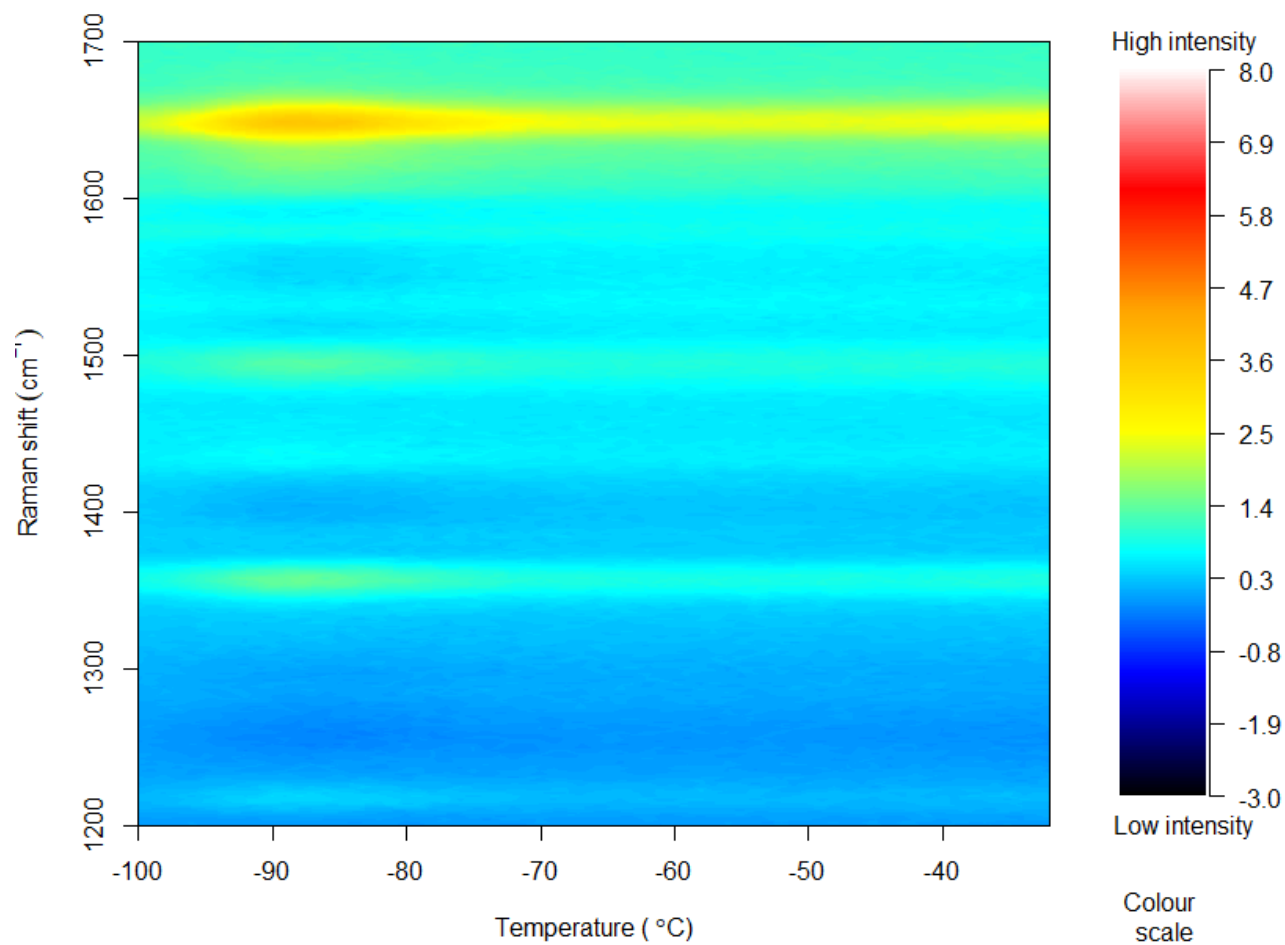


Figure C 3. Low temperature nifedipine repeat experiment 2 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

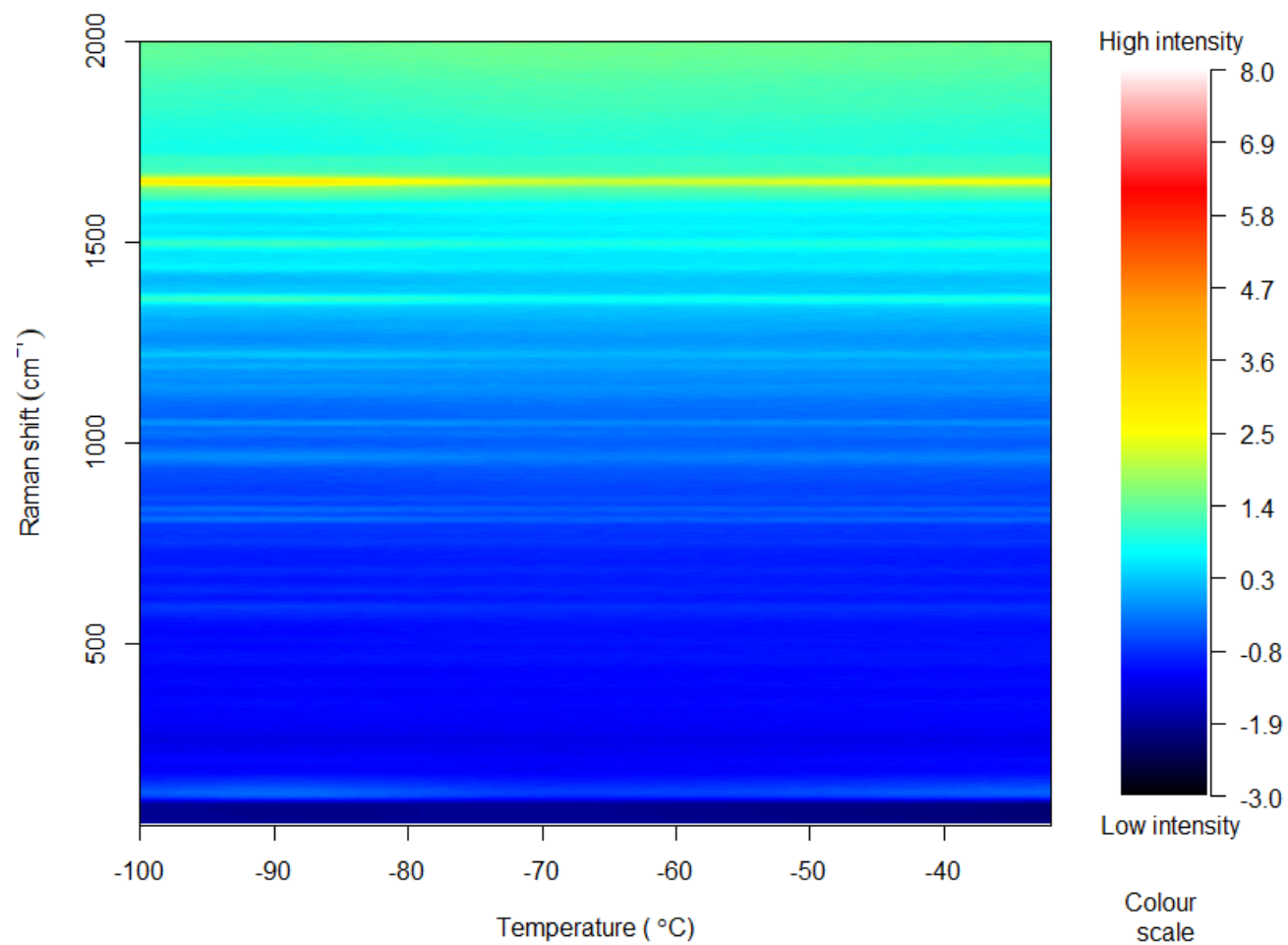


Figure C 4. Low temperature nifedipine repeat experiment 2 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ cooled from -30 to -100°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

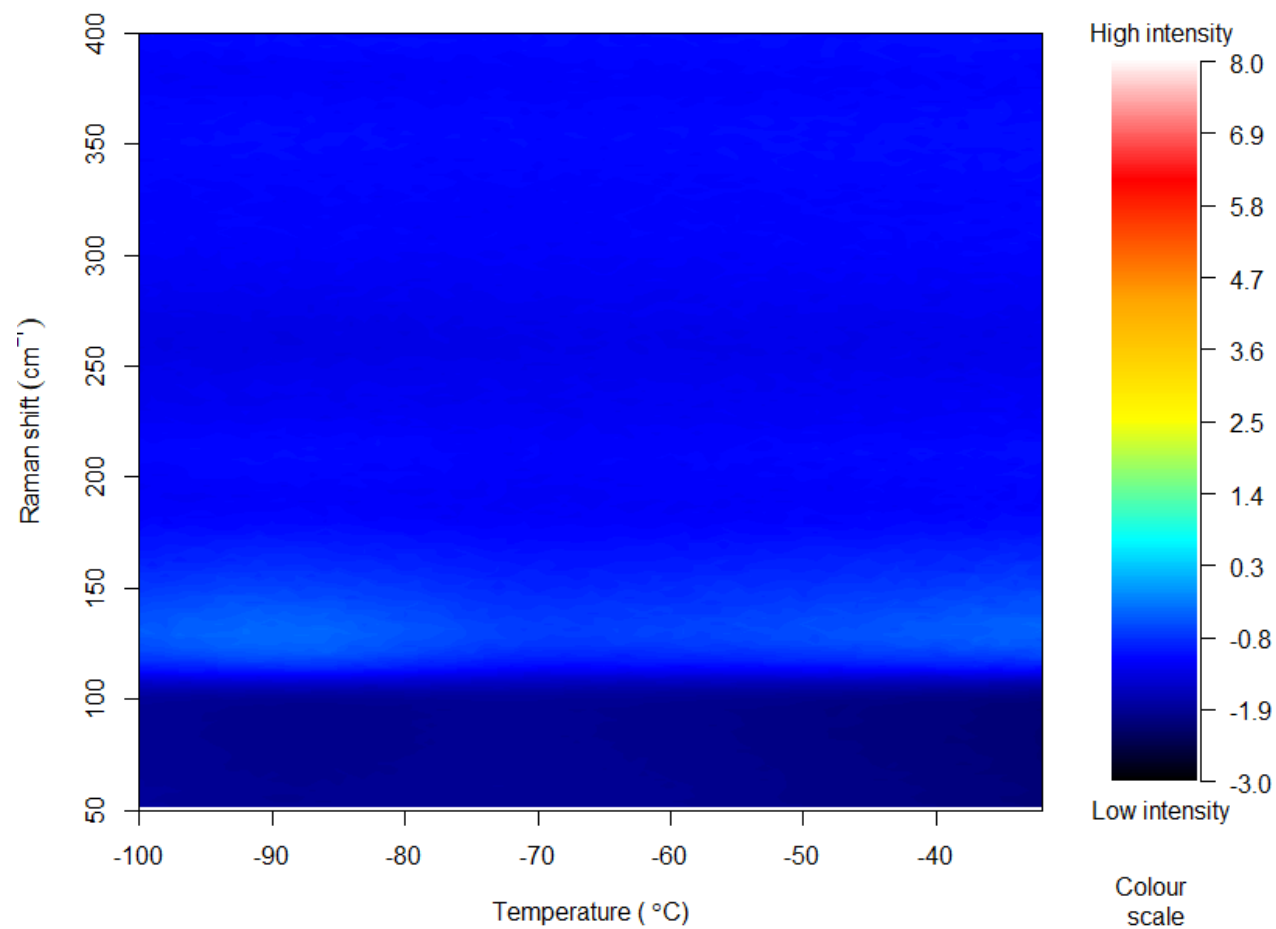


Figure C 5. Low temperature nifedipine repeat experiment 2 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ cooled from -30 to -100°C As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

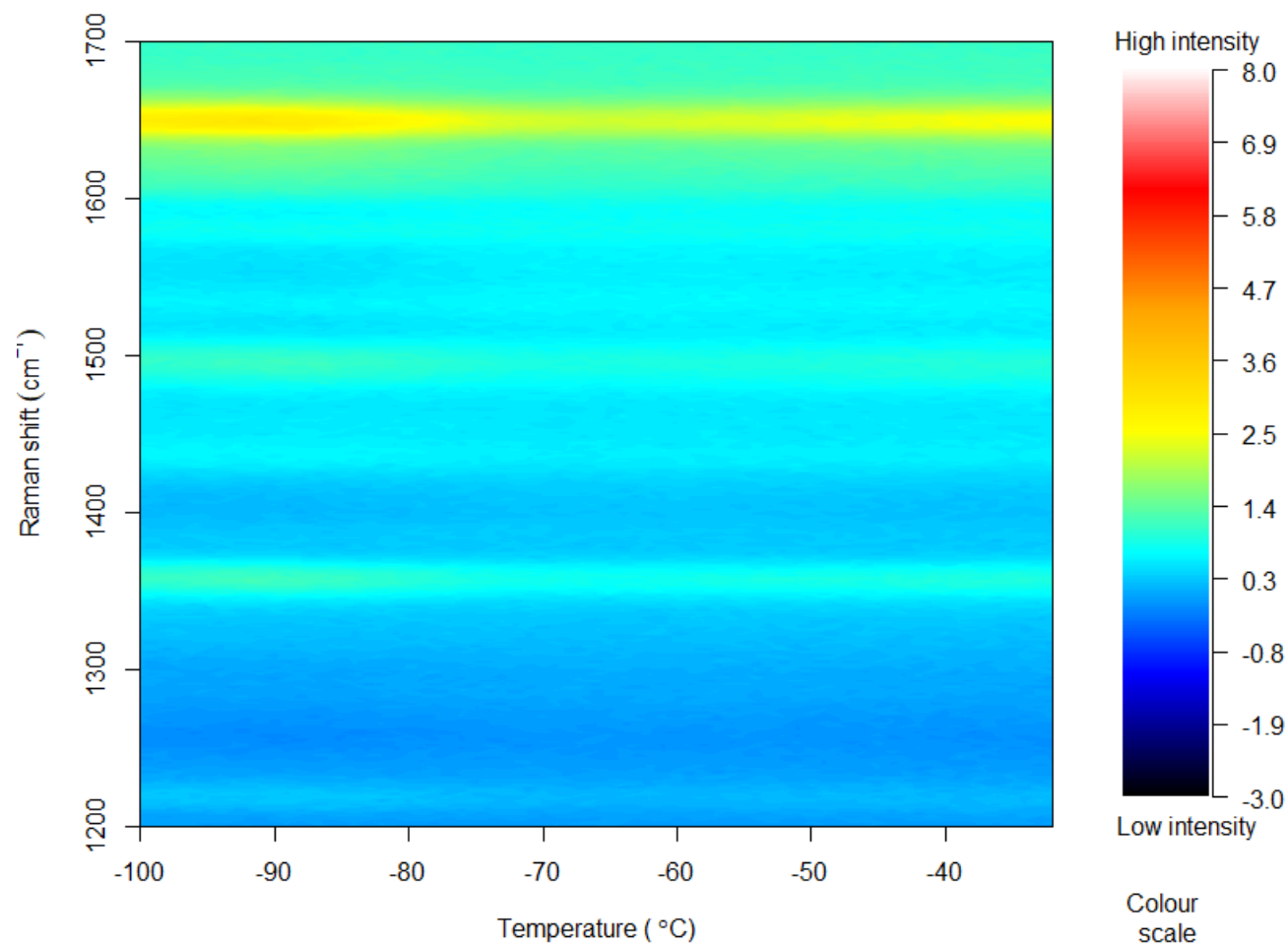


Figure C 6. Low temperature nifedipine repeat experiment 2 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ cooled from -30 to -100°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

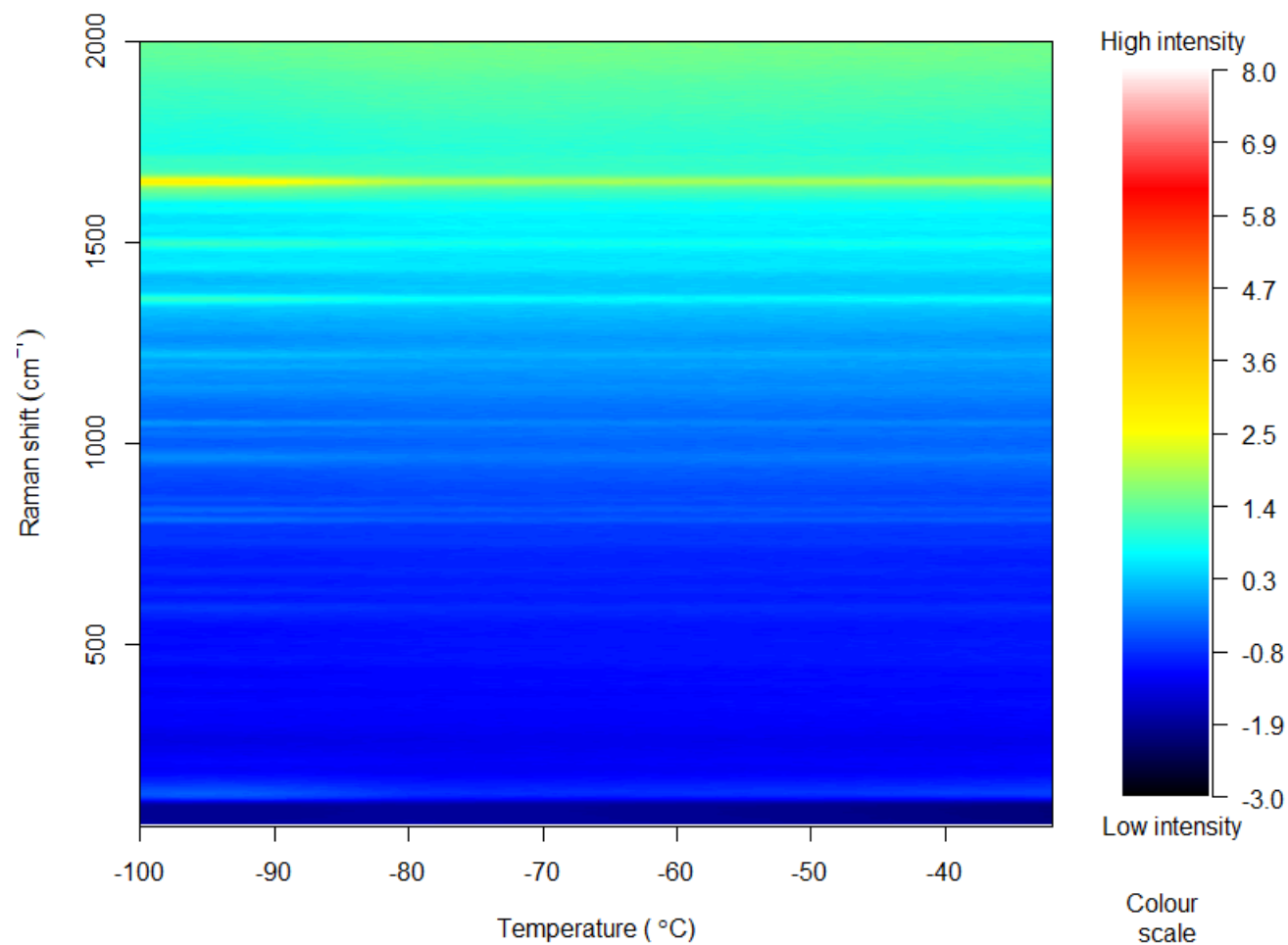


Figure C 7. Low temperature nifedipine repeat experiment 2 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm^{-1} heated from -100 to -30 $^{\circ}\text{C}$. As noted in the text, the sample is heated to 180 $^{\circ}\text{C}$ and cooled to 30 $^{\circ}\text{C}/\text{min}$. It is thought to be amorphous at low temperatures.

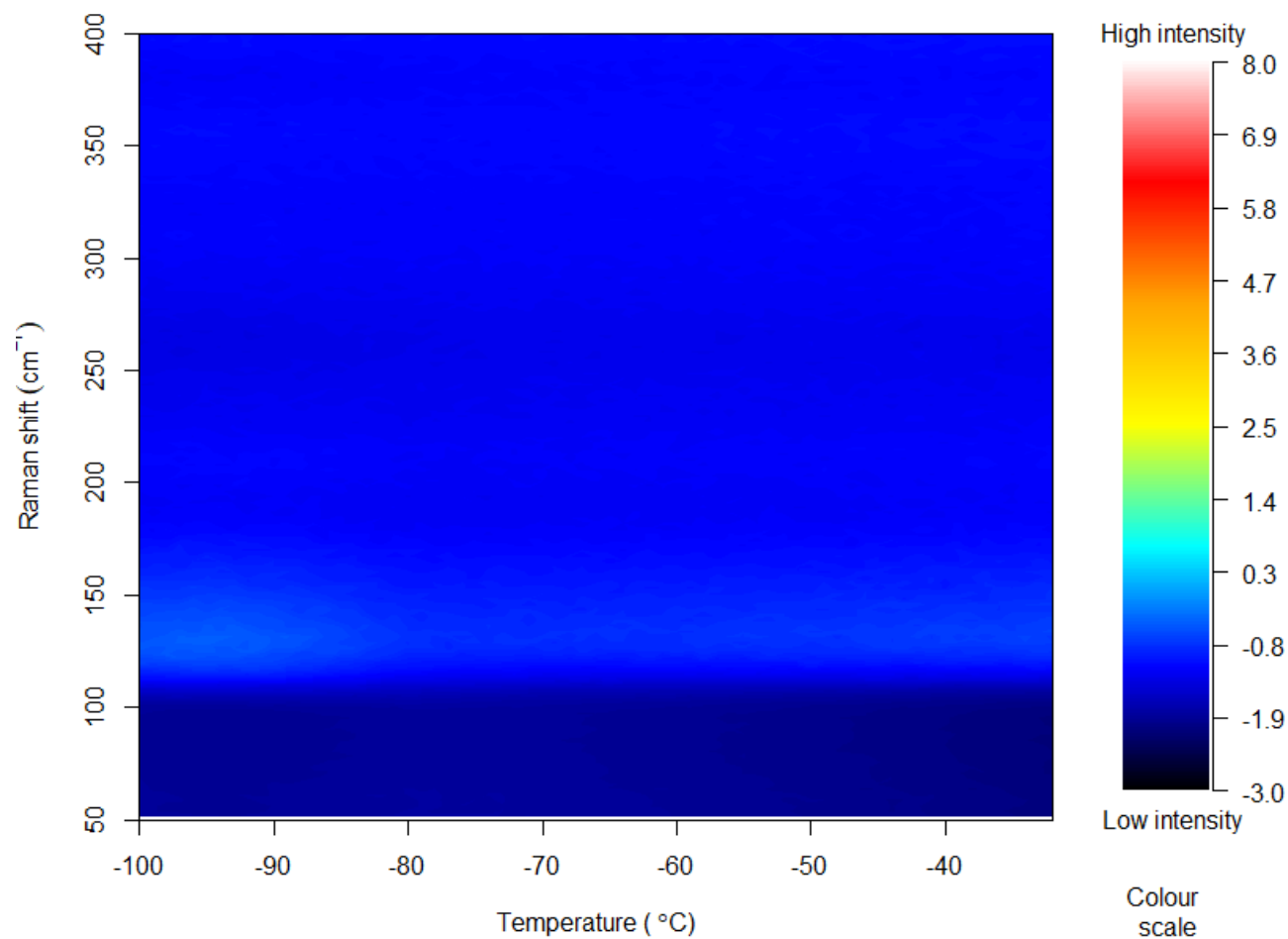


Figure C 8. Low temperature nifedipine repeat experiment 2 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

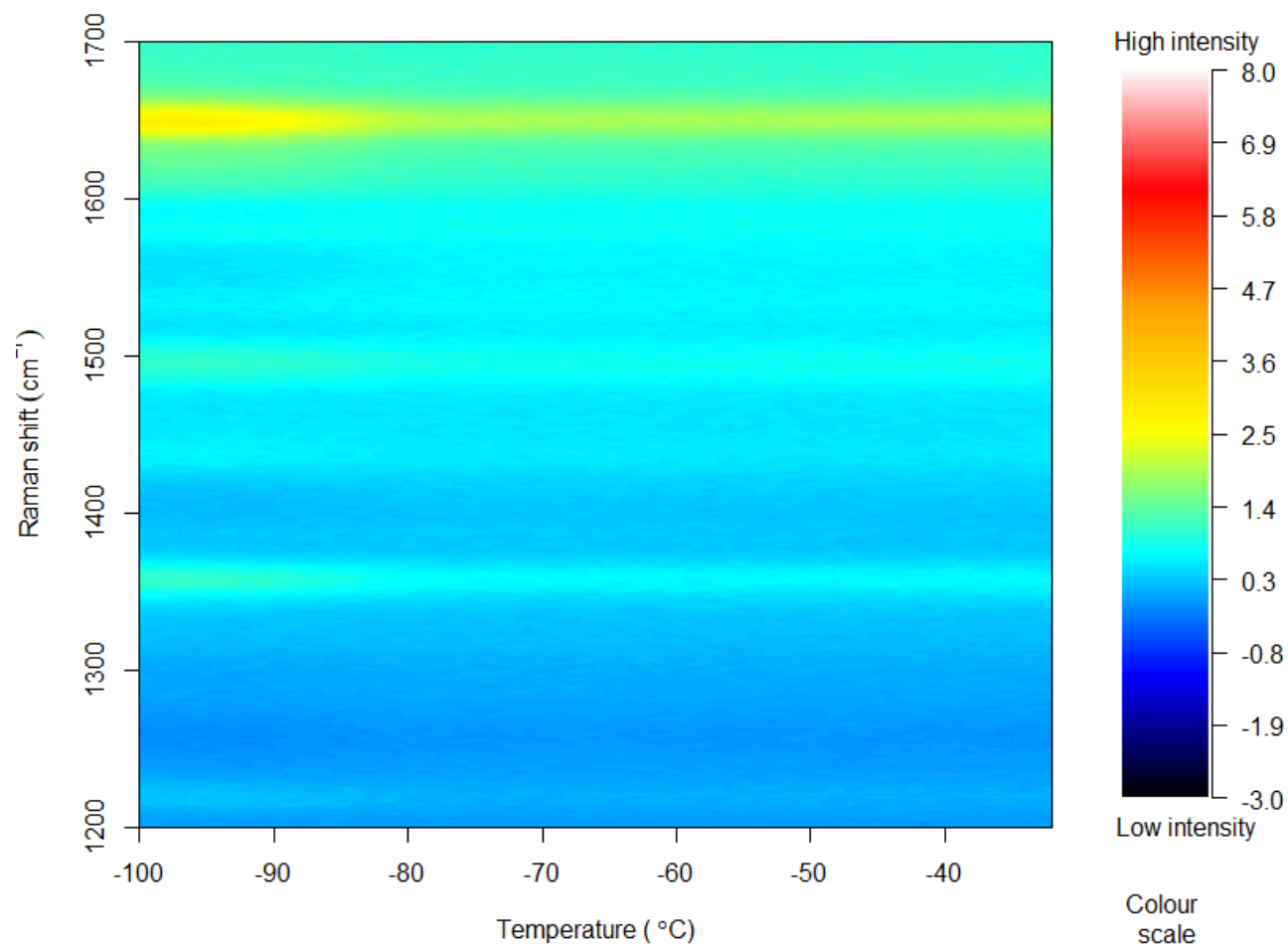


Figure C 9. Low temperature nifedipine repeat experiment 2 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 17000 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

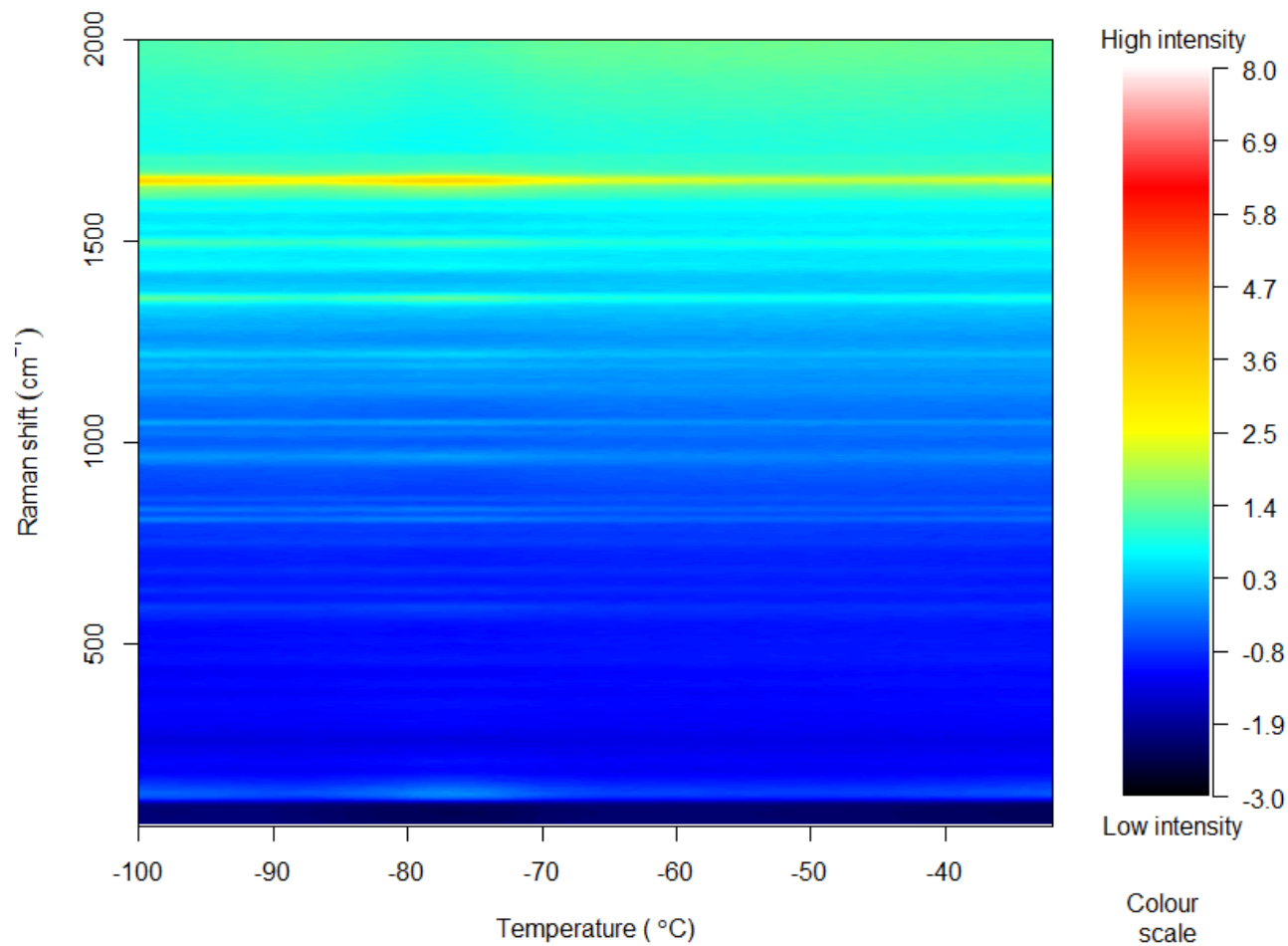


Figure C 10. Low temperature nifedipine repeat experiment 3 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

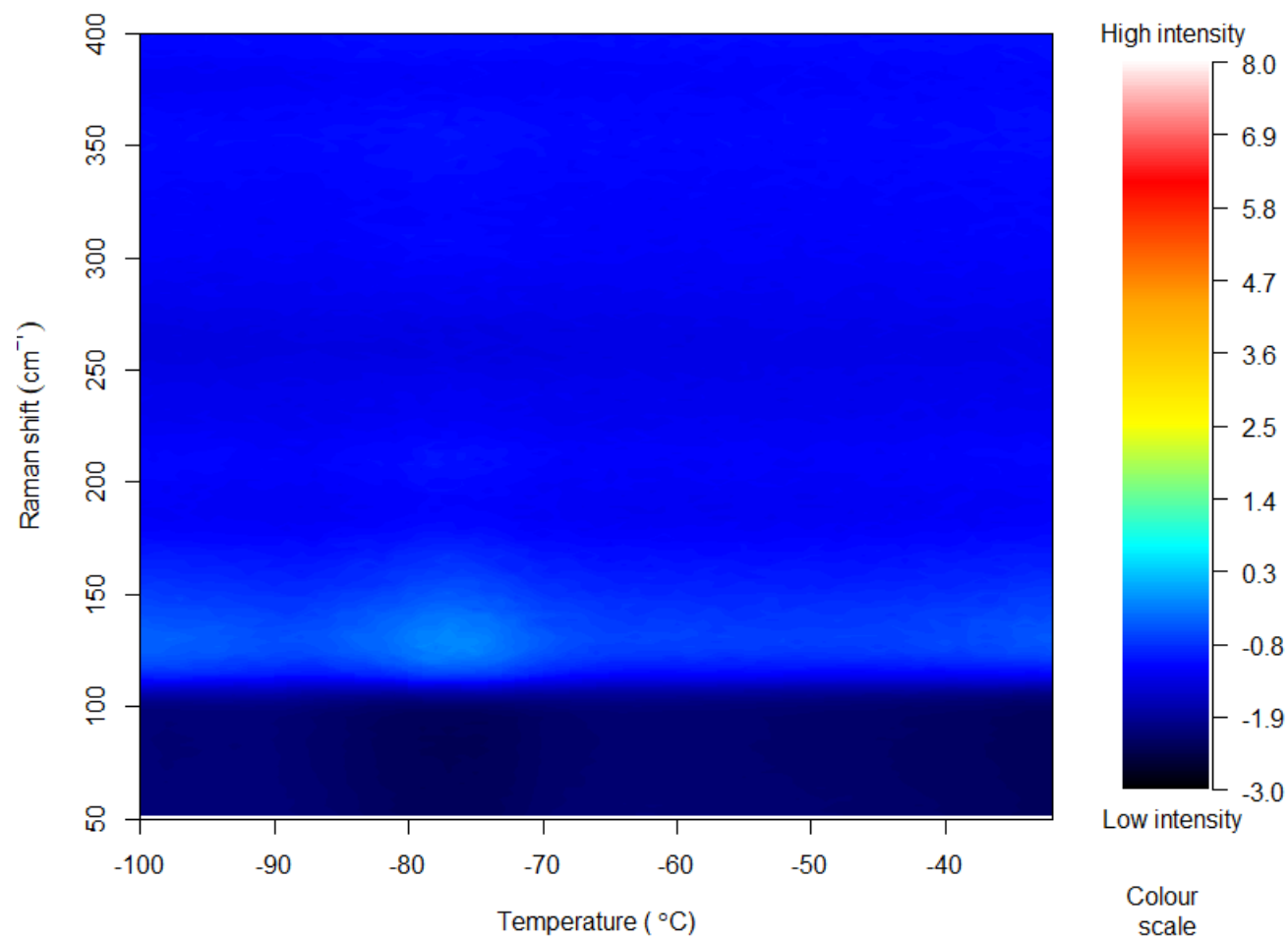


Figure C 11. Low temperature nifedipine repeat experiment 3 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

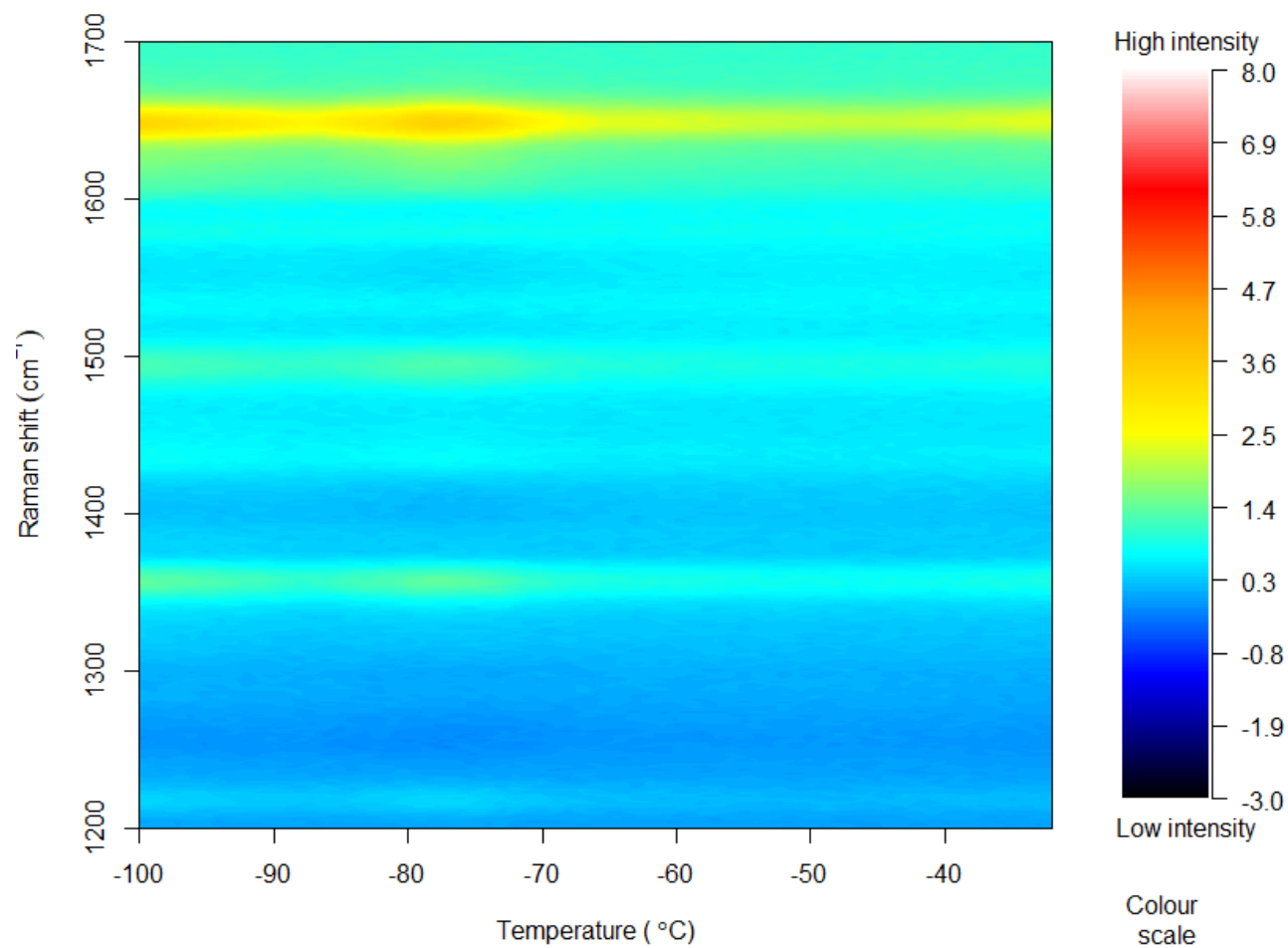


Figure C 12. Low temperature nifedipine repeat experiment 3 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

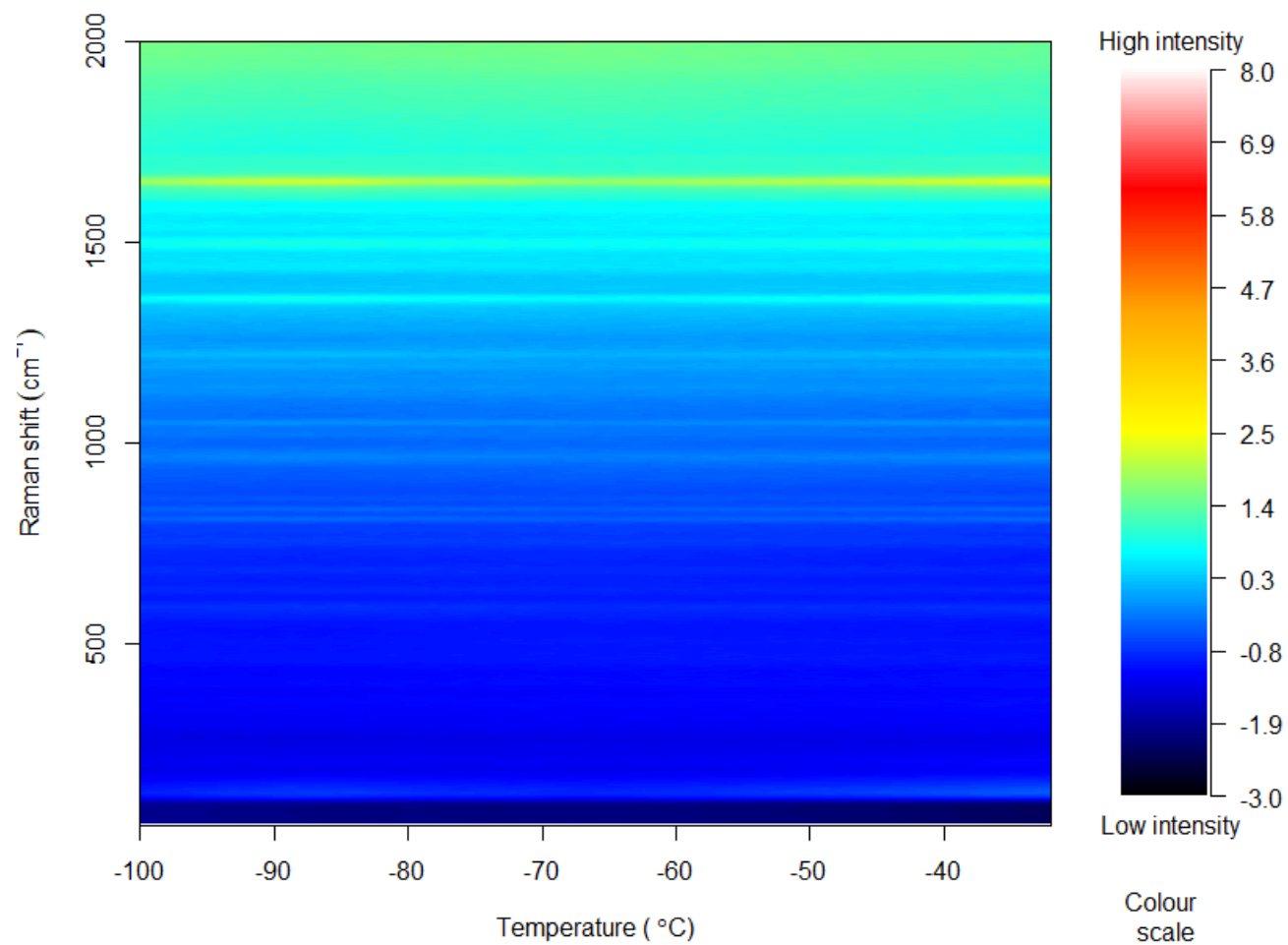


Figure C 13. Low temperature nifedipine repeat experiment 3 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm^{-1} cooled from -30 to -100 $^{\circ}\text{C}$. As noted in the text, the sample is heated to 180 $^{\circ}\text{C}$ and cooled to 30 $^{\circ}\text{C}/\text{min}$. It is thought to be amorphous at low temperatures.

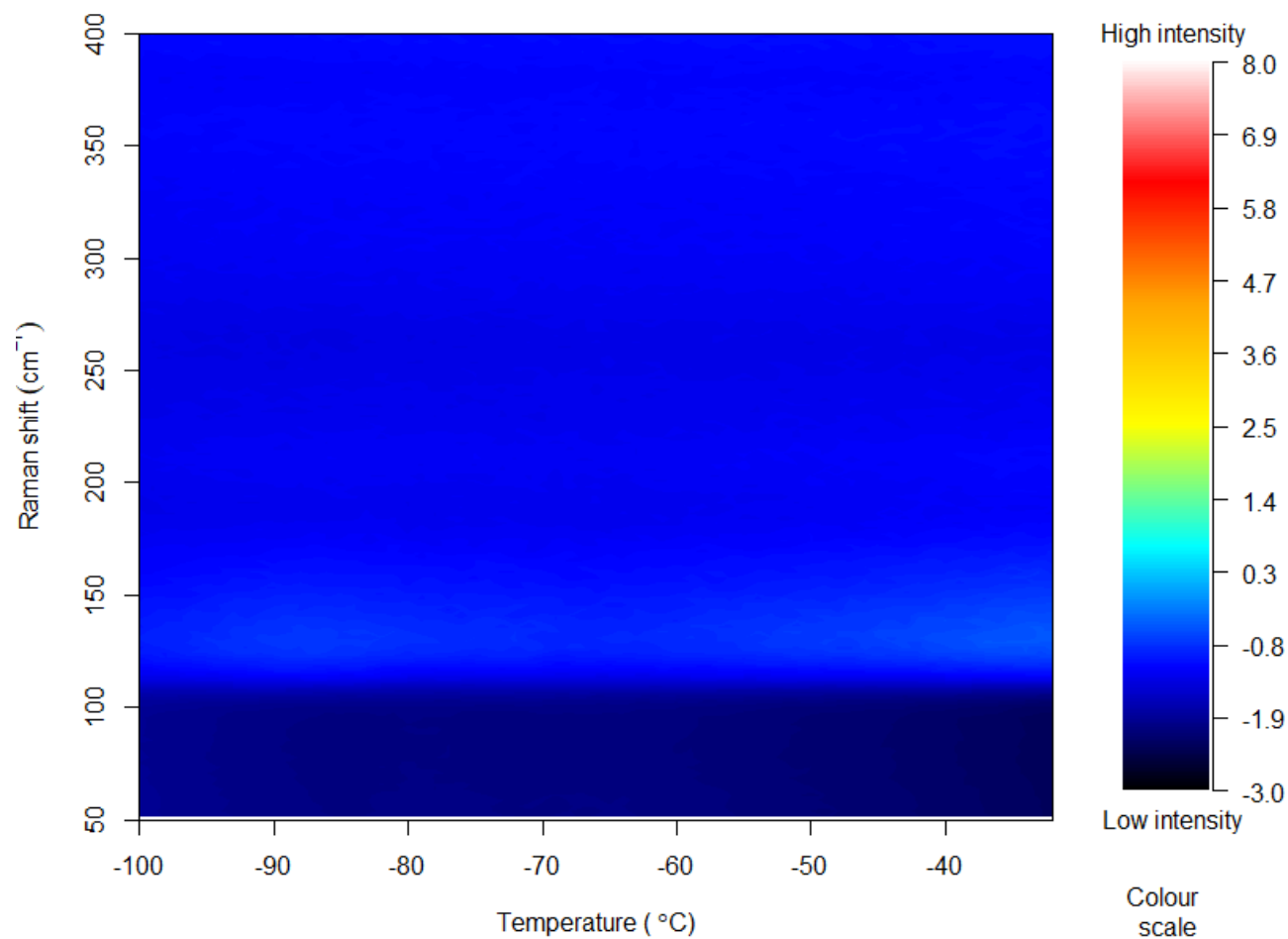


Figure C 14. Low temperature nifedipine repeat experiment 3 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ cooled from -30 to -100°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

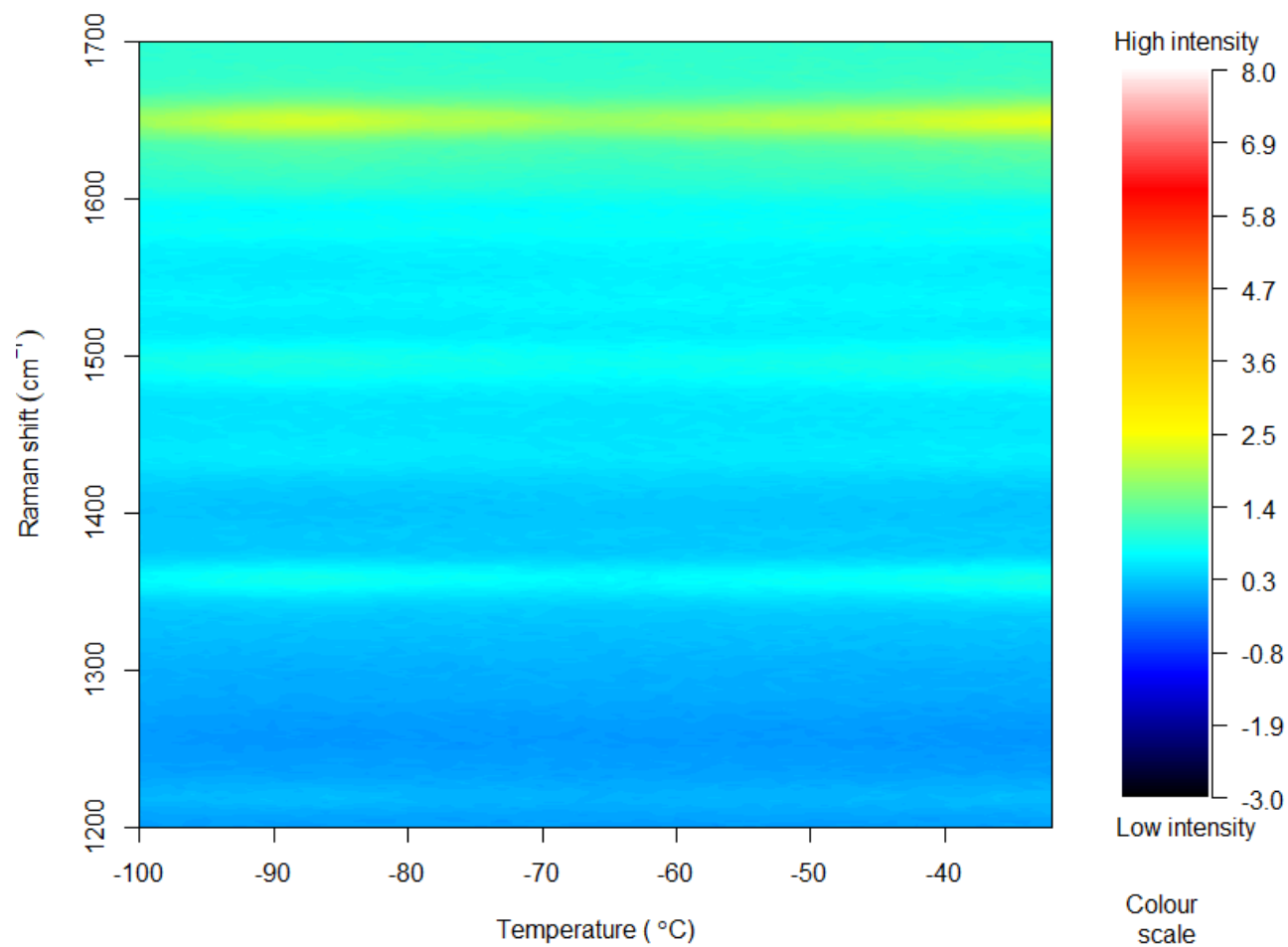


Figure C 15. Low temperature nifedipine repeat experiment 3 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ cooled from -30 to -100°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

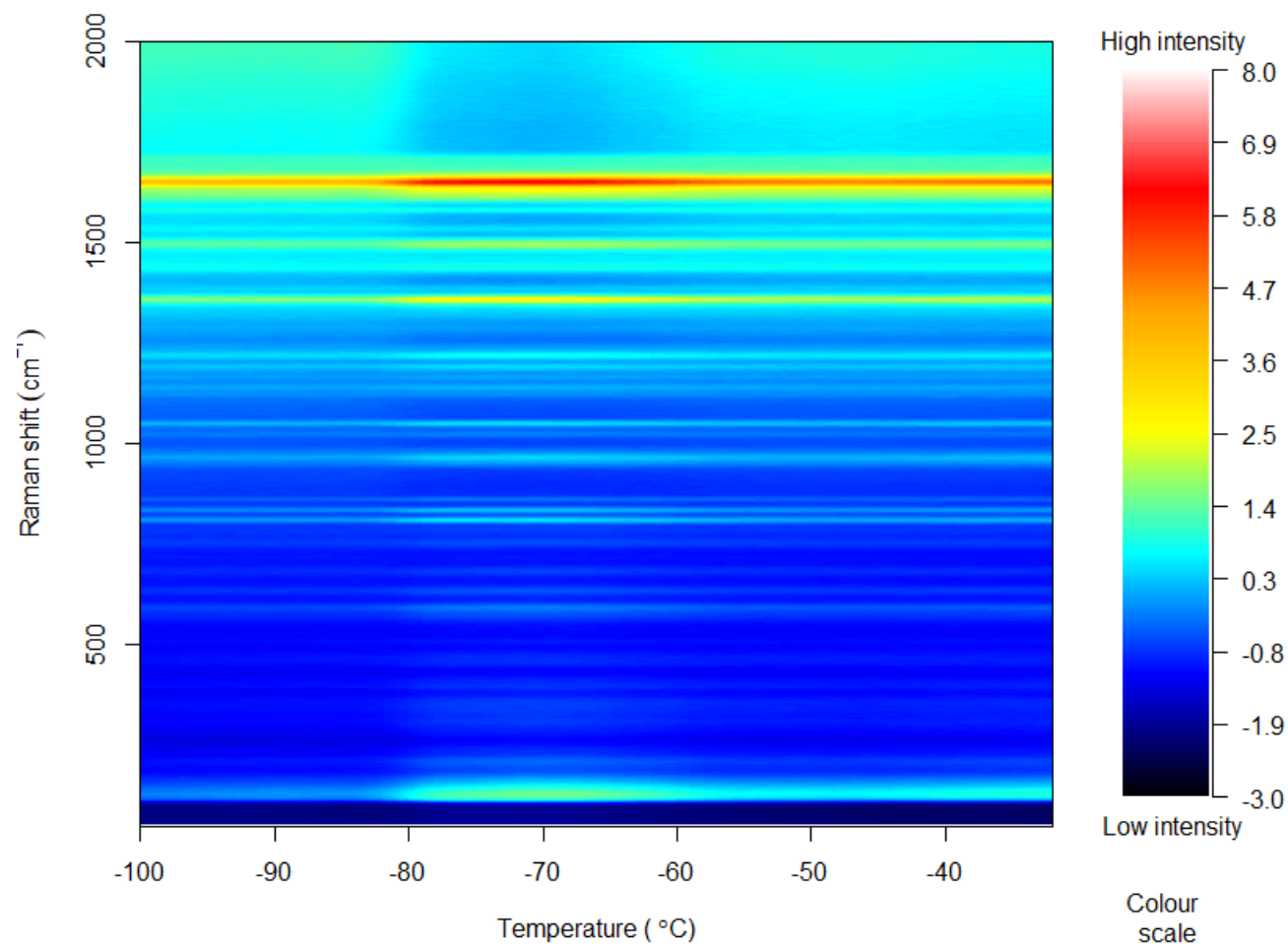


Figure C 16. Low temperature nifedipine repeat experiment 3 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

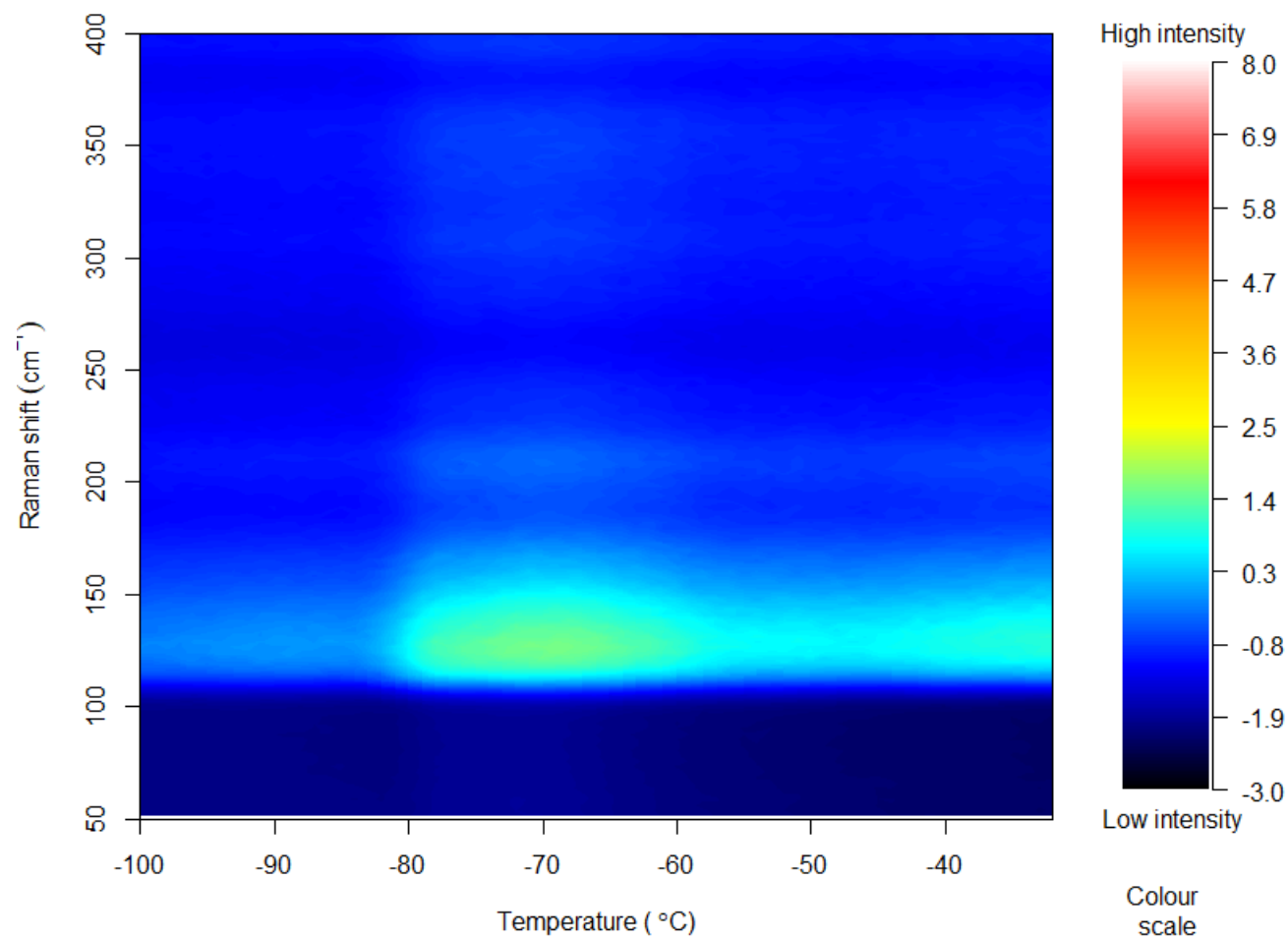


Figure C 17. Low temperature nifedipine repeat experiment 3 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

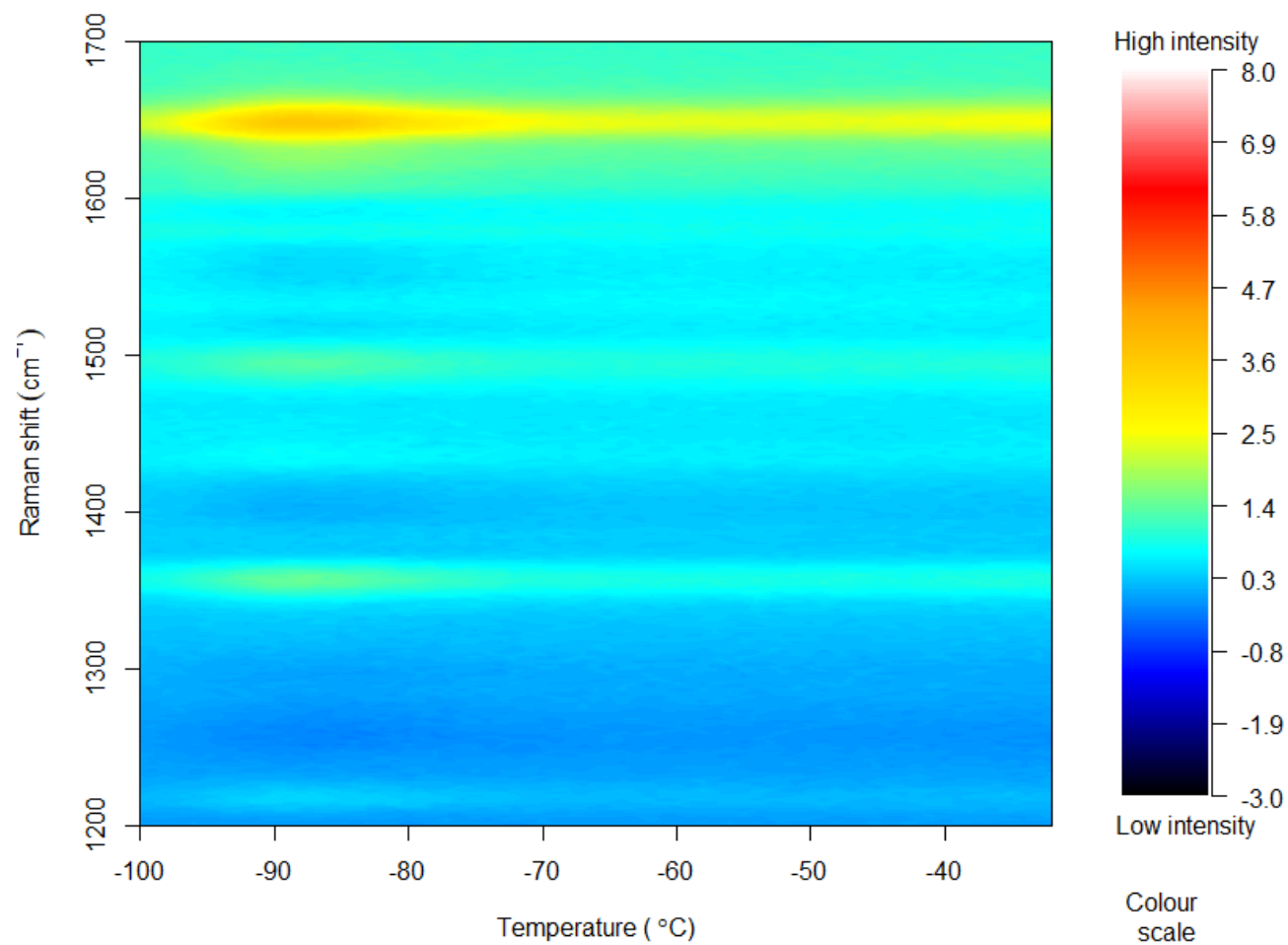


Figure C 18. Low temperature nifedipine repeat experiment 3 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to -30°C. As noted in the text, the sample is heated to 180°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

Appendix D

Supplementary information part IV

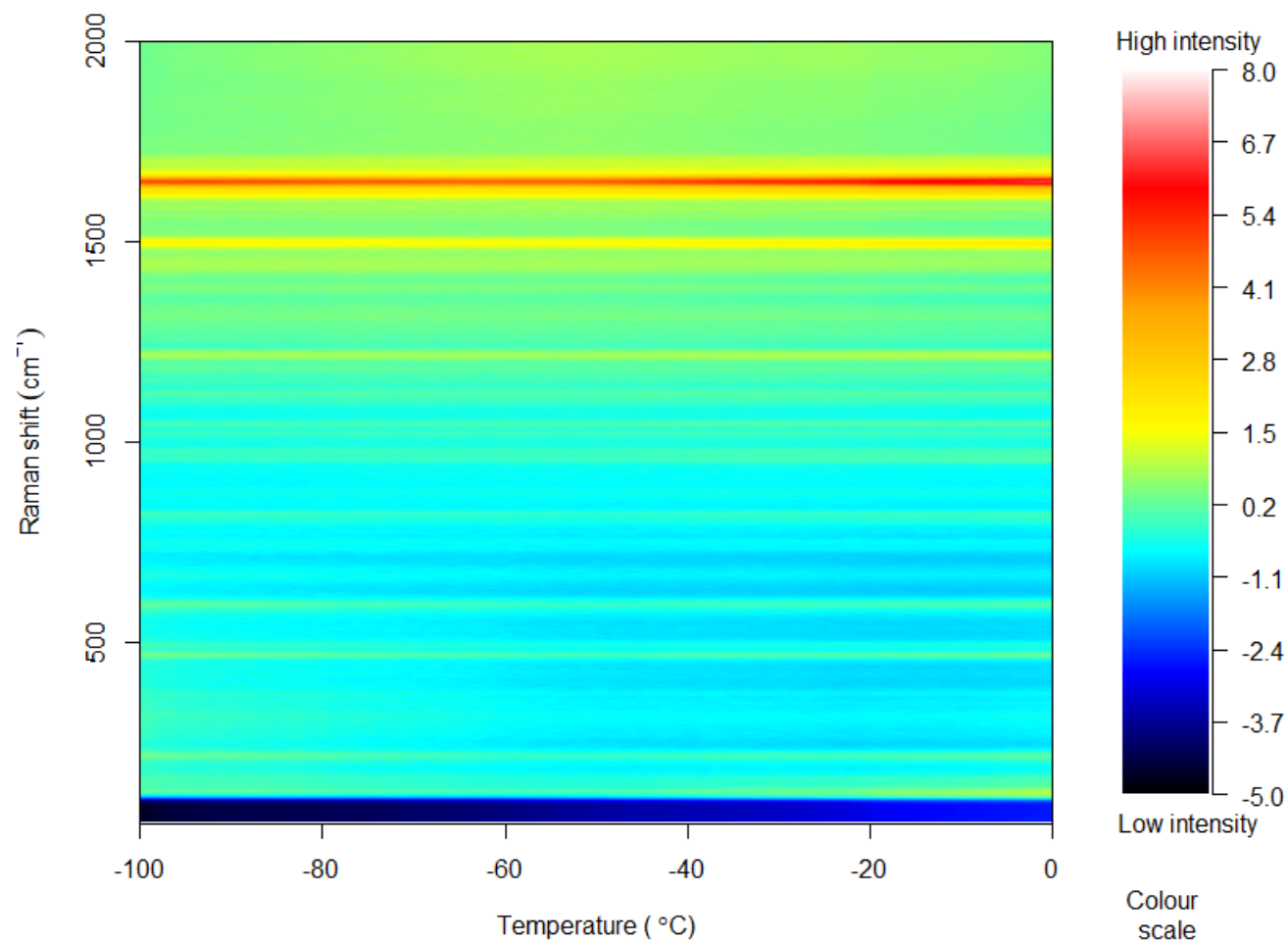


Figure D 1. Low temperature felodipine repeat experiment 2 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm^{-1} heated from -100 to 0 $^{\circ}\text{C}$. As noted in the text, the sample is heated to 150 $^{\circ}\text{C}$ and cooled to 30 $^{\circ}\text{C}/\text{min}$. It is thought to be amorphous at low temperatures.

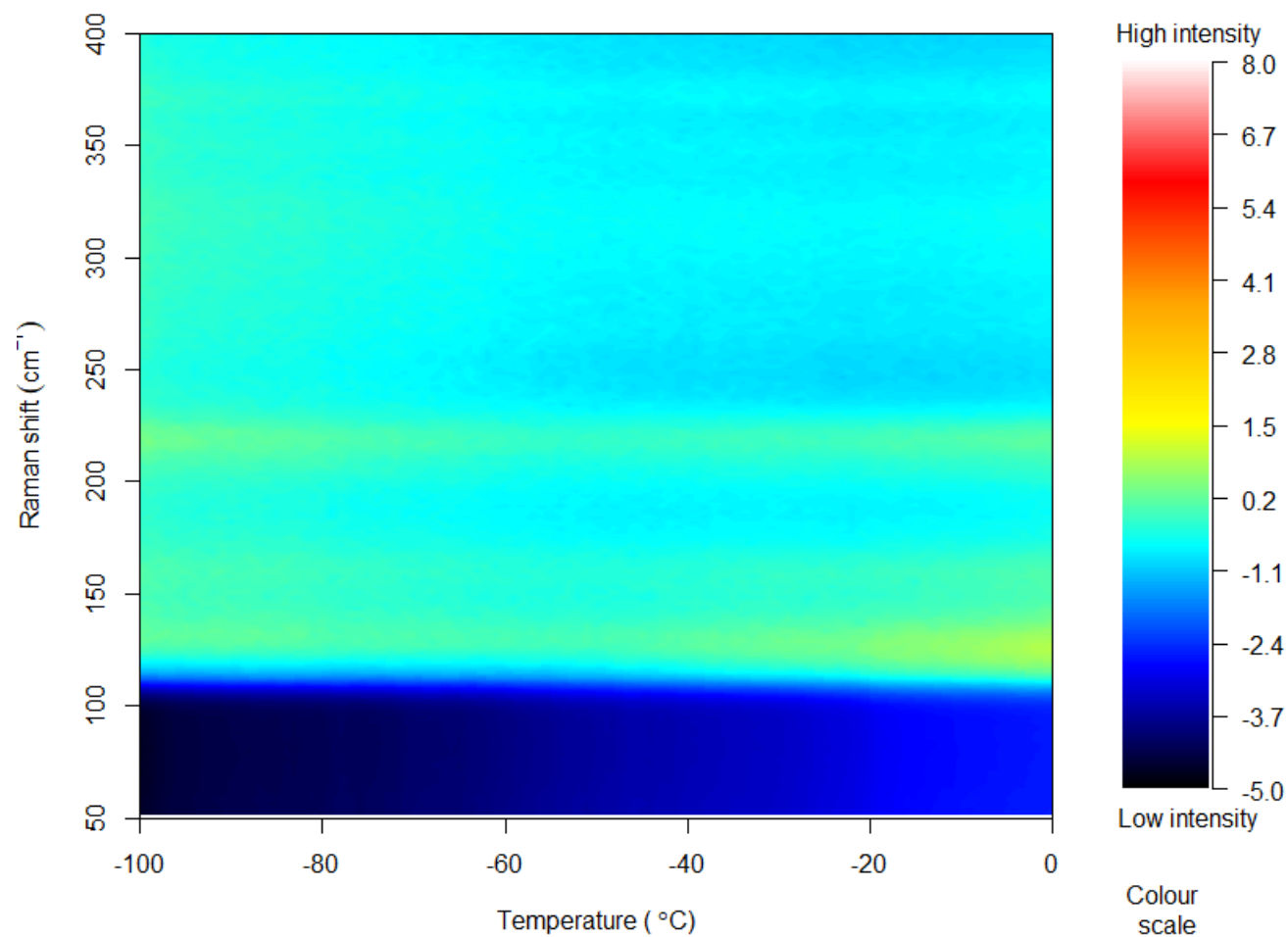


Figure D 2. Low temperature felodipine repeat experiment 2 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

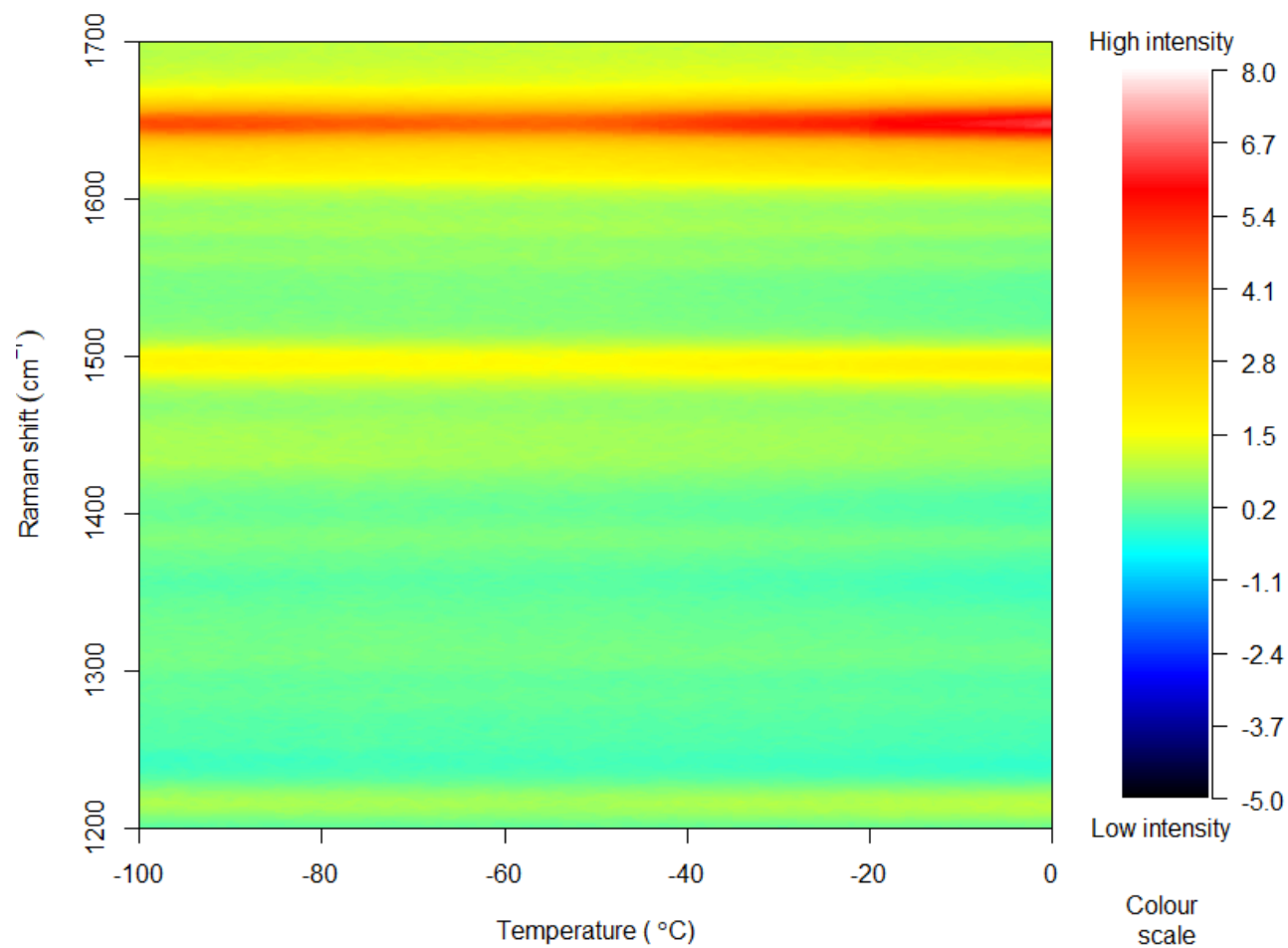


Figure D 3. Low temperature felodipine repeat experiment 2 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

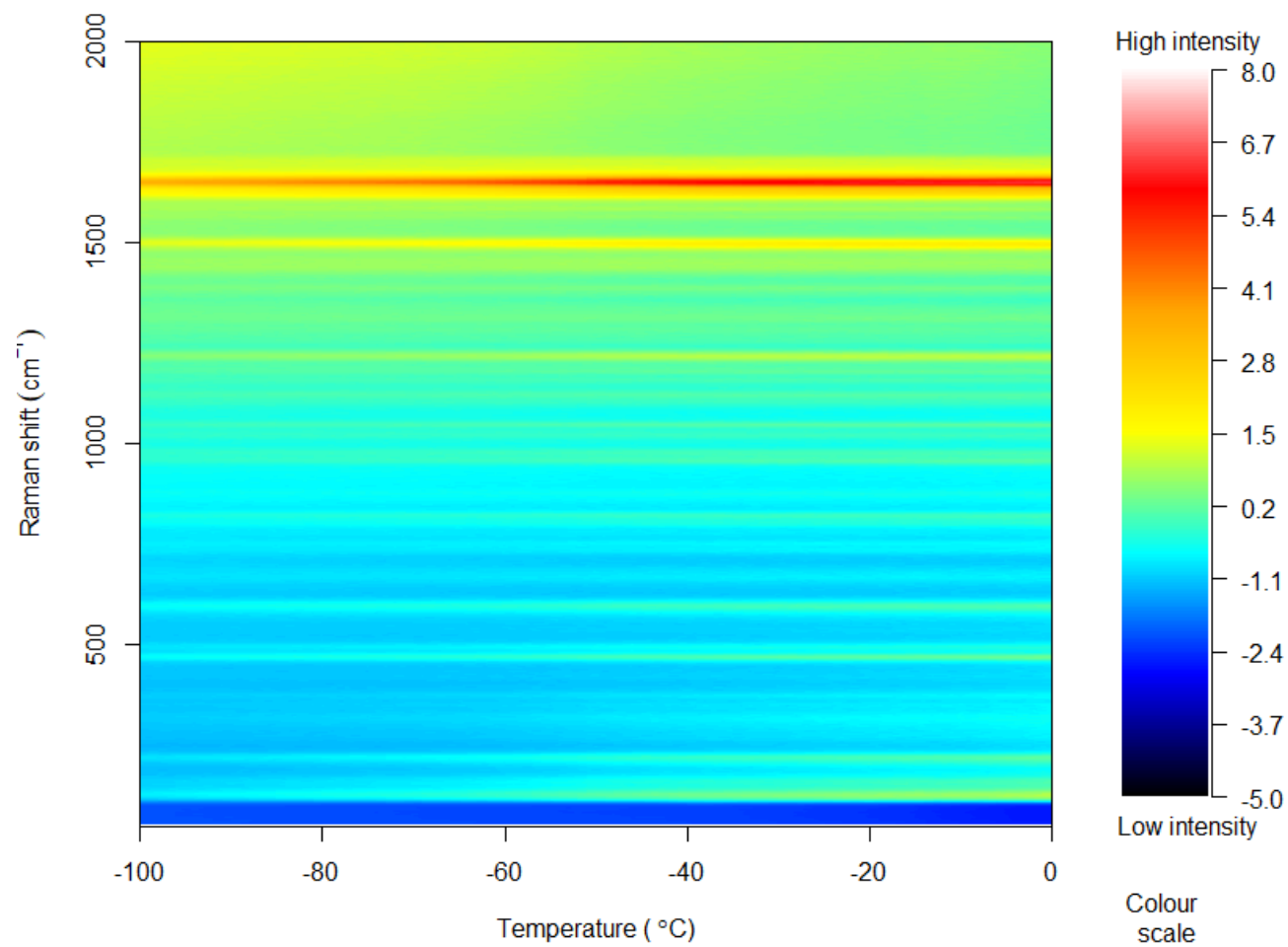


Figure D 4. Low temperature felodipine repeat experiment 2 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

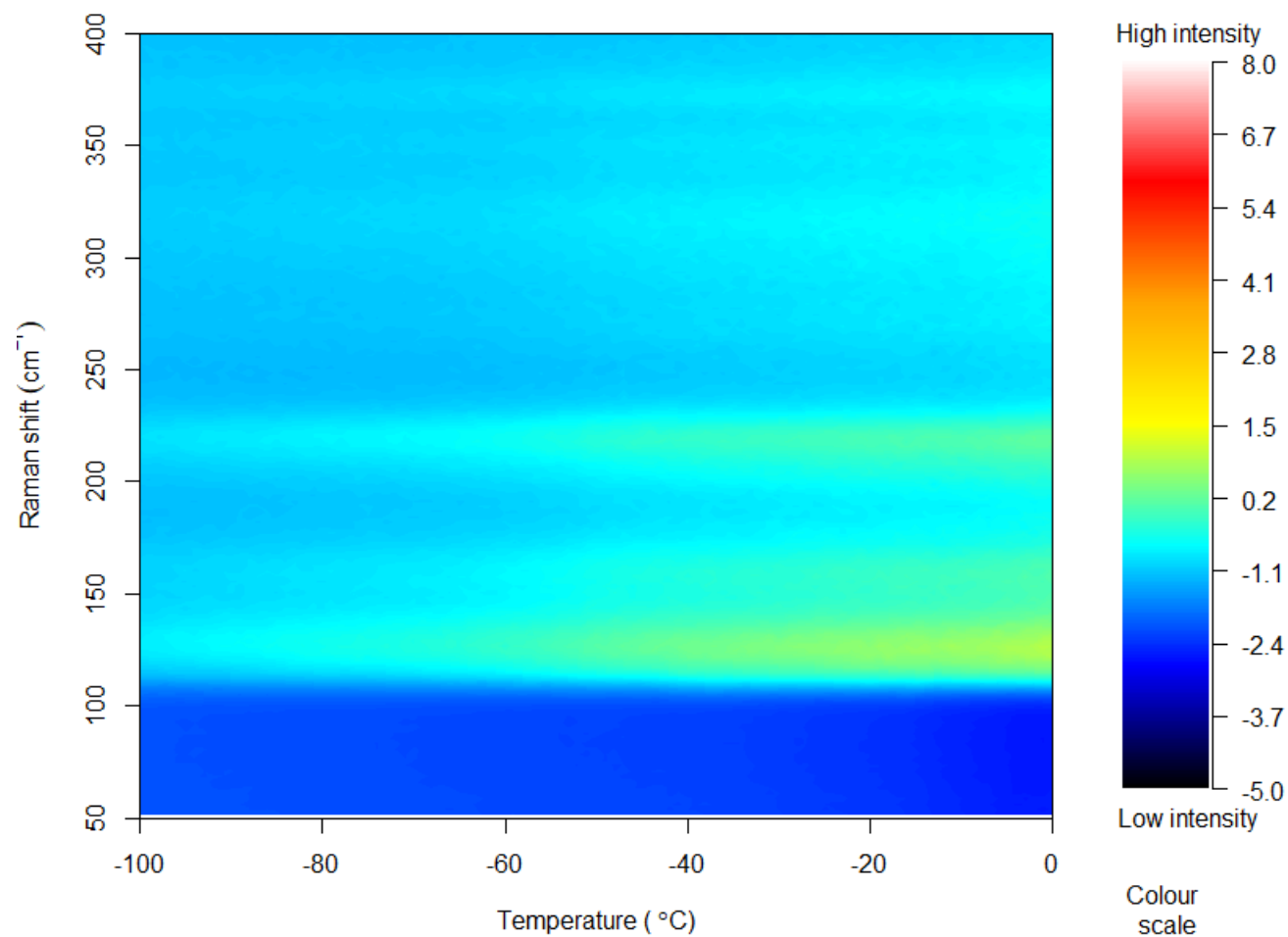


Figure D 5. Low temperature felodipine repeat experiment 2 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

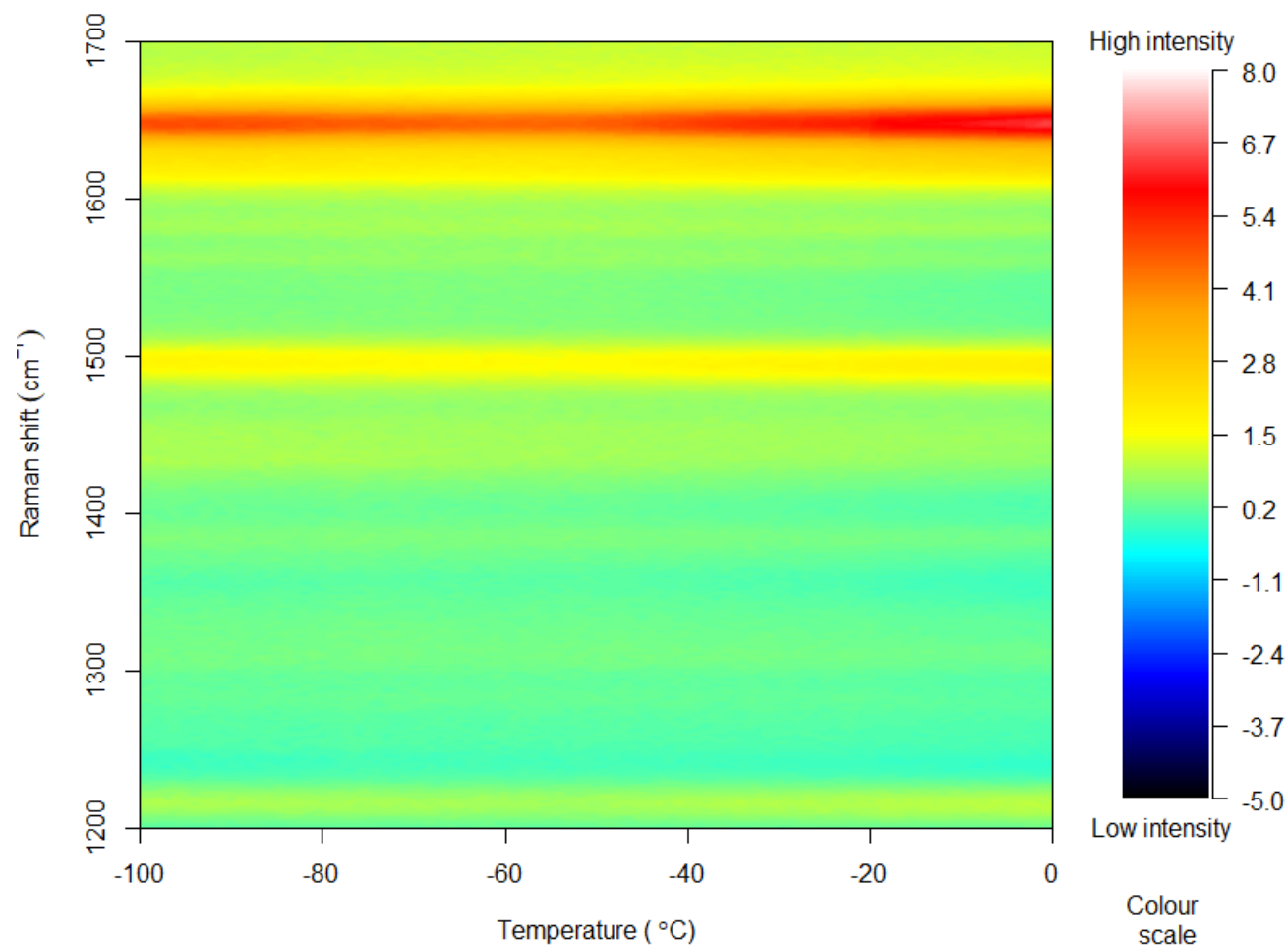


Figure D 6. Low temperature felodipine repeat experiment 2 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

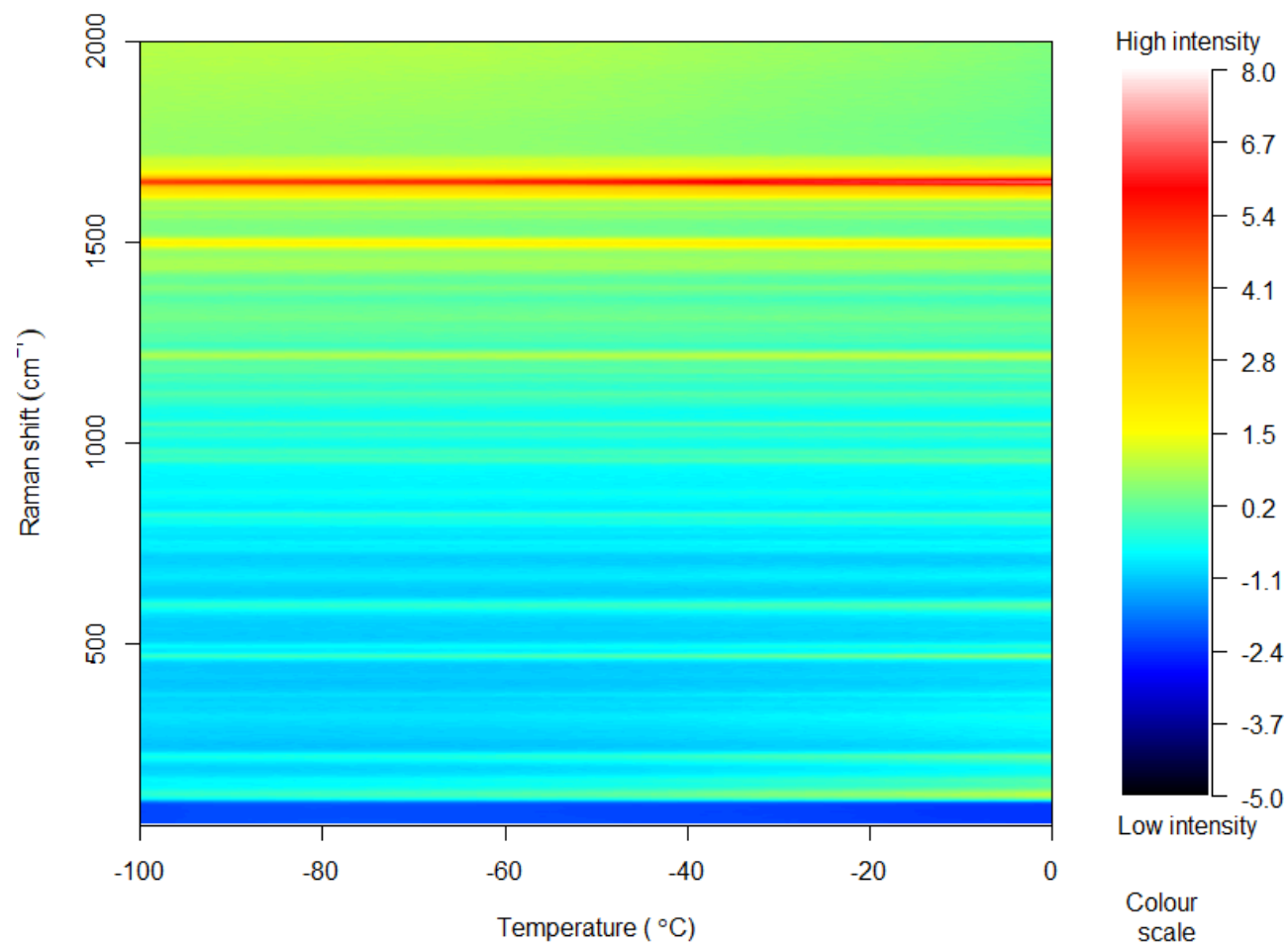


Figure D 7. Low temperature felodipine repeat experiment 2 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

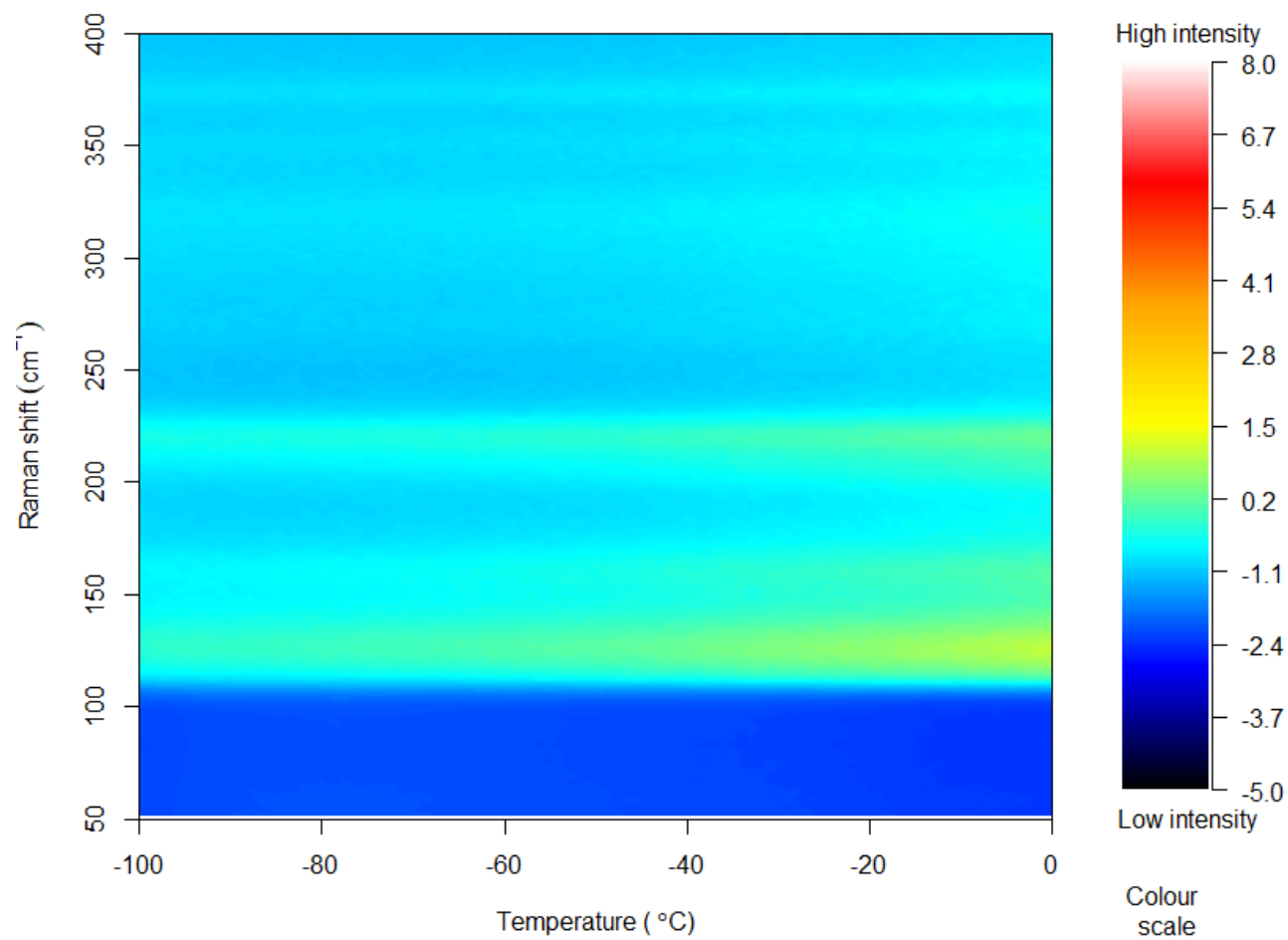


Figure D 8. Low temperature felodipine repeat experiment 2 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

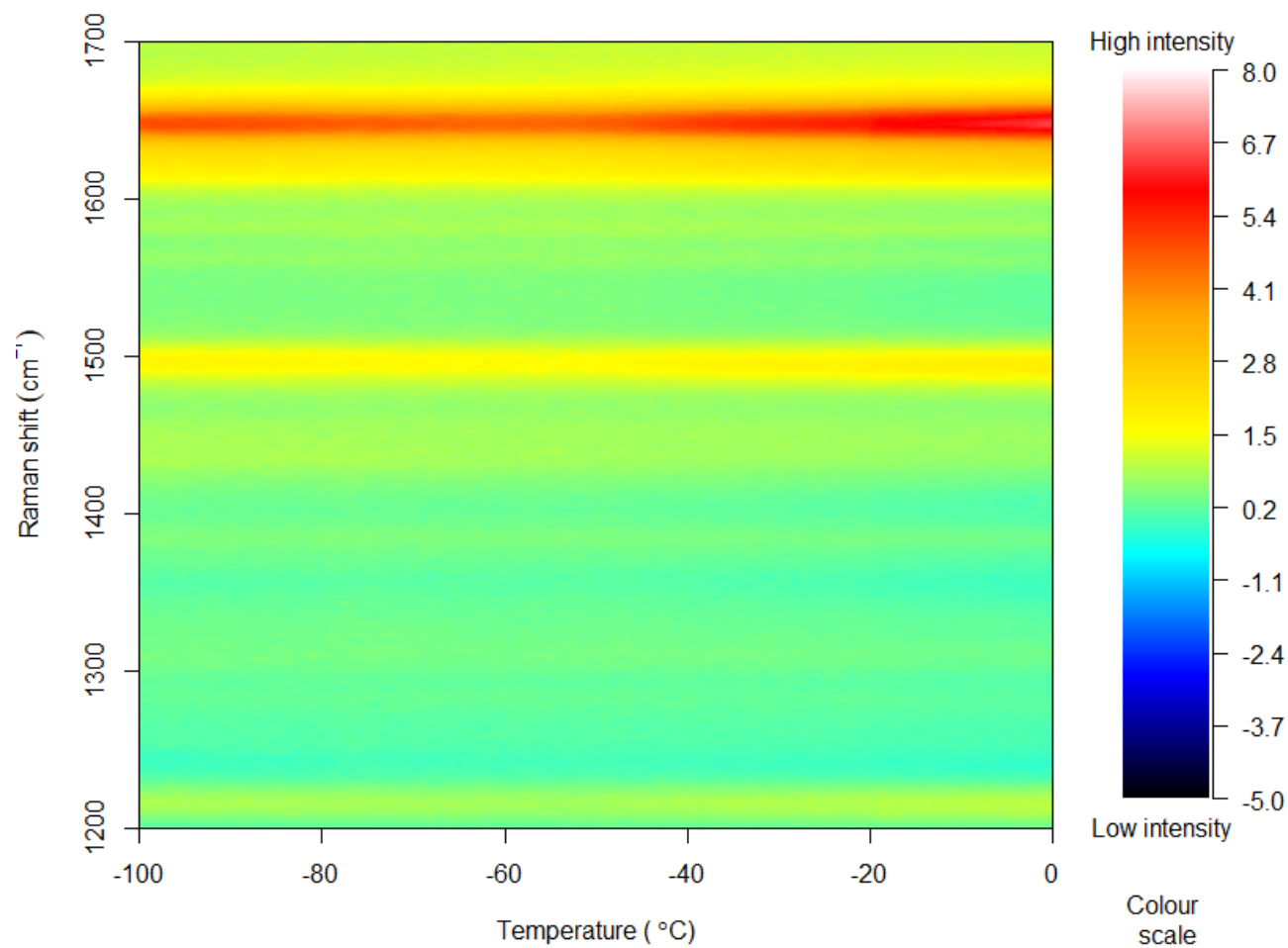


Figure D 9. Low temperature felodipine repeat experiment 2 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

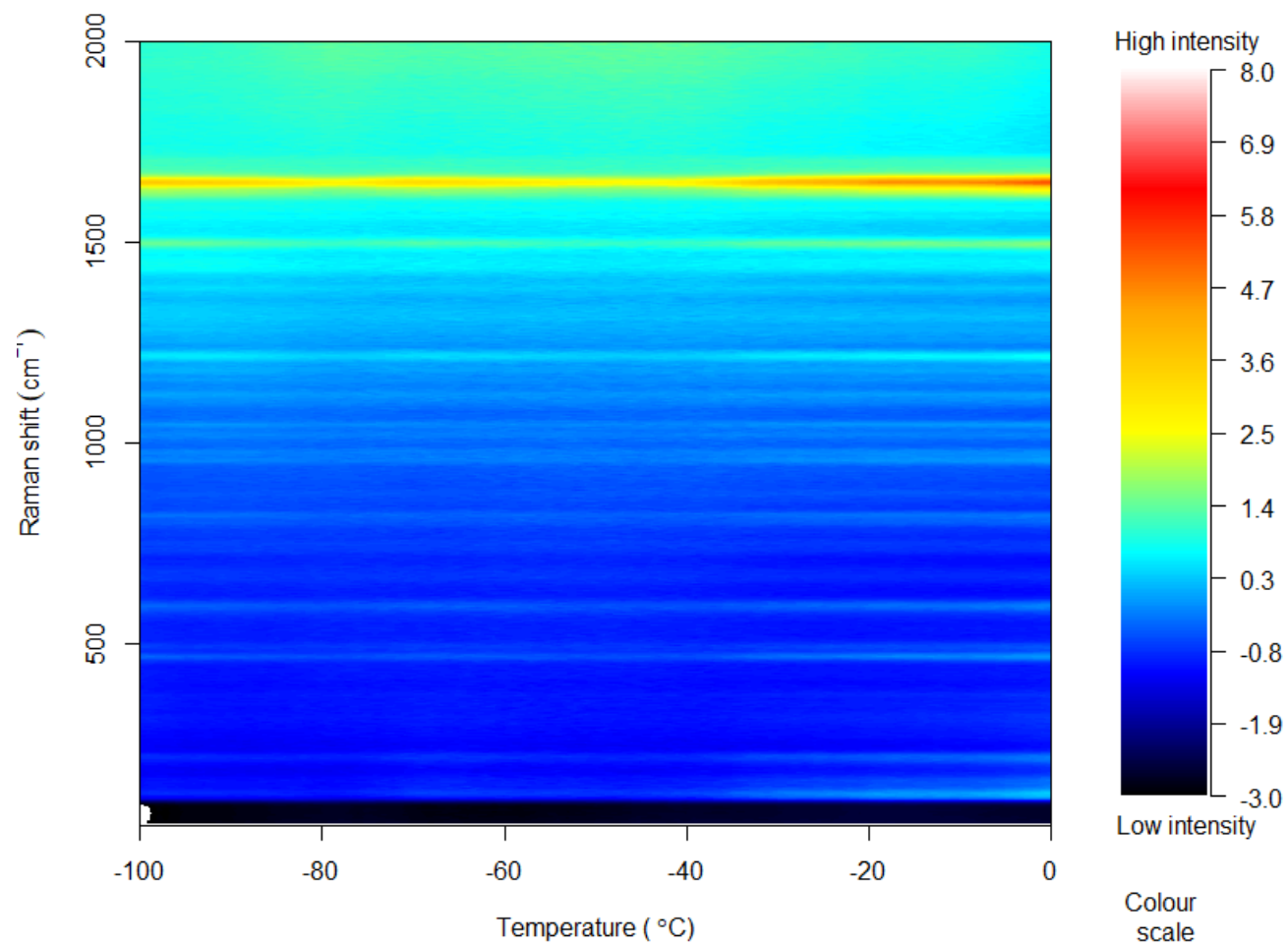


Figure D 10. Low temperature felodipine repeat experiment 3 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm^{-1} heated from -100 to 0 $^{\circ}\text{C}$. As noted in the text, the sample is heated to 150 $^{\circ}\text{C}$ and cooled to 30 $^{\circ}\text{C}/\text{min}$. It is thought to be amorphous at low temperatures.

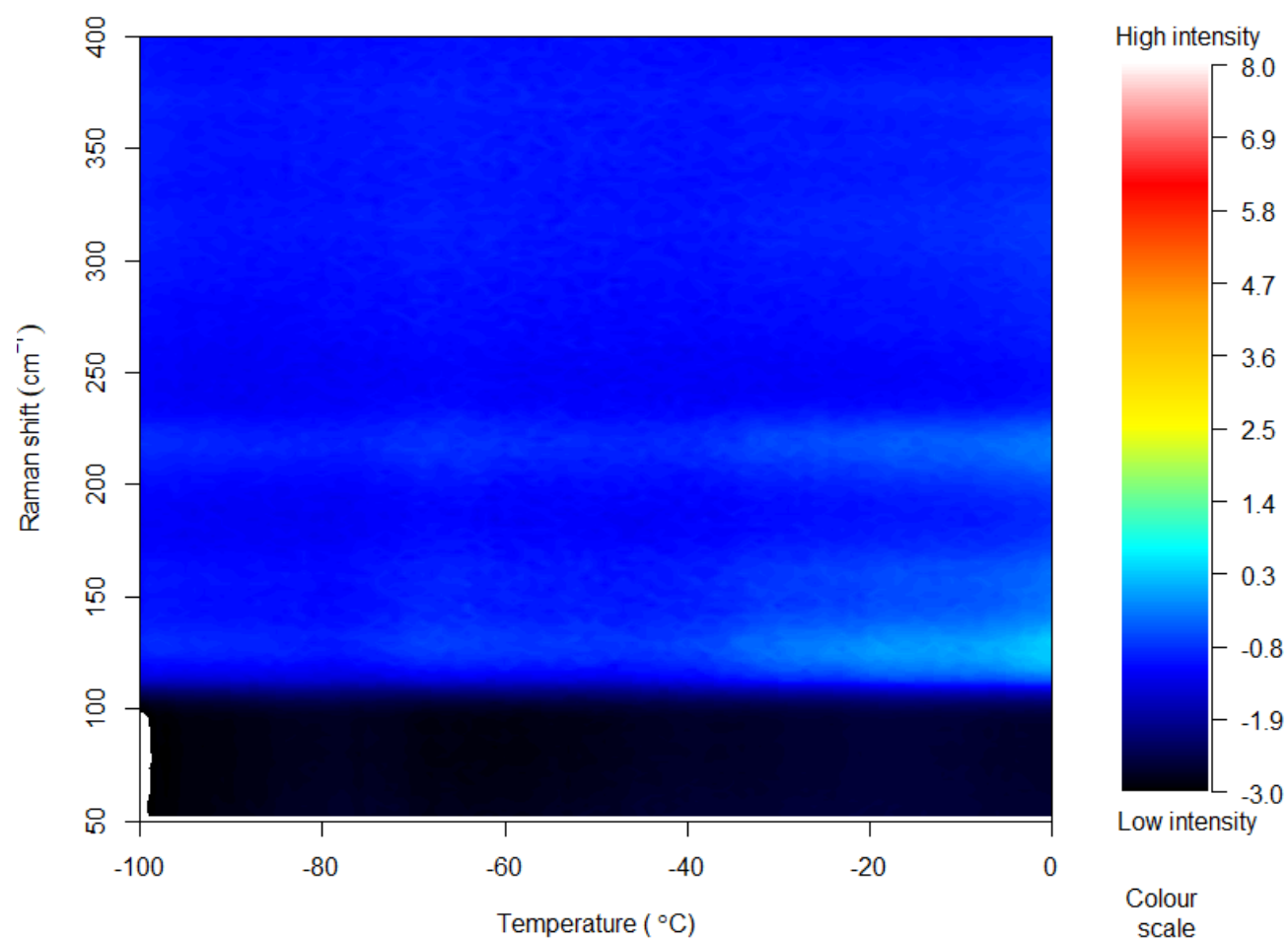


Figure D 11. Low temperature felodipine repeat experiment 3 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

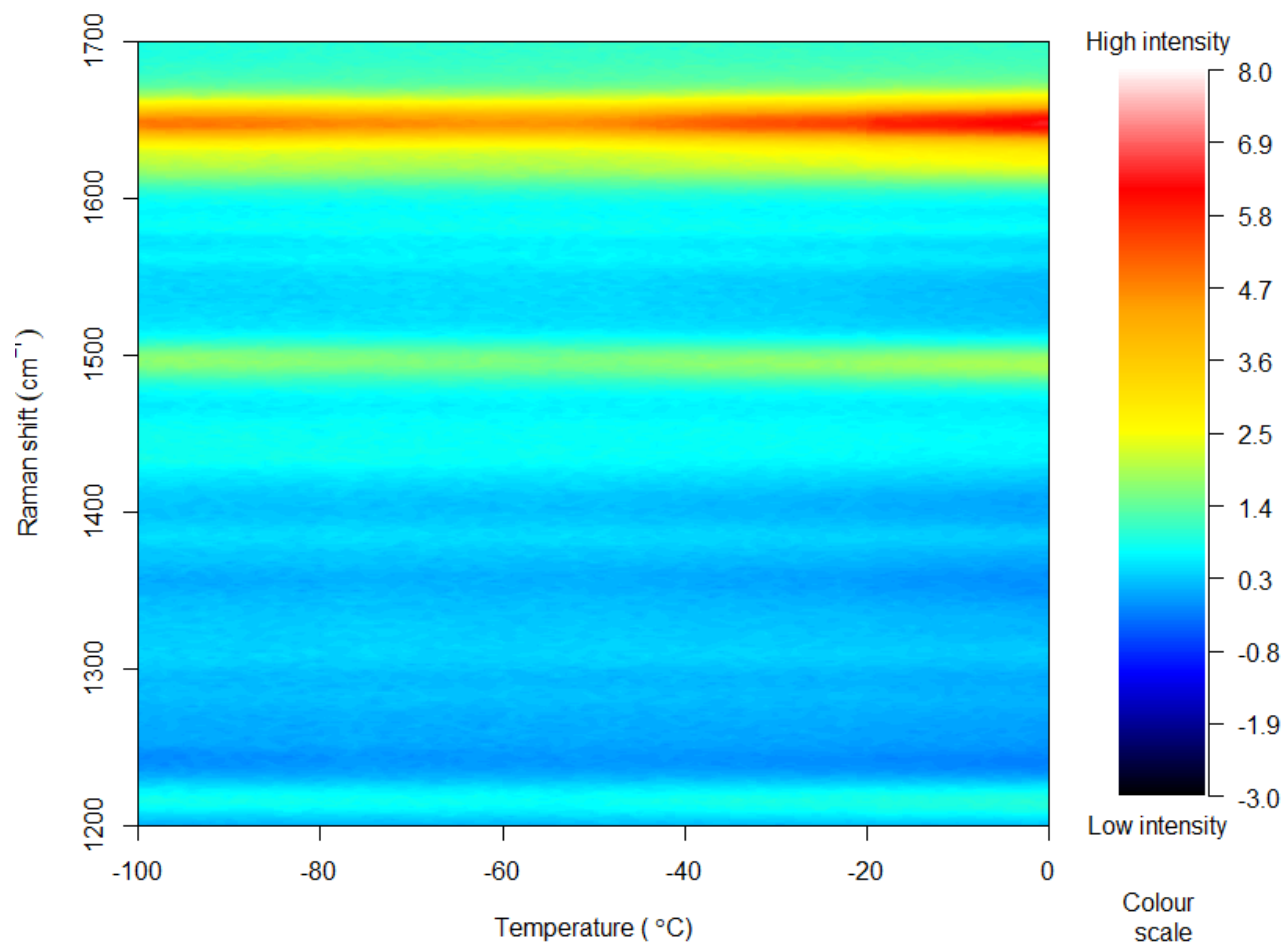


Figure D 12. Low temperature felodipine repeat experiment 3 (Cycle 1) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

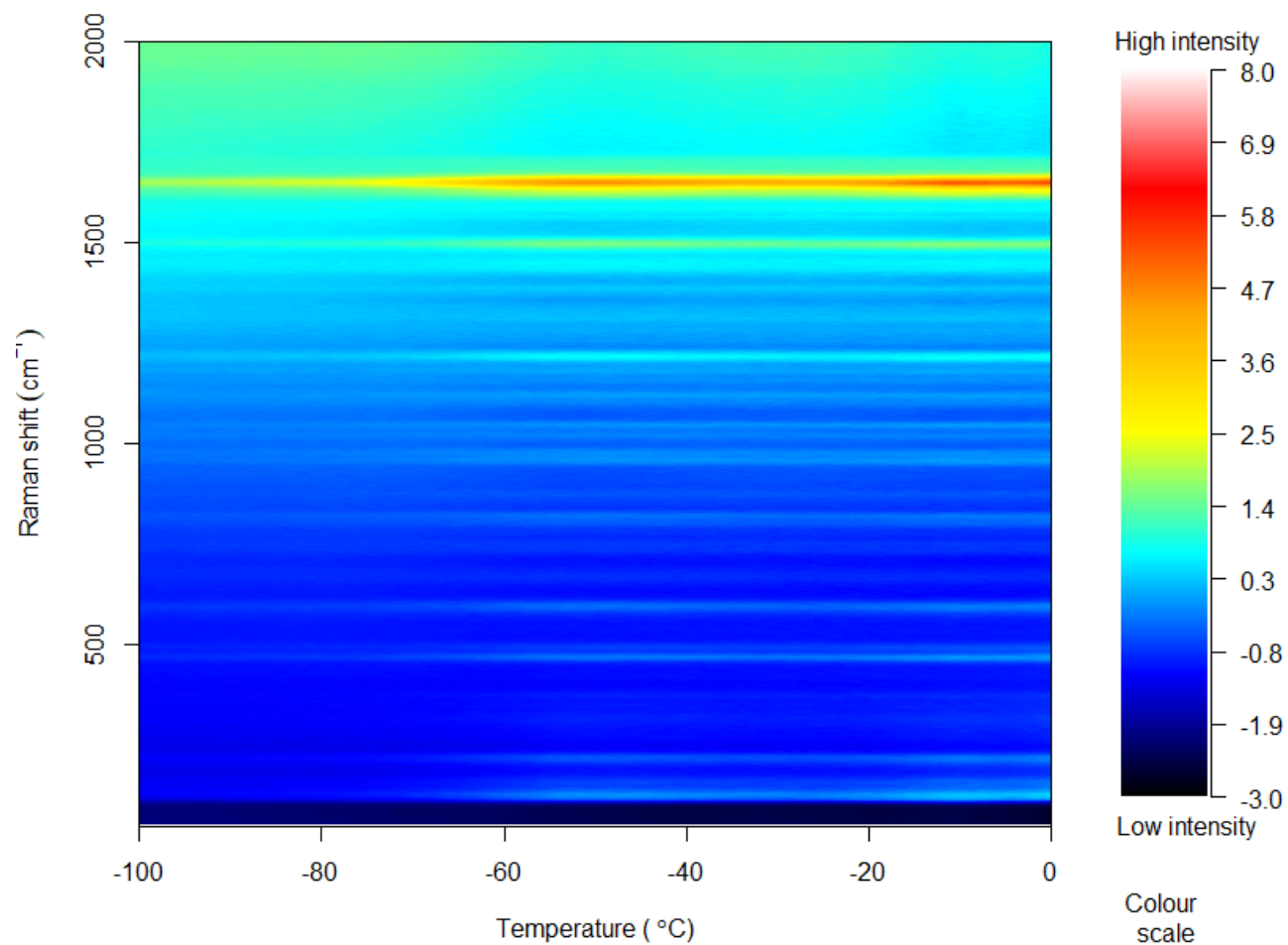


Figure D 13. Low temperature felodipine repeat experiment 3 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

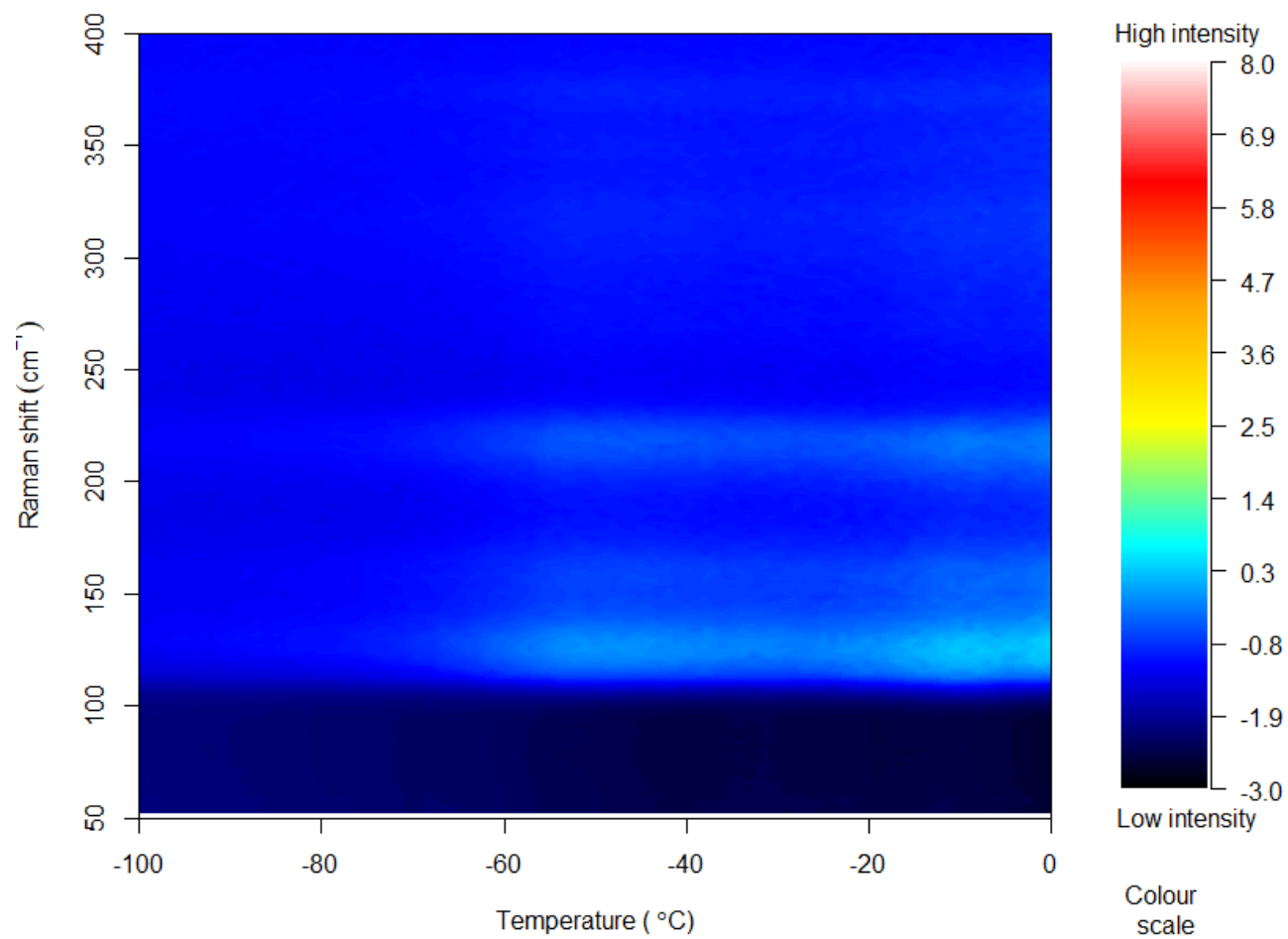


Figure D 14. Low temperature felodipine repeat experiment 3 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

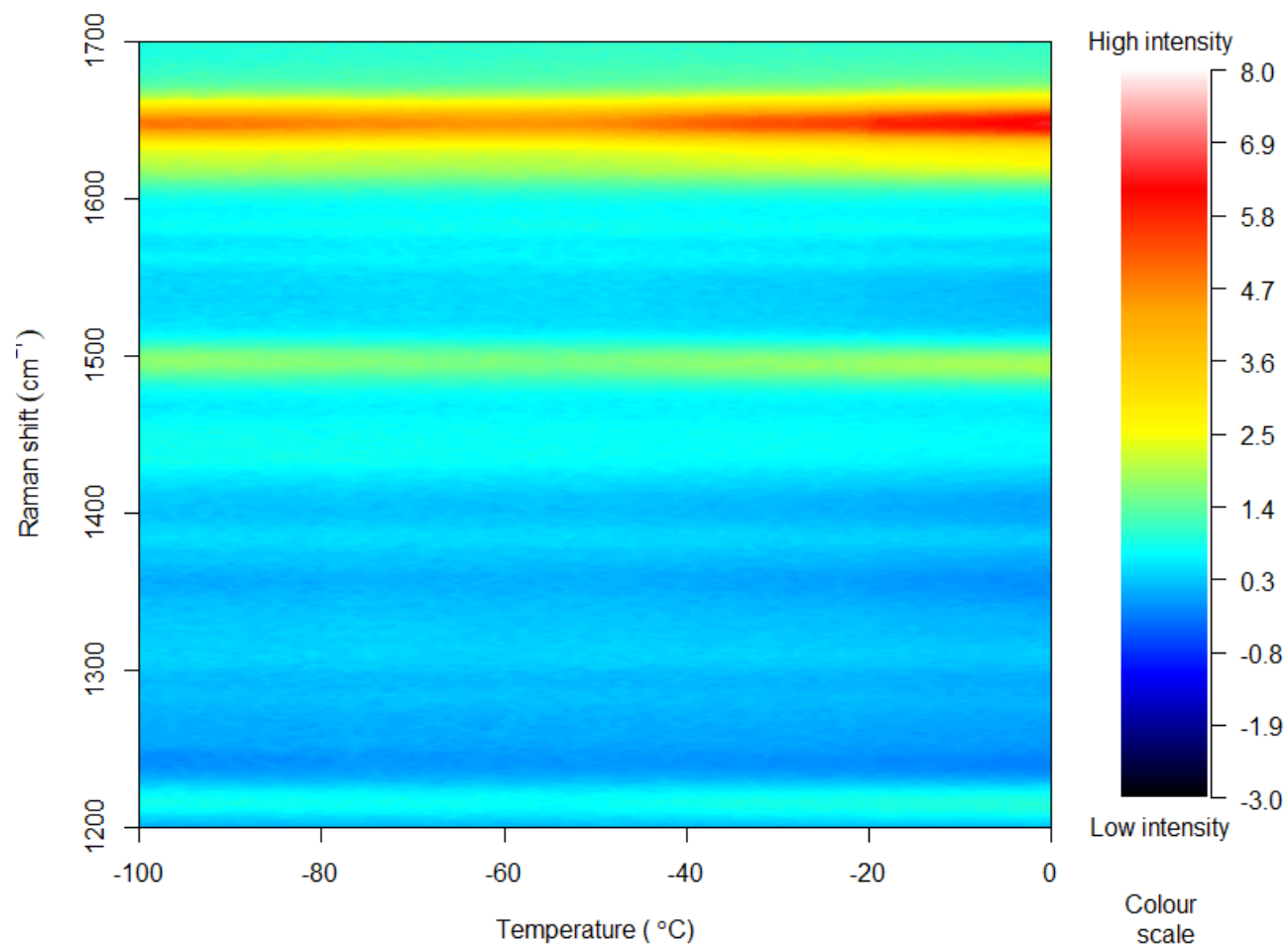


Figure D 15. Low temperature felodipine repeat experiment 3 (Cycle 2) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ cooled from 0 to -100°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

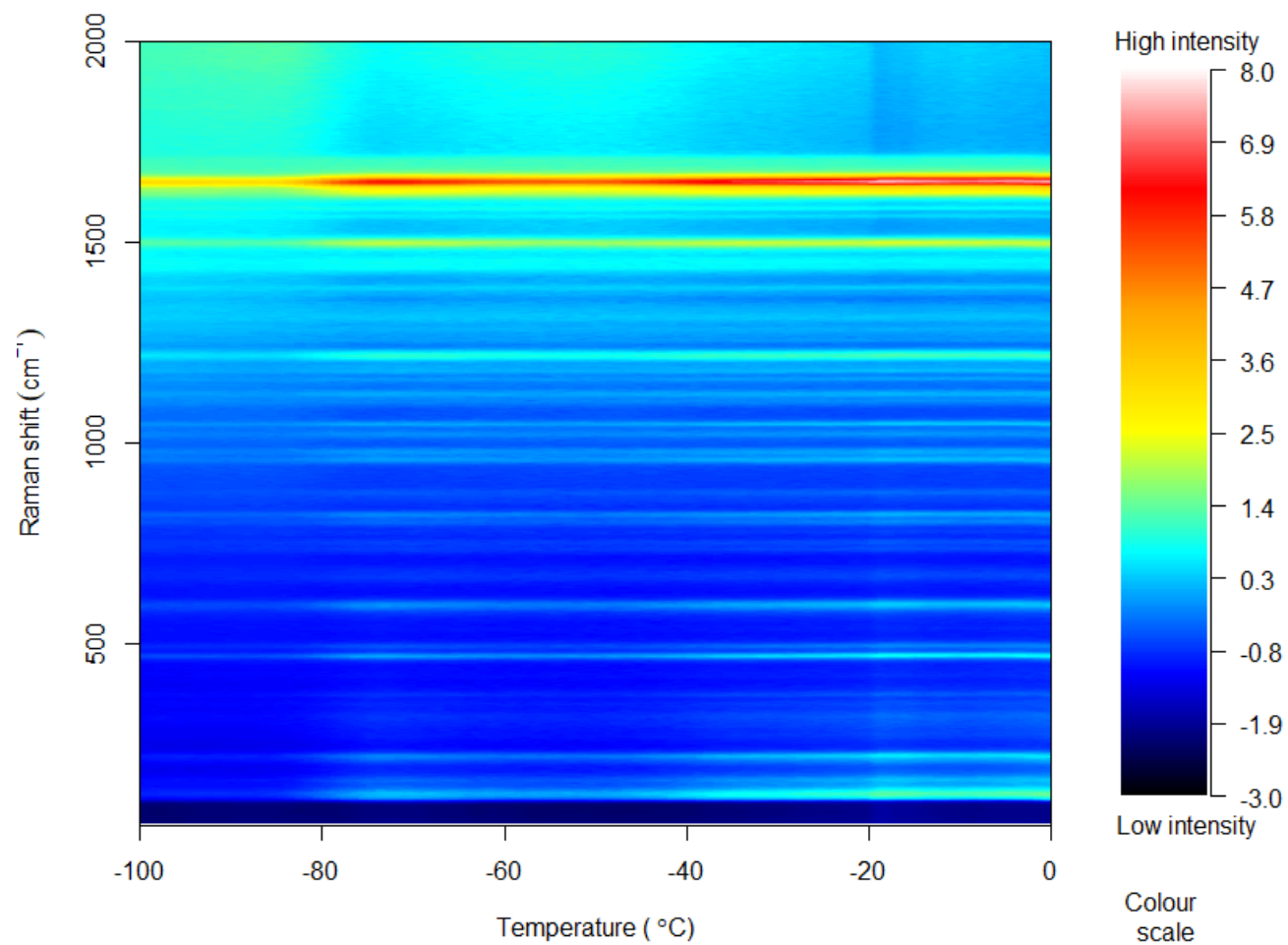


Figure D 16. Low temperature felodipine repeat experiment 3 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 2000 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

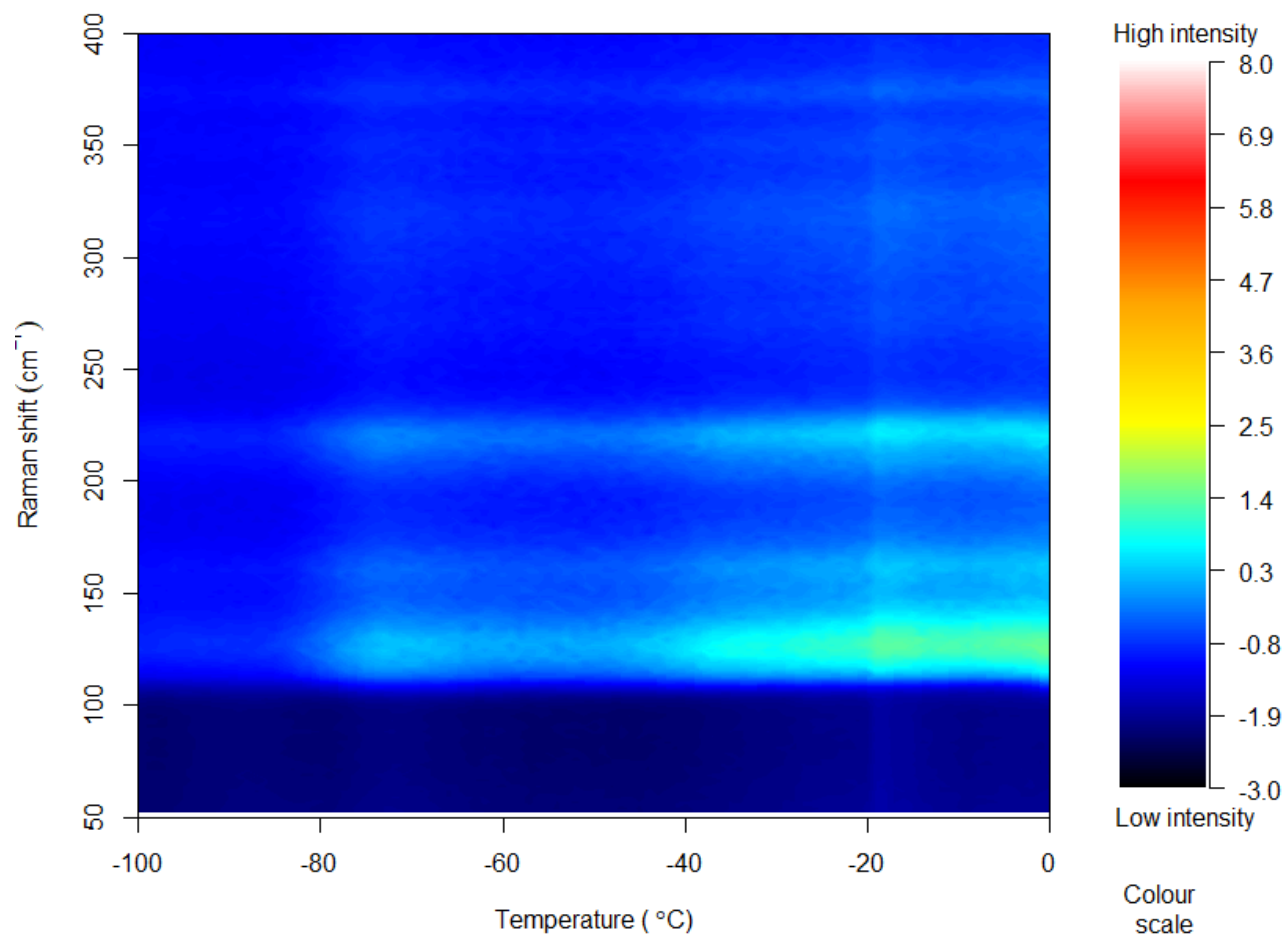


Figure D 17. Low temperature felodipine repeat experiment 3 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 0 to 400 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

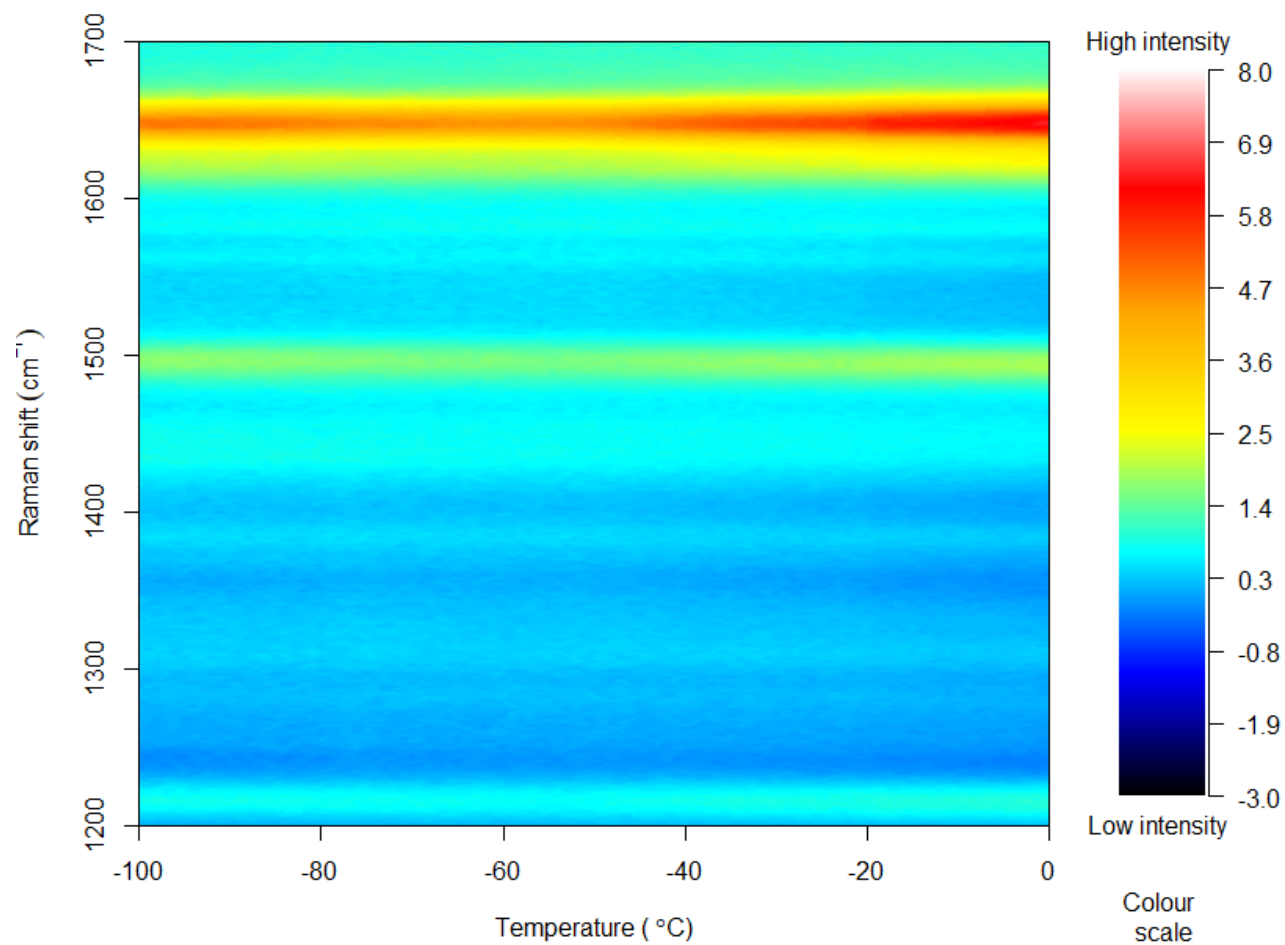


Figure D 18. Low temperature felodipine repeat experiment 3 (Cycle 3) - Heat map of variable temperature set up Raman spectra in the wavenumber range 1200 to 1700 cm⁻¹ heated from -100 to 0°C. As noted in the text, the sample is heated to 150°C and cooled to 30°C/min. It is thought to be amorphous at low temperatures.

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