First Author: Mustapha El Hadri

Order of Authors: Mustapha El Hadri; Abdelfettah Achahbar; Jamal khamkhami; Brahim Khelifa; C. Tran Le Tuyet; Vincent Faivre; Ouissam Abbas; Mimoun El Marssi; Fatiha Bougrioua; Serge Bresson

Dr. Serge BRESSON   28/10/14
Laboratoire de Physique des Systèmes Complexes
Université Picardie Jules Verne
17 rue Bataille
78500 Sartrouville
France

Email : sergebresson@yahoo.fr

Dear editor,

I'm pleased to submit you in your journal our revised article with a new title: "**Vibrational behaviour of Gelucire 50/13 by Raman and IR spectroscopies: a focus on the 1800 - 1000 cm^{-1} spectral range according to temperature and degree of hydration.**" I send you an electronic version.

We brought the following corrections:

```
It is clear from the Raman data that there are two transitions; one close
to a temperature of 35°C and another close to 44°C. But the figure 5
doesn't seem to reveal this aspect. During band fitting analysis, band
position and bandwidth of these four bands needs to be kept constant in
order to get meaningful transition points. This aspect is not considered in
the analysis (see T=46°C and T=38°C). In the case of alkane and PEG there
should be two Raman bands (for each molecule) assignable to crystalline and
molten states of these molecules. Hence there should be at-least four Raman
bands in the region between 1320 to 1260 cm-1. If one keeps the bandwidth
and position of these bands the same throughout the fitting, I would
anticipate a behavior as depicted below if the fittings were correct (clear
from the Raman data). (See the file uploaded through EES)
```

The reviewer is right. We thus changed the figure 4a) and the figure 5. On the figure 5, we presented the ratio of Area under the peak Area$_{1282}$/Area$_{1298}$ during the fusion from T = 26 °C to T = 50 °C (after 50°C, the peak at 1298 cm^{-1} disappears) as to suggest it to us the reviewer. We highlighted in yellow the changes in the text

```
This appears to match with the SAXS data. Also, it is a usual practice to
avoid first heating runs in thermal analysis. Hence the broad band that
appeared in the first heating run in SAXS measurements may not be
considered.  Importantly  it  does  not  appear  in  the  cooling  cycle.
```

The SAXS peak position at 0.15 (?) suggests an alkane spacing of 42 Å. Considering the high palmitate (C16 chain) composition in palm oil, a single carbon chain spacing would be less than 20 Å. Meaning, the triglycerides pack in an extended chain form giving a spacing that is double of single chain length. This agrees with the literature. Journal of the American Oil Chemists' Society May 1971, Volume 48, Issue 5, pp 245-247. The assignment of alkane melting point and PEG melting point are based on the comparison between SAXS and Raman data. A possible crystal packing of alkane chain could be as follows

We are agree with the reviewer

Yours sincerely,

Serge Bresson

1 **Vibrational behaviour of Gelucire 50/13 by Raman and IR spectroscopies: a**

2 **focus on the 1800 - 1000 cm^{-1} spectral range according to temperature and**

3 **degree of hydration.**

4

5 M. El Hadri[a], A. Achahbar[a], J. El Khamkhami[a], B. Khelifa[b,f], C. Tran Le Tuyet[c] ,

6 V. Faivre[c], O. Abbas[d], M. El Marssi[e], F. Bougrioua[f] and S. Bresson [f*]

7

8 [a]*Laboratoire de Physique de la Matière Condensée, Université de Tétouan, Maroc*

9 [b] *Faculté des sciences, Université d'Artois, Lens, France*

10 [c]*Equipe Physico-chimie des Systèmes Polyphasés, UMR CNRS 8612, Labex LERMIT*

11 *Université Paris Sud, France*

12 [d]*Walloon Agricultural Research Centre (CRA-W), Valorisation of Agricultural Products,*

13 *Department, Food and Feed Quality Unit (U15), 'Henseval Building', Chaussée de Namur 24,*

14 *5030 Gembloux, Belgium*

15 [e] *Laboratoire de Physique de la Matière Condensée, Université Picardie Jules Verne, 33 rue S[t]*

16 *Leu 80039 Amiens cedex, France*

17 [f]*Laboratoire de Physique des Systèmes Complexes, Université Picardie Jules Verne, 33 rue S[t]*

18 *Leu 80039 Amiens cedex, France*

19

20 **Abstract**

21 The present paper reports on physical and thermal properties of polyoxyethylene glycol

22 glycerides (Gelucire 50/13) used as sustained release matrix forming agent in pharmaceutical

23 applications. Gelucire 50/13 was essentially studied by Raman and IR spectroscopies according

24 to the temperature and the degree of hydration. The hydration behavior of this amphiphilic

25 excipient has been investigated with increasing water contents to study the behavior during

26 dissolution. In the spectral range 1800-1000 cm^{-1}, Raman and IR spectroscopies of Gelucire

27 50/13 were performed to characterize the contribution of its each components at room

28 temperature, with emphasis placed on the evolution of the CH_2 wagging and twisting, ν(C-C) and

[*] Corresponding author, Telephone: +33623301326,

E-mail adress: sergebresson@yahoo.fr (Serge BRESSON)).

ν(C-O) vibrational modes regions (1400–1000 cm^{-1}), along with analysis of the IR and Raman-active δ(CH$_2$) deformation region (1500-1400 cm^{-1}).

In comparison with temperature and degree of hydration, in the spectral range 1800 - 1000 cm^{-1}, the vibrational changes were directly correlated with conformational changes of the Gelucire structure. Overall, IR and Raman spectroscopy clearly demonstrated that the different functional groups studied could be characterized independently, allowing for the understanding of their role in Gelucire structure.

1 **Introduction :**

2 Gelucires are a group of amphiphilic excipients which have been widely studied as controlled

3 release matrices [1]. The incorporation of drugs into Gelucires has been reported to increase the

4 dissolution rate of poorly soluble drugs, often leading to improved drug bioavailability [2-5].

5 One compound from this group is Gelucire 50/13. This amphiphilic excipient, which has a

6 Hydrophilic-Lipophilic Balance of 13 and a melting temperature of 50 °C, consists of a mix of

7 mono-, di-, triacyl glycerol (around 20% in weight) and monoacyl polyoxyethylene glycols and

8 diacyl poly-oxyethylene glycols labeled, respectively, MPEG and DPEG. .The stability of the

9 crystalline structures and their interaction with water condition the drug release [6-8]. So, it is of

10 prime importance to characterize any possible structural evolution according temperature and

11 degree of hydration of the excipient prior to envisage pharmaceutical applications.

12 There have been a few studies of the thermal behavior of dry Gelucires [9,10], but, to our

13 knowledge, no systematic study of the hydratation behavior of Gelucire 50/13 . On the other

14 hand, numerous studies in IR and in AFM on hydrated PEG were realized [11-16]. From these

15 studies, we can lean to understand better the vibrational behavior of the hydrated gelucire. The

16 aim of this project was to investigate the vibrational behaviour of the Gelucire 50/13 according

17 the temperature and when exposed to water, at ambient temperature. Notably the state of

18 Gelucire 50/13 mixed with known amounts of water (from 0% to 40%) have been investigated.

19 The study of Gelucire by Raman and IR spectroscopies brings a lot of information about the intra

20 and intermolecular interactions and would facilitate the understanding of these with the aim of

21 improving the formulation of active products and then their efficiency in the body. Raman

22 spectroscopy is a vibrational spectroscopy technique that can provide a sensitive, relatively

23 quick, non-destructive means of probing molecular structure in solid and liquid lipids [17-20].

24 The most sensitive Raman and IR active features include the C-H and C-C stretching vibrations,

25 notably the ν(C-H) stretching region (3000-2800 cm^{-1}), ν(C-C) skeletal modes (1200-1000 cm^{-1}),

26 δ(CH$_2$) deformation region (1500-1400 cm^{-1}) and the t(CH$_2$) twisting region (1300-1250 cm^{-1}).

27 These regions provide insight into the degree of alkyl chain coupling, intramolecular motion, the

28 relative populations of *gauche* (distorted) and *trans* conformers as well as chain twisting and

29 bending. To understand better mechanisms involved in the structural modifications of the

30 gelucire according to the temperature, we can lean on the recent results obtained in Raman on a

31 2D Raman correlation study of PEG melting [21]. In the present article, we focus on the one

hand the Raman spectral characterization of the key transition phases of Gelucire and on the other hand, on hydrated Gelucire along with complementary Raman and IR analysis in the spectral range 1800-1000 cm^{-1}. Structural behaviour was confirmed using XRD.

Materials and Methods

Preparation of the Gelucire 50/13

Small pellets of Gelucire 50/13 were supplied by Gattefossé S. A. S., St Priest (France), and were analyzed without any special thermal treatment (as received). Gelucire 50/13 is synthesized by an alcoholysis/esterification reaction using as starting materials hydrogenated palm oil and PEG 1500, equivalent to approximately 34 monomer units -CH2-CH2-O-, and ended by two alcohol groups.

Preparation of the hydrated Gelucire 50/13

Chloroformic solutions of Gelucire 50/13 were prepared to homogenize the composition and were evaporated under a gentle stream of nitrogen to obtain lipid films. Residual organic solvent was eliminated under vacuum (<1mPa). The films were hydrated with adequate amount of water and mixed with vortex and ultrasonic water bath. Samples were prepared at least 24h before analysis in order to reach hydration equilibrium.

Raman spectroscopy

Raman spectroscopy was performed with a Jobin Yvon T64000 spectrometer equipped with a liquid nitrogen-cooled CCD detector for Gelucire according the temperature. The spectrometer, which provides a wavenumber resolution of < 3 cm^{-1}, was used in its backscattering configuration. A 514.5 nm Ar-ion laser source (power of $\approx$ 20 kW/cm^2) was focused on the CB surface with a spot size diameter of 1 μm. Spectra were recorded in parallel polarization where the incident and scattered light are parallel. No changes in sample temperature greater than ±0.01 °C were recorded. Scans of the 3100-800 cm^{-1} spectral region were acquired with 200 s scans.

FT-Raman and FT-IR spectroscopy

For the hydrated Gelucire, the FT-Raman and FTIR measurements were realised in the Walloon Agricultural Research Centre (CRA-W). FT-Raman and FTIR spectra were acquired on a Bruker

RAM II spectrometer coupled with Vertex 70 ATR-FTIR spectrometer. This instrument is equipped with a Nd :YAG laser (yttrium aluminium garnet crystal doped with triply ionised neodymium) with a length wave for the incident laser at 1064 nm (9398,5 cm^{-1}). The maximum of laser power is 1.5 W. The measurement accessory is pre-aligned, only the Z-axis of the scattered light is adjusted to set the sample in the appropriate position regarding the local point. The RAM II spectrometer is equipped with a liquid-nitrogen cooled Ge detector. FT-Raman spectra [4000-0] cm^{-1} and FTIR spectra [4000-600] cm^{-1} were collected with resolution of 1 cm^{-1} by co-adding 128 scans for each spectrum, and we use the sampling technique Attenuated total reflection (ATR) with a diamond and with a simple reflection in conjunction with infrared spectroscopy. The OPUS 6.0 software was used for the spectral acquisition manipulation and transformation.

X-ray diffraction (XRD)

X-ray diffraction patterns were acquired using a fine-focus Cu anode source (13 mA, 40 kV) and selecting the Cu Kα radiation with a wavelength λ of 1.54 Å. The incident beam was focused with a multilayer mirror (elliptic curvature, W/ Si, Osmic) and collimation achieved by slits placed before the sample. Wide-angle X-ray scattering (WAXS) analyses were performed using a position-sensitive linear gas detector set at 20° from the incident beam. The scattered intensity was reported as a function of the scattering vector $q = 4\pi \sin \theta/\lambda$ where θ is half the scattering angle. The repeat distances d, characteristic of the structural arrangements, were given by q (Å^{-1}) $= 2\pi/d$ (Å). Tristearin (β form) was used as standards to calibrate the detector. Samples were introduced into thin-walled glass capillaries (GLAS, Müller, Berlin, Germany) which were then placed in a specially designed temperature-controlled sample holder maintained at 20°C during the measurements. Igor 6.03 was used for data processing.

Results and discussion

Gelucire 50/13 is a mixture of mono-, di-, and triglycerides and mono-, diacyl polyethylene glycols. For glycerides mixture (approximately 20% in weight of the sample), the predominant fatty acid chains is the palmitostearic acid (C16 and C18), with approximately the same proportion of mono-, di- and triglycerides. However, the main part of the sample is composed of mono- and diacyl polyoxyethylene glycols (72% in weight) corresponding to 43% of DPEG and

29% of MPEG. The 8% remaining is free PEG 1500. At T = 20°C, Brubach *et al.* have observed three lamellar phases with different long spacing periods [22]. Therefore, one can observe a first lamellar phase with Bragg reflexion at 121.0 Å (it exists three orders 121.0, 62.5 and 41.3 Å) corresponding to a lamellar organization of MPEG, a second lamellar phase with Bragg reflexion at 90.0 Å (it exists two orders 90.0 and 45.0 Å) identifying to the PEG 1500 lamellar phase and a third lamellar structure observed at 49.5 Å assigned to the α form of triglycerides (see Fig. 1). These authors have studied the SAXS and WAXS evolution of Gelucire 50/13 during of the cycle of fusion-crystallization-fusion between 20 °C and 60 °C with a heating rate of 1°C/min. Their results are introduced on the Fig. 1. At 20 °C, as already mentioned, the Gelucire describes three lamellar phases with a long spacing of 121 Å (MPEG), 90 Å (PEG 1500) and 49.5 Å (triglycerides), the DPEG being interpenetrated in MPEG. These structures remain stable until 42°C. From 42 to 50° C, the structure of Gelucire comes down to those of MPEG and triglycerides in crystalline form. Between 50 and 54° C, only remain triglycerides. Gelucire is in liquid phase above 54°C. After cooling from 60∘Cto 20∘C with the same rate of 1∘C/min, they note that triglycerides crystallize the first ones to T = 42∘C, followed by PEG 1500, MPEG and DPEG at about 32∘C. [22]

At room temperature

The Raman and IR spectra of Gelucire 50/13 in the spectral range 1800-1000 cm⁻¹ at room temperature (T = 24 °C) were presented Fig. 2. We observe active modes at the same time in Raman and in IR (1064, 1145, 1240, 1280, 1306, 1455, 1470 and 1740 cm⁻¹), others modes only in IR (1107, 1306, 1329, 1344, 1359 and 1730 cm⁻¹) and others modes only in Raman (1130, 1298, 1440, 1480 and 1486 cm⁻¹). Their assignments of the observed modes in this spectral region are reported in table 1. All reported peak values were based on the maximum peak height of the unfitted spectra. Curve fitting and fitted frequency values labelled * were performed as per Bresson *et al.* [23]. On the Raman spectrum (see Fig. 2, we distinguish three well defined spectral regions : 1500-1400, 1350 – 1250 and 1150-100 cm⁻¹ whereas on the IR spectrum we observe four well defined spectral regions : 1800-1700 cm⁻¹, 1500-1400, 1380 – 1300 and 1150-100 cm⁻¹. Contrary to the X rays experiments on Gelucire 50/13 [22], it is not possible from the Raman spectra of the Gelucire to distinguish some vibrational modes which are exclusively connected to triglycerides, to the PEG 1500, to the MPEG or to the DPEG.

Raman behavior of Gelucire 50/13 according the temperature

Zachariah et al [21] have identified the specific Raman bands corresponding to different configurations of the PEG chain and the molecular structural dynamics of PEG melting. For us, the melting dynamics of PEG have been unambiguously explained along the C-O bond rotation coordinate. As the temperature of the system increases, the CH_2-wagging (1280 cm^{-1}) vibration becomes active. Then, the 1472 cm^{-1} CH_2 scissoring vibration, with considerable contribution from a backbone deformation $\delta(O-C-C) + \delta(CH)$, becomes active, and this provides flexibility to the chain. Subsequently, the 1124 cm^{-1} CH_2–rocking vibration, with a considerable C-O stretching contribution, becomes active and results in the bond rotation. The bond rotation results in the transformation of few TGT segments to GGG (or GGT or TGG) segments. Consequently the vibrations of segments containing gauche C-O conformations (at 1059 cm^{-1}) become active. In Gelucire 50/13, the PEG molecules coexist with the triglycerides, MPEG and DPEG molecules. So, the melting dynamics for Gelucire 50/13 are more complex.

To compare with results obtained by Brubach *et al*, we proceeded to the fusion and the crystallization of Gelucire 50/13 (with an increase in temperature and a cooling of 1°C/min). We present in Fig. 3 the Raman spectra of Gelucire 50/13 as a function of temperature in the case of fusion (Fig 3a) and cooling (Fig 3b) in the spectral ranges from 1000 to 1600 cm^{-1} with $\lambda = 514.5$ nm. From Figs. 3a) and 3b), we notice that after the gelucire re-crystallization we find the same Raman spectrum. According to the temperature, we notice changes of behavior in three spectral ranges: 1000-1200, 1200-1350, and 1400-1500 cm^{-1}. The most significant and quantifiable changes are in the two last spectral ranges. In the 1000-1200 cm^{-1}, we observe the appearance of a mode in 1124 cm^{-1} and a weakening of the peak in 1130 cm^{-1} from T=40°C when we heat Gelucire 50/13, and conversely when we cool the gelucire 50/13 from 60°C to 24°C. These results are in agreement with those of Zachariah concerning PEG [21]. Thus, It seems that we observe the C-O bond rotation of PEG within the gelucire.

In Fig. 4, we present the Raman spectra of Gelucire 50/13 adjusted by Lorentzian functions according to the temperature during the fusion (Fig 4a) and re-crystallization (Fig. 4b) between T = 26 °C and T = 60°C in the spectral range 1340–1220 cm^{-1} with $\lambda = 514.5$ nm (heating and cooling rate of 1 ° C / min). At T = 28°C, we note 5 vibrational modes at 1233, 1240, 1282,

1298, and 1306 cm^{-1}. We observe important changes for these 5 modes. We notice that two vibrational modes disappear during the heating of Gelucire: the mode at 1233 cm^{-1} towards 46°C and the mode at 1298 cm^{-1} towards 50 °C. At the same time, there is a 4 cm^{-1} shift towards lower frequencies upon the crystalline-liquid phase transition (T = 52°C) for the band at 1306 cm^{-1}. The other modes vary very slightly according to the temperature. Thus, we observe that below 48°C five vibrational modes exist in the spectral zone 1220-1330 cm^{-1}: 1233, 1240, 1282, 1298 and 1306 cm^{-1}. After 50°C, there are only 3 components corresponding to the modes at 1240, 1282, and 1302 cm^{-1}, all components of Gelucire being passed in liquid phase. Conversely, during the re-crystallization, the peak at 1298 cm^{-1} appears at the time of the crystallization of triglycerides in the structure of the Gelucire from T = 44 °C. From this temperature, the spectral range 1220-1320 cm^{-1} passes of 3 components in 4 components. Also, it is in T = 34 °C that PEG crystallizes and that appears the peak at 1233 cm^{-1}. We find then 5 components already observed at room temperature. From Figs. 4a and 4b, two transitions are clearly evident: melting of PEG chains and melting of alkyl chains.

In Fig. 5, we present on the one hand the ratio of Area under the peak $Area_{1282}/Area_{1298}$ during the fusion from T = 26 °C to T = 50 °C (after 50°C, the peak at 1298 cm^{-1} disappears) and on the other hand the intensity ratio I_{1472}/I_{1488} as a function of temperature for Gelucire 50/13 during the fusion from T = 26 °C to T = 60 °C. For the ratio of Area under the peak $Area_{1282}/Area_{12982}$, between 26°C and 34°C, the area ratio remains practically stable. Then, between 34°C and 42°C, we observe a light decrease of the area ratio due to a reduction in the influence of the peak at 1282 cm^{-1} corresponding to the fusion of PEG chains. After T=42°C, we observe a fast rise of this area ratio from 2.5 to 9.5 corresponding to a fast reduction of the area in intensity under the peak at 1298 cm^{-1} due to the fusion of alkyl chains. The evolution of this area ratio relates well the two transitions : the melting PEG chains at 35°C approximately and the melting alkyl chains at 44°C. For the intensity ratio I_{1472}/I_{1488}, between T = 26 °C and T = 36 °C, this ratio is characterized by a relatively stable intensity ratio of 0.8. Between T = 36 °C and T = 60 °C the ratio rises significantly and continuously to 1.7 marking the Gelucire melting point. This result is agree with the observations made within Raman PEG study [21]: the 1472 cm^{-1} CH_2 scissoring vibration, with considerable contribution from a backbone deformation δ(O-C-C) + δ(CH), becomes active when the temperature increases.

Vibrational behavior of hydrated Gelucire 50/13

Because of the polyoxyethylene part, such kind of excipients is very sensitive to hydration. The aim of this part is to investigate the behavior of the Gelucire 50/13 when exposed to water. Fig. 6 describes the effect of hydration on the x-ray diffractograms at wide-angles. In the dry state, the strong diffraction peaks at 4.61 and 3.80 Å show that the polyoxyethylene chains crystallize into a helical conformation as already proposed in the literature [22]. Interestingly, it is clear from the WAXS results that this helical organization totally disappears after hydration, even if the water content is low (20% w/w).

Fig. 7 shows the Raman spectra of Gelucire 50/13 (Fig. 7a) with $\lambda = 1064.0$ nm in the spectral range 1800-1000 cm^{-1}. And the IR spectra of Gelucire 50/13 (Fig. 7b) in the spectral range 1800-1400 cm^{-1} according to degree of hydration at room temperature.

Raman study

Changes were comparable with the study of Gelucire according to the temperature. Nevertheless, after 10% and 20% of hydration, some particular changes occur: the mode at 1400 cm^{-1} disappears after 10% of hydration whereas the modes at 1253 assigned to $\tau(CH_2)$ appears after 20% of hydration. These phenomena seem to indicate changes connected to applied new geometrical constraints of Gelucire with the presence of water molecules. As in the case of the study of Gelucire 50/13 according to the temperature, both spectral ranges 1500-1400 cm^{-1} and 1310-1220 cm^{-1} are sensitive to the changes of near environment: here in the increase of water molecules in the mixture Gelucire/water. In order to evaluate these changes, the intensity ratios I_{1298}/I_{1282} and I_{1440}/I_{1486} as a function of temperature for Gelucire 50/13 were described in Fig.8. Both intensity ratios quickly rise between 0% and 20% of hydration: for I_{1298}/I_{1282} the intensity ratio passes from 1.1 to 1.8 and for I_{1440}/I_{1486} from 0.7 to 1.6. Then, they have one different behavior after 20%, for I_{1440}/I_{1486} the rise remains slower but important. In the spectral range 1200-100 cm^{-1}, changes seem to be not significant.

IR study

In Fig. 7b), we note the appearance of a peak at 1645 cm^{-1} assigned to the water band. The intensity of this mode increases with the degree of hydration of Gelucire. The C=O stretching modes and CH$_2$ bending modes in the spectral range 1500-1400 cm^{-1} seem not to change significantly according the degree of hydration.

In contrary to the spectral range 1800-1400 cm^{-1}, the two spectral ranges 1400-1300 and 1200-1000 cm^{-1} are more sensitive to the degree of hydration as can be seen on Figs. 9a) and 9b). In Fig. 8, we present the IR spectra of Gelucire 50/13 adjusted by Lorentzian functions according to the degree of hydration in two spectral regions 1400-1300 and 1200-1000 cm^{-1}. We observe important changes on these two figures from 20% of hydration. In the spectral region 1200-1000 cm^{-1}, we notice that one vibrational mode at 1031 cm^{-1} appears from 20% of hydration. At the same time, three vibrational modes vary significantly: the mode at 1060 cm^{-1} shifts to 1075 cm^{-1} from 20% of hydration (+15 cm^{-1}), the mode at 1107 cm^{-1} to 1112 cm^{-1} (+5 cm^{-1}) and the mode at 1147 cm^{-1} to 1140 cm^{-1} (-5 cm^{-1}). The mode at 1095 cm^{-1} seems to vary very slightly. These results are introduced in the Fig. 10 representing the Raman frequency shift according to the degree of hydration in the spectral ranges 1400-1300 cm^{-1} and 1200-1000 cm^{-1}. Concerning the intensity of these modes, we remark that the intensity of the modes at 1095, 1145 and 1031 cm^{-1} seem to be constant more or less whereas the intensity of the mode at 1112 cm^{-1} decreases and the mode at 1075 cm^{-1} rises in intensity according to the degree of hydration.

Rozenberg e*t al* show that in IR spectroscopy the most noticeable changes for hydrated PEG-200 are observed for the wavenumber position and width of the components near 1068, 1104 and 1127 cm^{-1} assigned above to ν(C-OH), ν(C-O)$_{trans}$ and ν(C-O)$_{gauche}$ respectively at the water/PEG ratios 0.5:1.5 [11]. As for the intensities, while the total integrated band intensity is essentially constant, one notes a progressive increase of the 1104 cm^{-1} component (*trans* conformation) and a corresponding decrease of the 1126 cm^{-1} component (*gauche* conformation) with higher water concentration evident in the parallel growth of the water band near 1650 cm^{-1}. The changing ratio between the two conformations is the result of the whole H-bond structure changing when water molecules integrate into it. Water molecules have the effect of increasing the length of the H-bonded chain, letting the system to preferably relax to the energetically favored *trans* conformations. In contrast, the smallest changes are of the of the components near 1031 and 1142 cm^{-1} assigned to stretching (C-C) vibrations and deformational vibrations of (-CH$_2$-) groups. The most significant conclusion is that the changes of the parameters of the 1068 cm^{-1}

component, assigned to ν(C-OH), strongly indicate the participation of the OH-group in this process of water molecules joining the H-bond system of the PEGs [11]. Furthermore, Begum and Mastura [15] showed that the gauche state of the C-C bond, which is typical for the crystalline helical state, is retained when PEG is dissolved in water. Applied tension could drive a transition from a helix to an all-trans configuration, energetically less favorable in the absence of strain.

So, from these results, it seems that we observe the same phenomenon in the case of the hydrated Gelucire 50/13 but with frequency shifts for the modes assigned to ν(C-OH), ν(C-O)$_{trans}$ and ν(C-O)$_{gauche}$. For the Gelucire 50/13, the peak at 1075 cm^{-1} can be assigned to ν(C-OH) (+7 cm^{-1} in comparison with Rozenberg's results), the peak at 1095 cm^{-1} assigned to ν(C-O)$_{trans}$ (- 9 cm^{-1}) and the peak at 1112 cm^{-1} assigned to ν(C-O)$_{gauche}$ (- 14 cm^{-1}). The increase in intensity of the ν(C-OH) vibrations and the decrease of the contribution of ν(C-O)$_{gauche}$ vibrations for the Gelucire 50/13 according the degree of hydration seem to indicate that water molecules tend to join the existing hydrogen bonded structure of PEGs at the hydroxyl end groups.

If we focused on the spectral region 1400-1300 cm^{-1}, we can observe from the Fig. 9 similar changes from 20% degree of hydration. In the spectral region 1400-1300 cm^{-1}, we notice that one vibrational mode at 1349 cm^{-1} appears from 10% of hydration. At the same time, two vibrational modes vary slightly: the mode at 1342 cm^{-1} shifts to 1345 cm^{-1} from 20% of hydration (+3 cm^{-1}), the mode at 1360 cm^{-1} to 1356 cm^{-1} (-4 cm^{-1}). The mode at 1330 cm^{-1} seems to vary very slightly. These results are introduced in the Fig. 10 representing the Raman frequency shift according to the degree of hydration in the spectral ranges 1400-1300 cm^{-1} and 1200-1000 cm^{-1}. Concerning the intensity of these modes, we remark that the intensity of the modes at 1330 and 1356 cm^{-1} seem to be constant more or less whereas the intensity of the mode at 1344 cm^{-1} decreases and the mode at 1349 cm^{-1} rises in intensity according to the degree of hydration.

Begum and Mastura [15] showed that the relative population of the *gauche* conformation around the C-C bond increases significantly with increasing water fraction in PEGs whereas the *trans* conformation around C-O bond increases. Furthermore, Venkataraman and Vasudevan [13] indicate that the CH2 wagging modes in the IR spectrum between 1170 and 1380 cm-1 progress when temperature increase. It seems to indicate that *trans* conformers are dominant in the methylene chain. In comparison with our results, it will seem that in this spectral region, the

mode at 1344 cm^{-1} can be dominated by the ν(C-O)$_{gauche}$ vibrations and the peak at 1349 cm^{-1} to the CH$_2$ wagging vibrations.

Conclusion

In this work, we obtained the vibrational behavior of Gelucire in the spectral range 1800-1000 cm^{-1} at room temperature and according to the temperature and the degree of hydration. In each case, we have assigned all vibrational modes. From the Brubach's results obtained by X-ray diffraction and DSC as a function of temperature, we looked for markers of changes in crystal structures and in liquid phase among the vibrational modes of Gelucire.

For Gelucire, a complete study temperature (fusion and re-crystallization of the sample) enabled us to confirm the sensitivity of Raman vibrational modes compared to the previous conformational structure of Gelucire. By this study, we show that when the temperature of the system increases, the CH$_2$-wagging (1280 cm^{-1}), the 1472 cm^{-1} CH$_2$ scissoring vibration vibration and the 1124 cm^{-1} CH$_2$–rocking vibration become active for Gelucire 50/13. These results are similar to those obtained by Zachariah in the case of 2D Raman correlation study of PEG melting.

The study according to the degree of hydration confirms that the vibrational modes in the spectral range 1800-1000 cm^{-1} are very sensitive to environmental changes especially in IR spectroscopy: we observe the appearance of new peaks at 1253 cm^{-1} in Raman spectroscopy, 1031 and 1349 cm-1 in IR vibrations after 20% of hydration, and some variations of intensity ratios indicating an increase of the *trans* conformation around C-O bond and of the CH2 wagging vibrations. The distinct liquid water band near 1645 cm^{-1} (Fig. 7b) in IR spectroscopy may be an independent measure of the water content in such studies.

This suggested that techniques such as Raman spectroscopy should be used in concert with established techniques such as XRD to properly characterize and better understand the Gelucire 50/13 structure.

References

[1] A.M. Mouricout, D. Gerbaud, C. Brossard, D. Lefort des Ylouses, *S.T.P. Pharma*, 6, (1990) 368-375

[2] F. Damian, N. Blaton, L. Naesens, J. Balzarini, R. Kinget, P. Augustijns, G. Van den Mooter, Eur. J. Pharm. Sci., 10, (2000) 311-322

[3] J.M. Gines, M.D. Veiga, M.J. Arias, A.M. Rabasco, *Int. J. Pharm.,* 126, (1995) 287-291

[4] M.K. Gupta, D. Goldman, R.H. Bogner, Y.C. Tseng, *Pharm. Dev. Technol.,* 6, (2001) 563-572

[5] B. Perissutti, F. Rubessa, F. Princivalle, *S.T.P. Pharma Sci.*, 10, (2000) 479-484.

[6] F. Damian, N. Blaton, R. Kinget, G. Van den Mooter, *Int. J. Pharm.,* 244, (2002) 87-98

[7] N. Jeanmaire-Wolf, S. Segot-Chicq, G. Cohen, D. Wouessidjewe, *S.T.P. Pharma*, 6, (1990) 103-109.

[8] W. Sutananta, D.Q.M Craig, J.M. Newton, *Int. J. Pharm.,* 110, (1994) 75-91

[9] W. Sutananta, D.Q.M. Craig, J.M. Newton, *Int. J. Pharm.,* 111, (1994) 51-62

[10] D.Q.M. Craig, J.M. Newton, *Int. J. Pharm.*, 74, (1991) 33-41

[11] M. Rozenberg, A. Loewenschuss, Y. Marcus, *Spectrochimica Acta Part A* 54 (1998) 1819-1826

[12] N.V. Venkataraman, S. Vasudevan, *J. Phys. Chem. B* 105 (2001) 1805-1812

[13] V.V. Naik, S. Vasudevan *J. Phys. Chem. C* 115 (2011) 8221-8232

[14] M.W.A. Skoda, R.M.J. Jacobs, J. Willis, F. Shreiber, *Langmuir*, 23 (2007) 970-974

[15] R. Begum and H. Matsuura, *J. Chem. Soc., Faraday Trans*., 93(21) (1997) 3839-3848

[16] F. Oesterhelt,M. Rief, H.E. Gaub, *New J. Phys.* 1, (1999), 6.1-6.11

[17] Y.R Hu, J.K. Liang, A.S. Myerson, L.S. Taylor, *Industrial & Engineering Chemistry Research* 44 (2005) 1233-1240

[18] G. Schoukens, K. De Clerck, *Polymer* 46 (2005) 845-857

[19] S. Bresson, M. El Marssi, B. Khelifa, *Vib. Spec.* 40 (2006) 263-269

[20] E. Da Silva, S. Bresson, D. Rousseau, *Chem. Phys. Lipids,* 157 (2009) 113-116

[21] A. Z. Samuel and S. Umapathy, *Polymer Journal* 46 (2014) 330-336

[22] J.B. Brubach, M. Ollivon, V. Jannin Malher B., C. Bourgaux, P. Lesieur and P. J. Roy, *Phys. Chem B*. 108 (2004) 17721-17729

[23] S. Bresson, M. El Marssi, B. Khelifa, *Chem Phys Lipids., 134*, (2005) 119-129

Figures captions

Fig. 1. Evolution of SAXS and WAXS patterns with the temperature for (a) the fusion, (c) the crystallization, of Gelucire 50/13 at 1 °C/min. (b, d) Position and intensity (size of the symbol) of each peak observed in SAXS and WAXS [22].

Fig. 2. Raman and IR spectrum of Gelucire 50/13 in the spectral regions from 1800 to 1000 cm^{-1} at room temperature.

Fig 3. Raman spectra of Gelucire 50/13 (a) and (b) in the spectral region from 1600 to 1000 cm^{-1}, as a function of temperature. The temperature is increased or decreased with a rate of 1 °C/min.

Fig. 4. Raman spectra of Gelucire 50/13 fitted by Lorentzian curves as a function of temperature during the fusion (a) and recrystallization (b) between T = 26 °C and T = 60°C in the spectral region 1340–1220 cm^{-1} (heating and cooling rate of 1° C/min) with λ = 514.5 nm.

Fig. 5. the ratio of Area under the peak Area$_{1282}$/Area$_{1298}$ during the fusion from T = 26 °C to T = 50 °C and Intensity ratio I$_{1472}$/I$_{1488}$ as a function of temperature during the fusion from T = 26 °C to T = 60 °C.

Fig. 6. WAXS pattern of dry and hydrated Gelucire 50/13 (20 and 40%).

Fig. 7. Raman and IR spectra of Gelucire 50/13 as a function of degree of hydration at room temperature in the spectral region 1800–1000 cm^{-1} with λ = 1064.0 nm for Raman vibrations and in the spectral region 1800–1400 cm^{-1} for IR vibrations.

Fig. 8. Raman intensity ratios I$_{1298}$/I$_{1282}$ and I$_{1440}$/I$_{1486}$ as a function of degree of hydration from 0% to 40% at room temperature.

Fig. 9. IR spectra of Gelucire 50/13 fitted by Lorentzian curves as a function of degree of hydration from 0% to 40% at room temperature in the spectral region (a) 1250–950 cm^{-1} and in the spectral region (a) 1380–1320 cm^{-1}.

Fig. 10. IR frequency shifts of Gelucire 50/13 as a function of degree of hydration at room temperature in the spectral region 1400–1000 cm^{-1}.

Highlights

Vibrational behavior of Gelucire in the spectral range 1800-1000 cm^{-1} at T = 24°C

Vibrational behavior of Gelucire according to the temperature and degree of hydration

For the first time, we have assigned all vibrational modes of the Gelucire 50/13

These modes in this spectral range are very sensitive to environmental changes

Figure 1
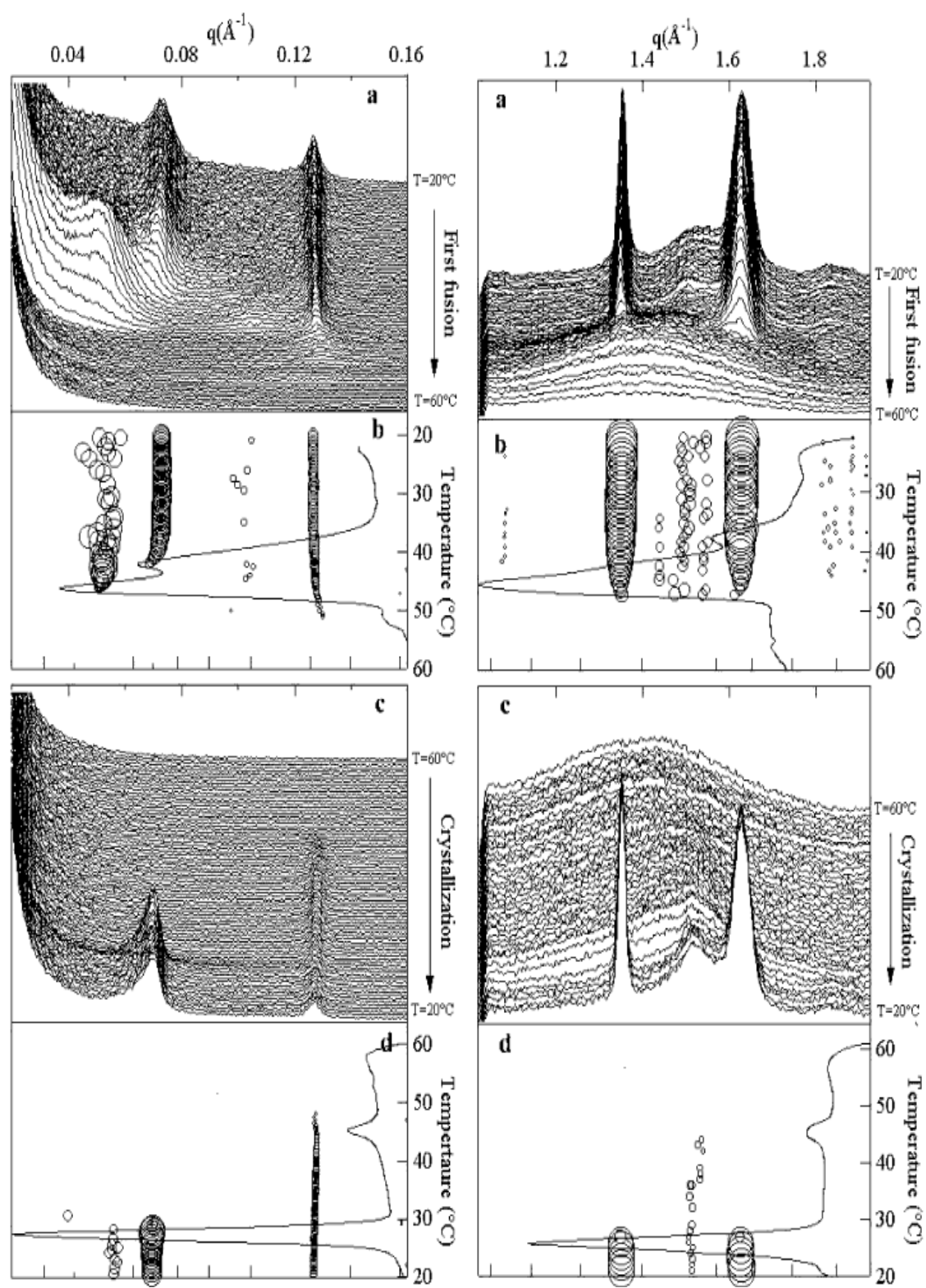
Fig. 1

Figure 2
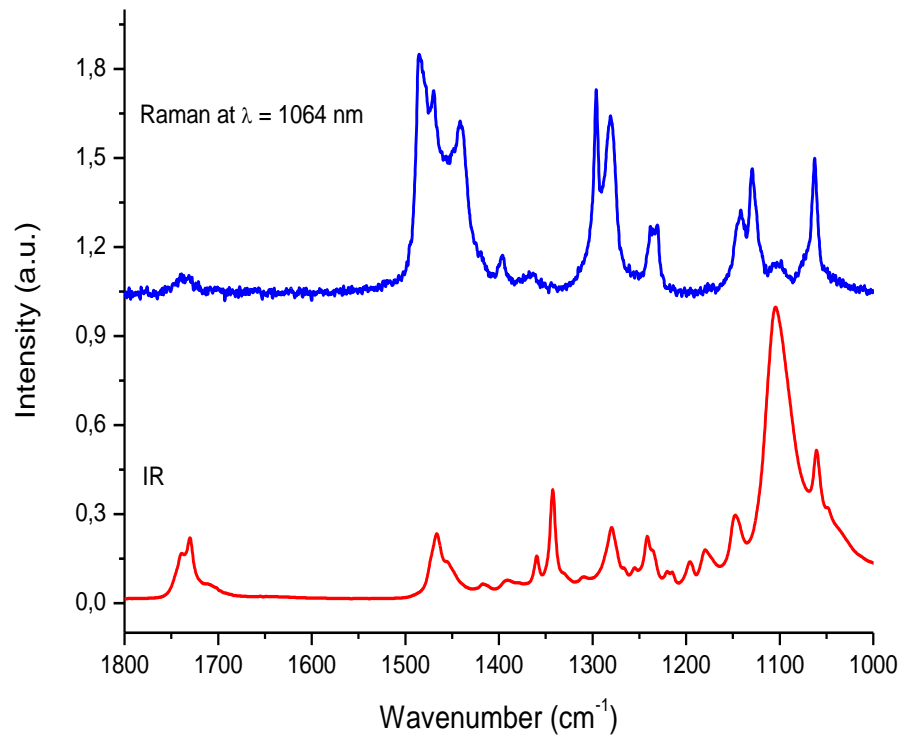
Fig 2

Figure 3
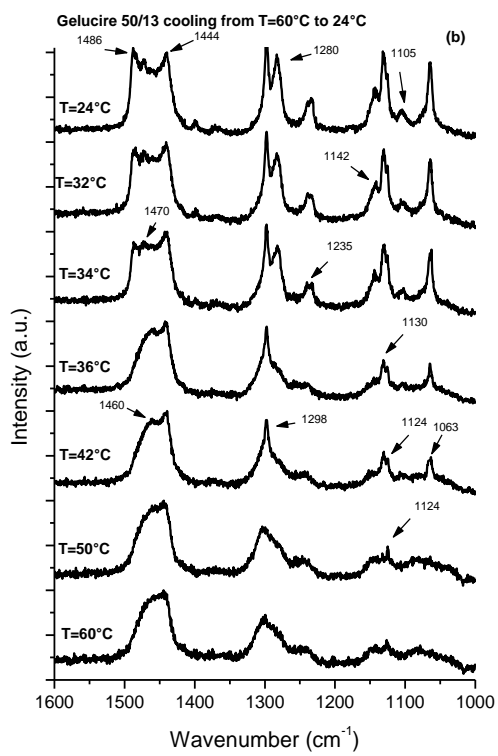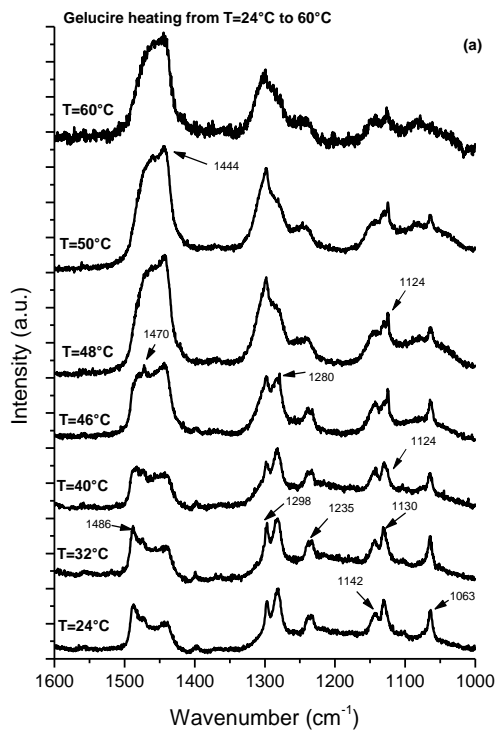
Fig 3.

Figure 4
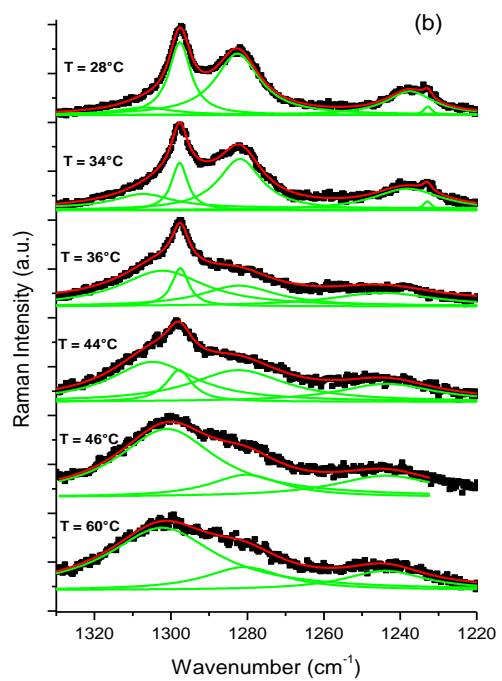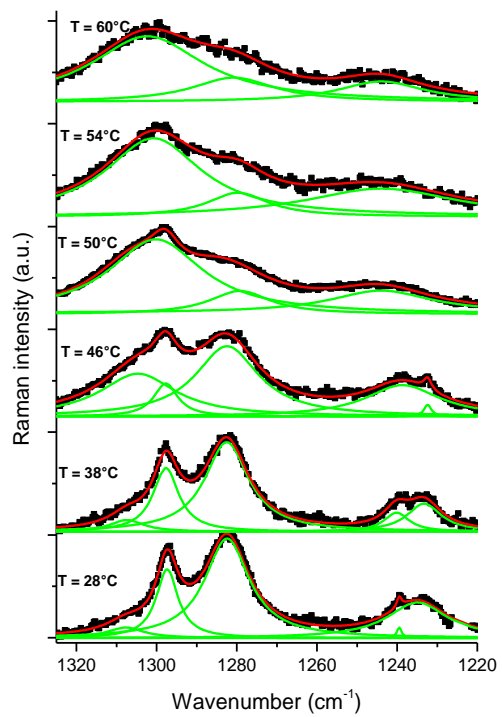
Fig. 4

Figure 5
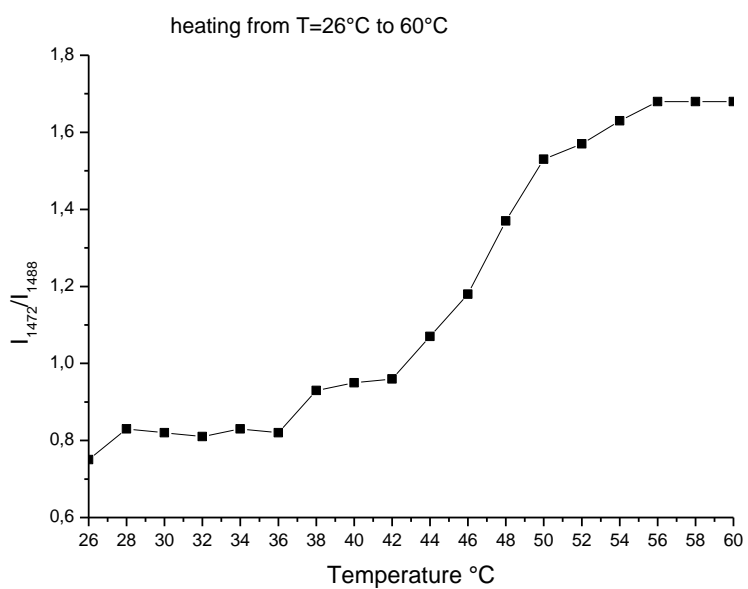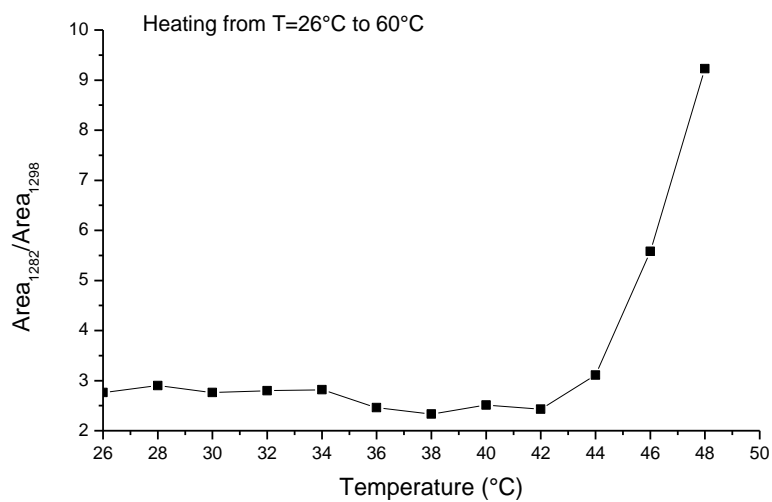
Fig 5.

Figure 6
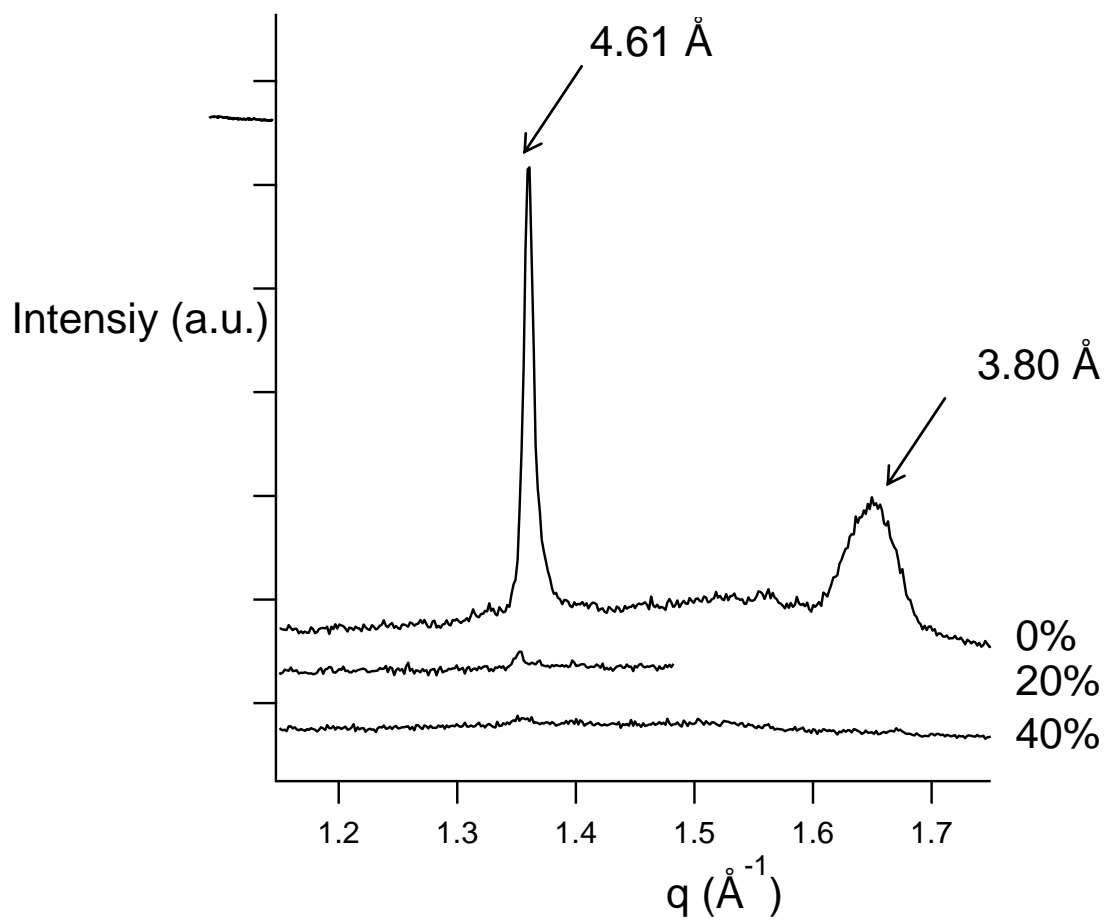
Fig. 6

Figure 7
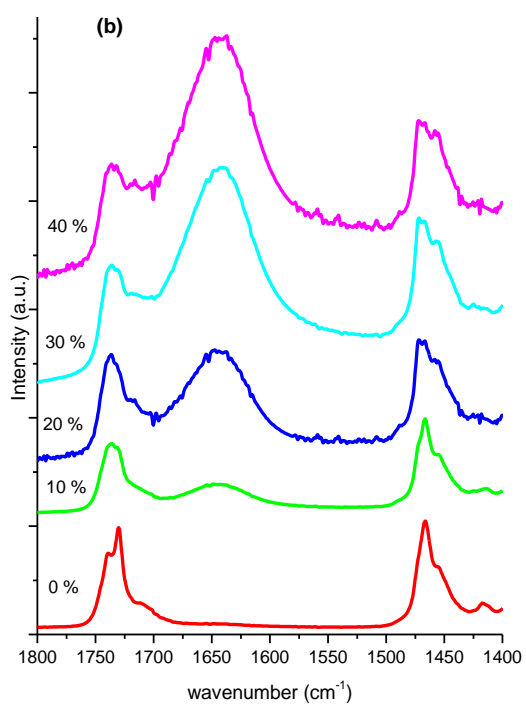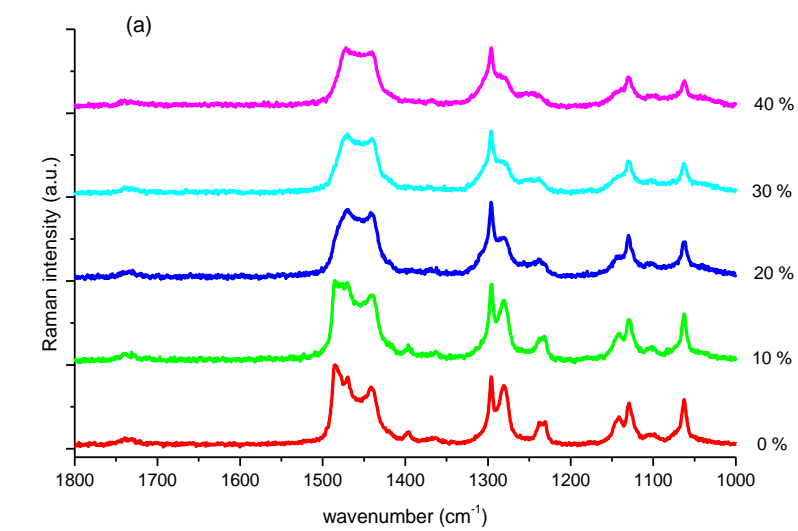
Fig 7

Figure 8

Fig. 8

Figure 9

(a)

40 %

30 %

20 %

10 %

0 %

Intensity (a.u.)

1200   1150   1100   1050   1000   950

wavenumber (cm^{-1})

(b)

40 %

30 %

20 %

10 %

0 %

Intensity (a.u.)

1380   1370   1360   1350   1340   1330   1320
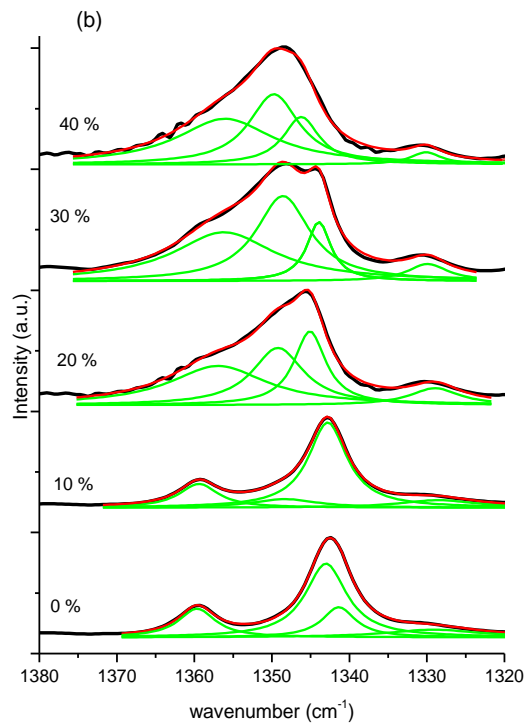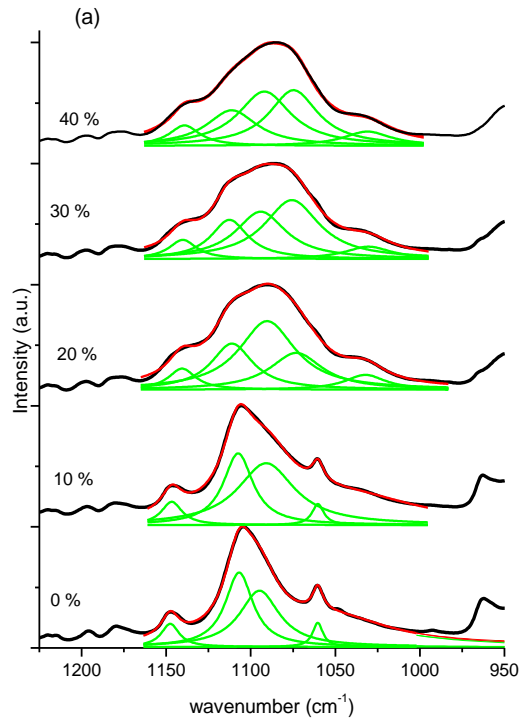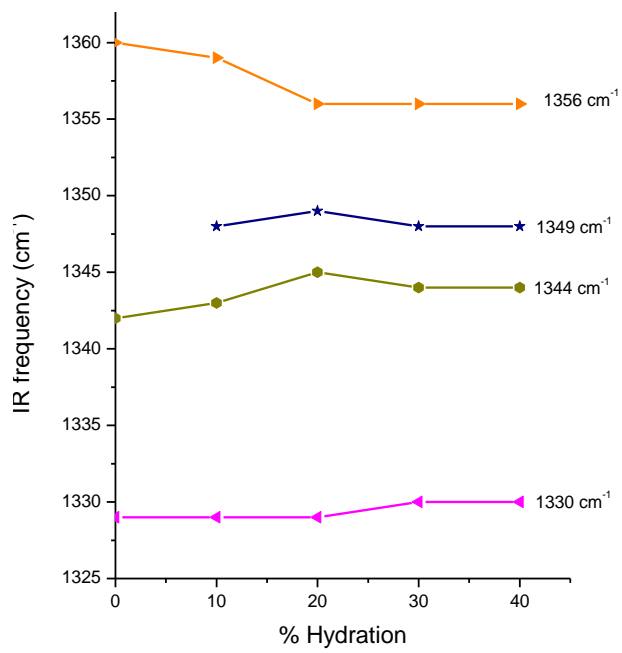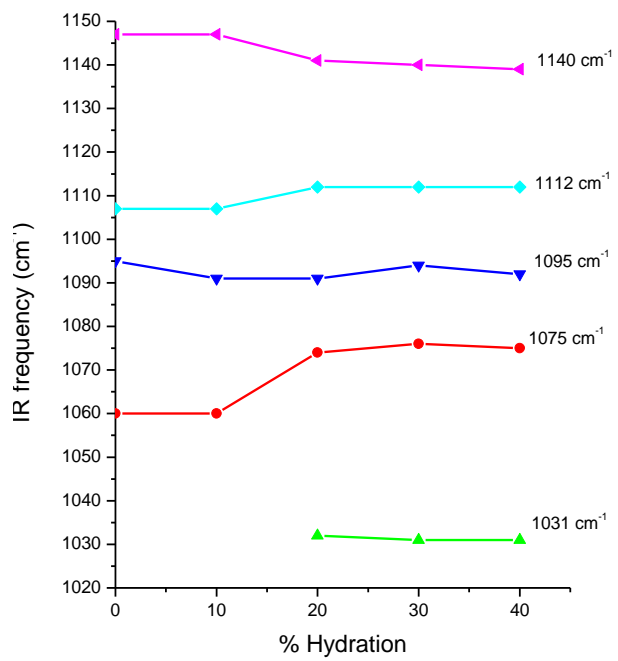
wavenumber (cm^{-1})
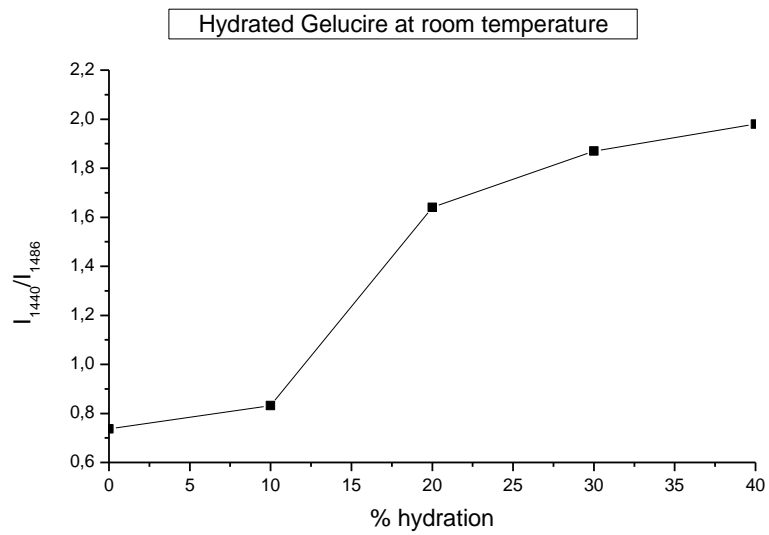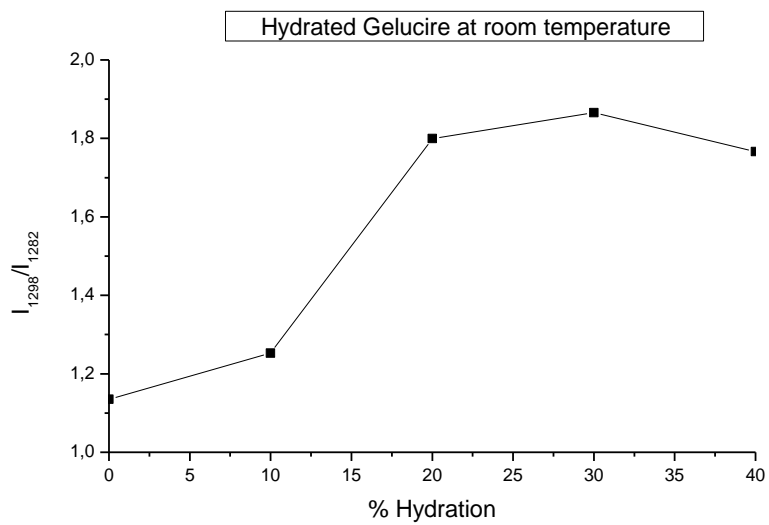
Fig. 9

Figure 10

Fig. 10

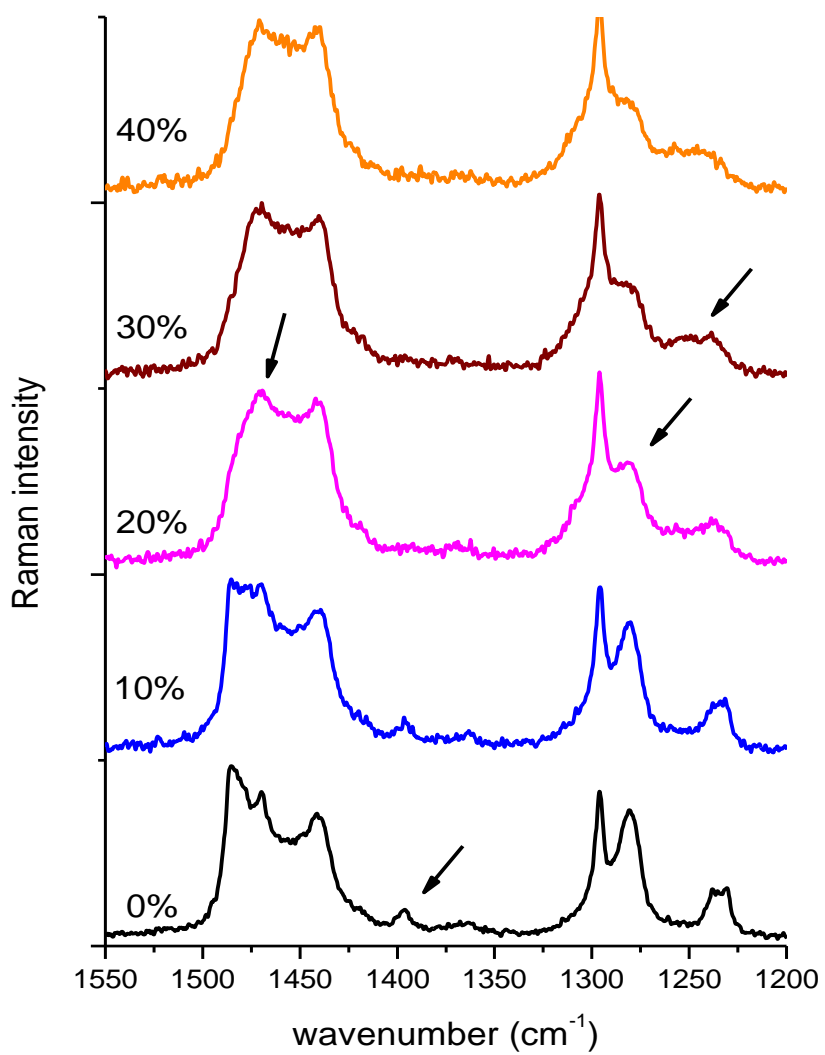Effects of the degree of hydration on Gelucire 50/13 Raman spectra

Table 1

Table 1: Assignment of IR and Raman bands for Gelucire 50/13 at room temperature in the spectral range 1800-1000 cm^{-1}. vw = very weak; w = weak; m=medium, s = strong; sh = shoulder; ν = stretching; δ = bending (in-plane deformation); τ = twisting ; w = wagging; σ = scissoring, T = trans; G = gauche; * = fitted value.

IR frequency (cm^{-1})	Assignments	Raman frequency (cm^{-1})	Assignments
1060 (m)	ν(C-C)$_T$[12]	1064 (s)	ν(C-C)$_T$[12] + ν(C-O)$_G$[21]
1095 (sh)		1104 (w)	ν(C-C)$_G$ [12]
1107 (vs)	ν(C-O)$_T$ [11]		
		1130 (s)	ν(C-C)$_T$ [12]
1147 (m)	δ (-CH$_2$-) [11]	1145 (m)	δ (-CH$_2$-)
1177 (w)			
1196 (vw)			
1219 (vw)		1233 (w)	w(CH$_2$) [12]
1240 (m)	w(CH$_2$) [12]	1240 (w)	w(CH$_2$) [12]
1279 (m)	w(CH$_2$) [12]	1282 (s)	w(CH$_2$) [21]
		1298 (m)	τ(CH$_2$) [12]
1306 (vw)	ν(C-C)$_T$[15]	1306*(sh)	τ(CH$_2$) [27]
1329 (sh)	ν(C-C)$_T$[15]+ w(CH$_2$) [13]		
1344 (s)	ν(C-C)$_G$[12]]+ w(CH$_2$) [13]		
1359 (m)	ν(C-C)$_G$[12]+ w(CH$_2$) [13]		
1385 (vw)			
		1400 (vw)	
1415 (vw)		1420 (vw)	δ (CH$_2$) [12]
		1440 (m)	δ (CH$_2$) [12]
1454 (w)	σ (CH$_2$) [12]	1456* (sh)	δ (CH$_2$) [12]
1465 (m)	σ (CH$_2$) [12]	1471* (m)	δ (CH$_2$) + σ (CH$_2$) [12,21]
		1480* (sh)	δ (CH$_3$) +δ (CH$_2$) [12]
		1486 (s)	δ (CH$_3$) +δ (CH$_2$) [12]
1730 (m)	ν(C=O) [27]	1738 (w)	ν(C=O) [27]
1740 (m)	ν(C=O) [27]		