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Crystallisation and melting behaviour of poly (3-hydroxybutyrate) in dilute solution: towards an understanding of physical gels

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Abstract

The purpose of this paper is to examine the thermodynamics of physical gelation by presenting some results gathered on gels of stereo-regular poly hydroxybutyrate (PHB). The investigation has been carried out in dilute solutions mainly by high sensitivity scanning calorimetry through the use of two solvent systems. Gels were formed under several different experimental conditions to clarify the thermo-reversibility of the gelation–dissolution process. Some peculiarities of the polymeric features in the gel phase are discussed in view of the recent assessment of the implication of chain stiffness as the triggering factor for gelation versus crystallisation. © 1998 Elsevier Science B.V.

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1. Introduction

Over the last few years the peculiar phenomenological behaviour of polymeric gel phases has provoked increasing scientific and practical interest in the fundamental aspects of the structure and formation mechanism of these intriguing systems. As a matter of fact, even the definition of gel needs careful thought [1,2]. The most common definition [3] of polymeric gels is often given for cross-linked chains and excludes the reversible gels which are so important in biological systems and which, more recently, are also well known for the synthetic polymers. Most of these gels are thermo-reversible, while others are controlled by chemically reversible non-covalent bonds. Interest in physical gels and their formation mechanism lies mainly in the 'quasi'-equilibrium state and the co-existence of 'quasi-ordered' regions surrounded by a 'quasi-fluid' system, and, therefore, in the possibility of tuning the composition and properties of these materials. The insistence of the term 'quasi' stresses the ill-defined structural and phase characteristics of the gel.

It is now accepted that the property which univocally defines a gel is its visco-elastic behaviour. In fact, the visco-elastic spectra obtained by rheological measurements can provide the rank of the interactions from the entangled state to the weak-gel or strong-gel phase [2]. A further differentiation between weak and strong gels is given by the large deformation behaviour; strong gels break and can be re-formed only after a new gelation cycle, whereas weak gels are thixotropic and re-form after a standing time. Given

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the role of the interactions in solution [4], which eventually determine the topological constraints at the basis of the gel properties, it is of fundamental importance to study the gel formation in dilute solution in order to understand the molecular origin of the structural and dynamic features of the polymeric chains in this supramolecular organisation. The main aim of this paper is to provide some thermodynamic and structural (i.e., conformational) insight to the problem of the formation of gel phases. The ultimate aim of the present research would be to deduce semi-empirical 'selection rules' in order to predict the conditions for the gel formation and the gel properties.

2. Solution conformation and gelation properties of PHB

Independently of the final macroscopic features of the gel phase, the conformation in the crystalline and amorphous states is one important characteristic which determines the morphology and other physical properties of the stereo-regular polymers possibly undergoing physical gelation under controlled conditions. The crystallisation of poly hydroxybutyrate (PHB) has recently been studied in some detail, while the conformation of amorphous polymers (solid or in solution) has been studied to a relatively smaller extent. The crystalline structure of poly(D-(-)-β-hydroxybutyrate), PHB, shows a 2_1 helical conformation [5] in the monoclinic crystals. Conformational analysis and statistics have been carried out on segments of PHB chains in the isotactic configuration according to methods of molecular mechanics and by using Monte Carlo methods [6]. The potential energy of the torsional angles has been evaluated as a function of torsional variables with the contribution of non-bonding and coulombic potentials. While the energy profile for the –O–CH– rotation is characterised by two narrow minima distorted from the trans and gauche (-) position, some greater conformational freedom arises from the rotation about the $-CH-CH_2$ and the $-CH_2-CO-$ bonds, although no minima are found for the trans-conformation of the latter rotation. These findings provide reasonable interpretation for the solid-state helical conformation, but also suggest an intrinsic tendency of the solvated polymer to adopt some preferential local conformations [7] which may resemble the most stable one in the solid state. In addition to these studies on conformational and solution properties, out of mere serendipity, fluorescence bands of PHB were detected which were a function of the monomer composition [8] in the co-polymeric samples of poly(D-(-)-β-hydroxybutyrate–hydroxyvalerate), co (HB and HV). The preliminary observation was that chloroform, the most common solvent for poly (hydroxyalkanoates), was not able to generate fluorescence properties, whereas the polymers were fluorescent in dimethylsulfoxide (DMSO), dimethylformamide (DMF) and trifluroethanol (TFE). The spectroscopic key of this phenomenon concerns the interaction with the solvent and the molecular conformation of the polyhydroxybutyrate. PHB seems to be fluorescent in solvents where chain stiffness and/or aggregation is thought to be increased, as detected, for example, by solution viscosity. Spectral results in TFE have been unambiguously interpreted as an intrinsic property of the co-polymer chemical composition [8], while the presence of polymer aggregates was detected in the other solvents, such as DMF and DMSO. In these solvents PHB forms aggregates and eventually at higher concentration undergoes a gel phase which may be considered as an interference with the fluorescence phenomenon. The common fluorescent behaviour in all the three solvents is a proof that it derives from a property of the polymer itself and not from the gel phase.

A preliminary characterisation of the gel phase has already been carried out with several experimental approaches aiming at elucidating the phenomenological aspects of the kinetics and the thermodynamics of this process [9,10]. Both the calorimetric and dielectric properties of 1% PHB in DMF have confirmed the presence of an extensive crystalline phase of PHB which forms reproducible compact nanoparticles. It is worth mentioning that, on cooling dilute solutions of PHB in DMF from temperatures above 100°C to room temperature, the optical activity of the solution (0.1%) deviated towards negative values of rotation with a sigmoidal shape, monitoring the ordering of the chains. The formation of widely dispersed aggregated structures was also shown by the change in the viscosity as a function of time measured on rapidly cooled solutions. The most relevant result was given by the heat associated with the disruption of the gel phase which was measured by differential scanning calorimetry (DSC). Several scanning cycles were shown to be reproducible on both of the two melting endotherms on heating and on the gelation exotherm on cooling, displaced by a marked hysteresis.

Many of these characteristics place the PHB biogels in a category which is quite different from that of the other biopolymeric gels studied so far [2] and closer to those formed, for example, by linear amylose chains. Here we specifically wish to report some features of the PHB gel formation and dissolution, for which some enlightening aspects, not previously reported in the literature, have been found.

3. Experimental

3.1. Materials

The sample of PHB used in this work, 'Biopol-GO8', was originally supplied by the Biopolymer Division ICI (UK) and labelled as pure PHB. The PHB received, after purification [9], had a molecular weight, M_W, of 3.4×10^5 da, determined by light scattering and, indirectly, by viscosity measurements. A value of 2.74 has been determined for the molecular weight distribution by HP–SEC by using polystyrene standards.

Solvent-grade N,N-dimethylformamide (DMF) and N-methyl-2-pyrrolidone (NMP) were the chosen gel-forming solvents and used without further purification.

Sample solutions were prepared by dissolving the polymer in an appropriate amount of solvent, gradually raising the temperature up to 110°C, under stirring [9]. The formation of a self-standing gel occurred upon cooling solutions with PHB concentration higher than 1% w. More diluted samples still yielded opalescent solutions because of aggregate formation.

3.2. Rheological measurements

Rheological measurements were carried out with the rheometer Rheometrics RFS 8500, equipped with a software for data acquisition and processing (Rhios, version 4.2.2).

The measurements were performed in dynamic frequency sweep conditions, at controlled strain (10%), in the frequency range 5×10^{-2}–10^2 rad s. The geometry was parallel plates with 50 mm diameter and 1 mm gap.

3.3. Calorimetric measurements

A highly sensitive MicroDSC Setaram microcalorimeter was used. The two cells were filled with the fluid sample and with the reference solvent, particular care being given to the weight balance of the two cells. Calorimetric measurements were run under different experimental conditions by varying the temperature programme and the scanning rate. Accessible temperatures ranged between 10°C and 97°C and scanning rates from 0.05 to 0.5 degree $\min^{-1}$. Nominal temperatures have been reported without calibration for the different scanning rate. For the crystallisation either the scanning mode or the isothermal one was possible.

The output signal was recorded as data file through a Burr–Brown interface on a PC. The data files were analysed using commercial software (Origin, MicroCal, USA).

4. Results

4.1. Visco-elastic results for gel definition

As stated in Section 1, the only features which can univocally define a gel are their visco-elastic properties. The PHB gel in DMF was formed in situ in the rheometer cell at a constant temperature of 40°C. The formation of the gel was followed by the departure of the elastic modulus G' as a function of the resting time (after about 1 h the gelation process was completed). The visco-elastic spectrum of PHB–DMF (1%) in the frequency range 5×10^{-2}–10^2 is reported in Fig. 1. The visco-elastic spectrum resembles a typical gel system with both G'' and G' nearly frequency-independent and with $G'>G''$ in the whole range of frequency accessible with conventional instrumentation, while η^* steadily decreases with the frequency. On the contrary, for a non-gelling system a predominantly liquid-like behaviour with $G'<G''$ and a strong frequency dependence of both G' and G'' ($G'\propto\omega^2$ and $G''\propto\omega$) would have been expected, while the complex viscosity η^* should be nearly constant at low and high frequencies and should show a power-law

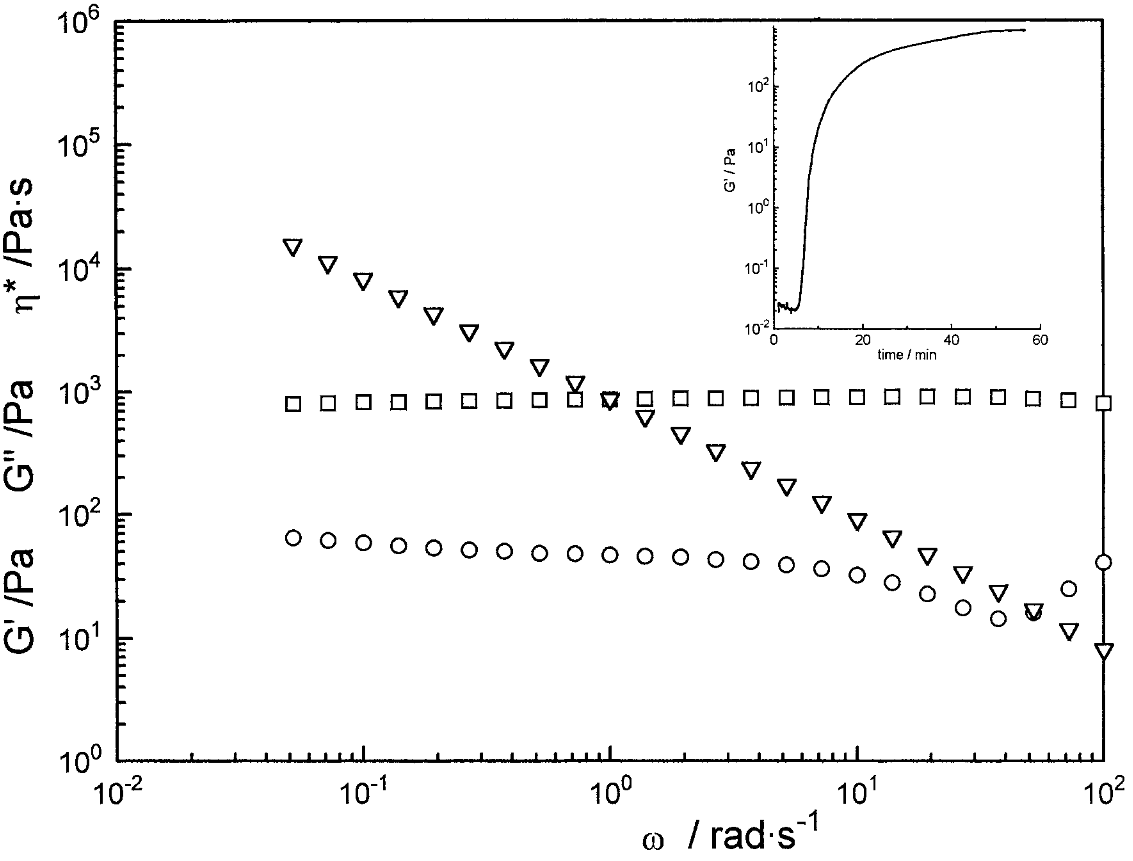

Fig. 1. Viscoelastic spectra [(□): G', (○): G'', and (▽): η^*] of the gel of 1% PHB in DMF (formed in situ in the rheometer at 40°C). The insert shows the time evolution of G'.

decrease as the frequency increases in the medium regime.

The flow curves of PHB–DMF give an indication of the existence of an apparent yield stress (apparent infinite viscosity), at low shear rate, without a Newtonian plateau. The remarkable shear thinning behaviour can be quantified in terms of the higher slope (≈ -1) in the power-law region. This behaviour, in some instances between that of the true gels and the so-called 'weak gel', likely reflects the occurrence in the polymer network of some weak non-covalent intermolecular forces, in addition to the true topological constraints. The visco-elastic spectra of weak gels are similar to those of true gels indicating that non-transient supramolecular structures also occur in the former systems. However, under shear the former flow while the latter rupture suggesting a different strength and/or extent of the interactions between the chain segments.

4.2. Calorimetric results

The presence of crystallisation and melting peaks in the calorimetric profiles has been used to define the gel–sol phase transition of PHB in dilute solutions of DMF and of NMP. The thermal transitions have already been ascribed [9] to the formation and dissolution of an extensive crystalline phase, which is responsible for the (thermo-reversible) gelation phenomenon. Due to the complexity of the system and the involved phenomena, the influence of different factors had to be identified by appropriate analysis. Various measurements were carried out in different experimental conditions in order to gain an insight into phase transitions and related kinetics. Most of the calorimetric data refer to systems prepared with a defined protocol and analysed in the micro-calorimeter with a scanning rate β=0.5 K min^{-1}, by following the conditions described in Section 3. Under these circumstances, all scans show two melting endotherms on heating and one crystallisation exotherm on cooling as exemplified in Fig. 2. Although the size, shape and position of the peaks depend on the thermal history of the sample, the composition and the experimental conditions described below, it is clear that some interesting features can be grasped from the temperature data reported in Fig. 3.

Our data of T_{m1} and T_{m2} for both PHB/DMF and PHB/NMP mixtures in the dilute range of polymer concentration do not seem to be affected by the difference in the solvents, whether DMF or NMP. This fact is quite unexpected since the two solvents have different physico-chemical properties, unless they have to be considered in the PHB system as a mere dispersing agent. The only difference between the two solvents emerges from the data of crystallisation where a slight decrease is observed for the T_c data of PHB in NMP with respect to the same data in DMF. Apparently, this seems to be a phenomenon related to the kinetics of crystallisation in the two solvents, due to differing viscosity, and not to the stability of the once-formed crystallites of PHB.

The dependence on the composition of T_{m1} and T_{m2} (although, they are not true equilibrium values) should follow the equation on the melting point depression of a pure crystallisable polymer as a function of the solvent volume fraction:

$$(T_{\mathrm{m}}^{-1} - T^{\circ}-1_{\mathrm{m}}) = RV_{\mathrm{u}}(\Delta H_{\mathrm{u}}V_1)^{-1}(\phi_1 - \chi\phi_1^2) \tag{1}$$

where ϕ_1 is the volume fraction of the solvent, T_m, the melting temperature of the polymer at the composition ϕ_1, $T^{\circ}{}_m$, that of the pure polymer, ΔH_u, the enthalpy of melting of the structural unit of the polymer, V_u and V_1, the molar volumes of the polymer structural unit and of the solvent and χ is the Flory interaction parameter, which is also dependent on composition and temperature [11]. While a more extensive characterisation of the whole phase diagram of the PHB/DMF system has also been carried out and is discussed in another paper (Fabri et al., to be submitted), let it simply be mentioned that the data of T_{m1} and T_{m2} in the dilute range of concentration have been plotted with a linear dependence with the square root of composition (as percent weight of polymer). The linearity of the melting temperatures versus the square root of the PHB composition may be fortuitous, but it may also be derived from a compensation of the concentration dependence of Eq. (1) in the square root term or simply be due to the range of composition investigated (from 0.075% to 10% w/w).

An inspection of dependence of the thermal profiles of PHB under different experimental conditions is instructive for the understanding of the growing mechanism of the polymer crystalline fraction.

4.3. Cooling scans

A quantitative analysis of the kinetic process by using calorimetric and dielectric results is in progress (F. Bordi et al., in preparation). Some experimental evidence is reported here which is relevant to the presentation and interpretation of the resulting heating scans.

The cooling scans of the PHB/DMF or PHB/NMP mixtures from a high temperature (above the melting peak) are characterised by a symmetric bell-shaped exothermic curve (Fig. 4), typical of an ordering process in dilute systems. However, when non-isothermal crystallisation is carried out at several scanning rates there are two modifications: firstly, at higher scanning rates the crystallisation peak shifts towards lower temperatures; secondly, a small endothermic peak appears at the onset of the exothermic crystallisation peak (Fig. 4). Before going ahead with the phenomenological description of the other results, let us consider the hypothesis that the endothermic peak is ascribed to a liquid–liquid phase demixing (LLPD). This hypothesis is based on the following results: (i) the LLPD endothermic peak always occurs at the onset of the crystallisation peak, (ii) the heat effect associated to the LLPD peak seems almost independent of the polymer concentration and (iii) the heat effect associated to the LLPD peak increases with increasing cooling rates.

These results indicate that by more or less rapid cooling (cooling rates, $\beta<-0.5$ K min^{-1}) there is a temporary co-existence of one polymer-rich liquid phase and one polymer-diluted liquid phase, having in mind that the phenomenon has only been recorded

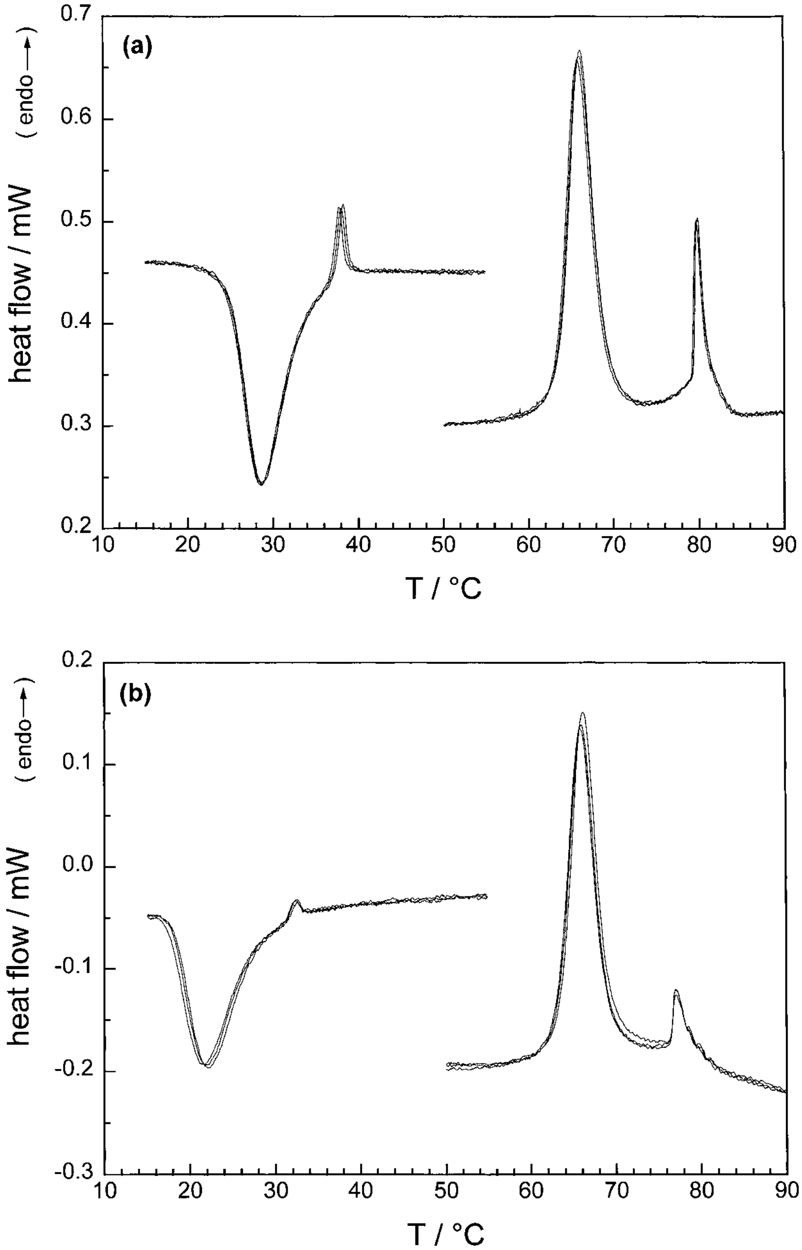

Fig. 2. Melting scans of PHB in DMF (a) and of PHB in NMP (b) recorded at scan rate 0.5 K min^{-1} with high sensitivity DSC. Polymer concentration is 1% w.

on original polymer concentrations below 5%. Higher concentrations would require higher scanning rates, but the small endothermic effect is completely masked by the large exothermic crystallisation heat. The liquid–liquid phase separation is, therefore, the triggering process which induces the crystallisation in the polymer-rich liquid phase when a moderately dilute solution (less than 5%) is cooled [12]. In principle, isothermal heat of mixing measurements should be carried out to prove that the extent of the

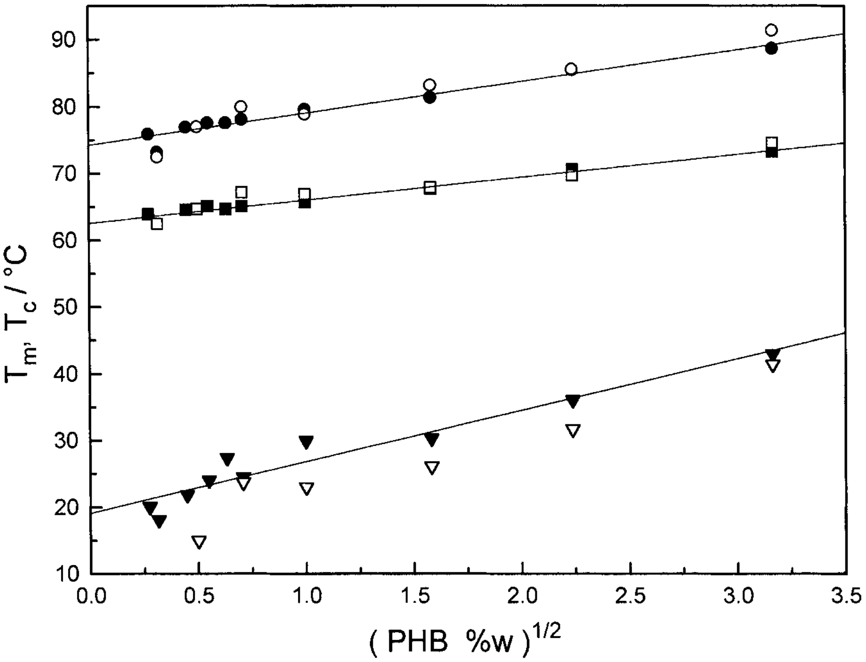

Fig. 3. Dependence of the melting [(□■): T_{m1}, and (○●): T_{m2})] and gelling-crystallization [(▽,▼): T_c] temperature as a function of the square root of polymer concentration. Full symbols refer to PHB in DMF, open symbols to PHB in NMP.

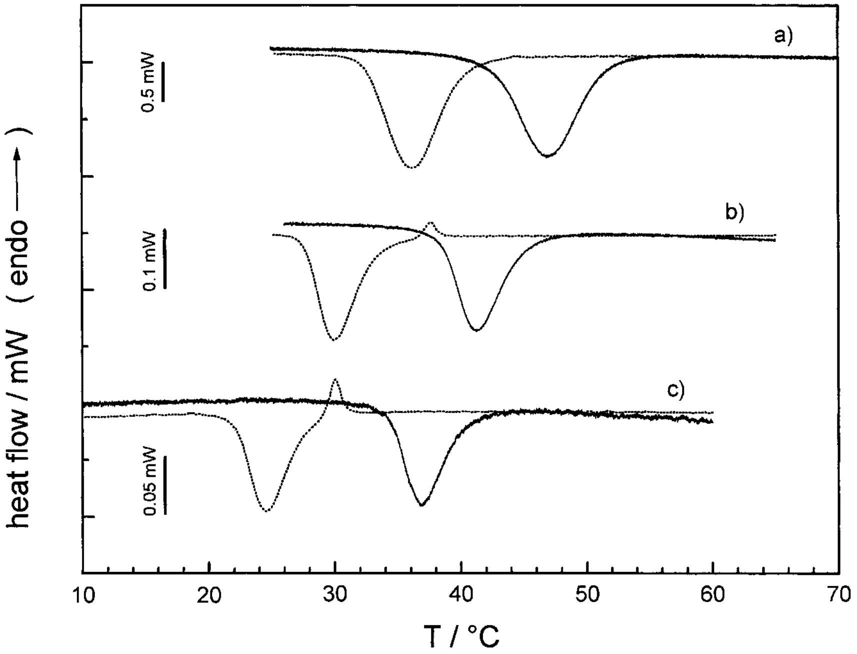

Fig. 4. Cooling scans of PHB gels at low concentration: (a) 5% w, (b) 1% w, c) 0.5% w; scan rate 0.5 K min^{-1} (dotted line) and 0.05 K min^{-1} (solid line). The signals were normalised for the actual polymer concentration.

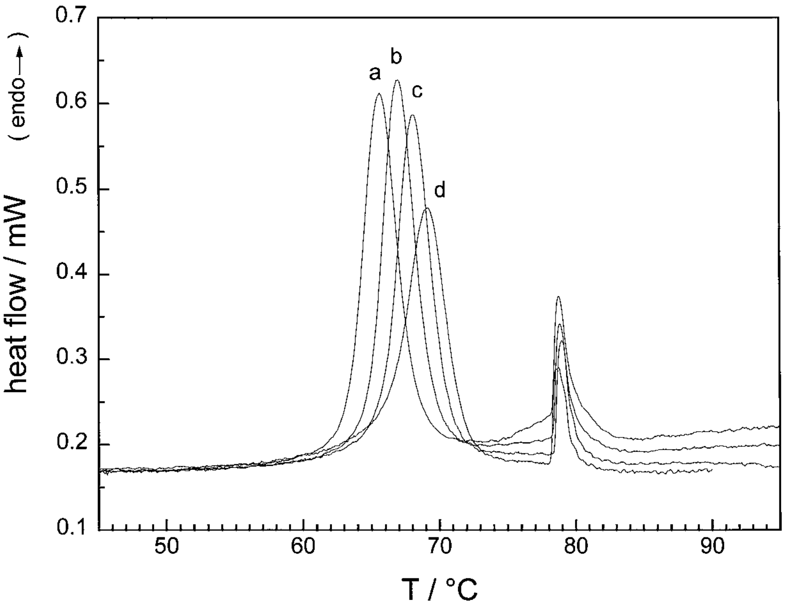

Fig. 5. Dependence of the gel melting temperatures on the cooling scan rate. The heating scans were recorded with a constant heating scan rate of 0.5 K min^{-1} following cooling scan rates of 0.5, 0.2, 0.1, 0.05 K min^{-1} (curves a, b, c and d, respectively). PHB 1% w in DMF.

endothermic peak is related to the $\Delta_{\text{demix}}H$ of the two liquid phases.

4.4. Heating scans

According to the current understanding of the polymer crystallisation processes occurring at different crystallisation temperatures, one would expect some modification of the melting profile of the polymer fraction crystallised under different temperature conditions. As a matter of fact, the results of Fig. 5 only partially satisfy this expectation, if the melting scans are recorded under constant conditions (β=0.5 min^{-1}). Samples crystallised at high scanning rates (i.e., at lower temperatures, with the appearance of the pre-crystallisation exothermic peak) show a lower melting temperature, and the melting T_{m1} changes by almost 5°C by changing the cooling rate by a factor of 10 (cooling rates range from β=−0.5 to −0.05 K min^{-1}). Surprisingly enough the other sharp peak, characteristic of a higher stability morphology (T_{m2}), does not change position (Fig. 5).

On the other hand, two more observations are reported to validate the difference observed between the two melting peaks. When heating is carried out at different scanning rates (from 0.5 to 0.1 K min^{-1}) on samples crystallised under β=−0.5 K min^{-1}, the first peak remains practically unchanged (Fig. 6), while the second peak moves towards higher temperatures, a phenomenon clearly ascribed to an annealing process favouring the more stable morphology. However, this annealing is possible only after the first melting has occurred producing disordered chains which are evidently in an under-cooled metastable liquid phase. More than 20 min are necessary, under these circumstances, to allow these non-crystalline chains to grow on the surface of the more stable crystals. Parallel to the shift towards higher temperatures, the area of this peak appears to increase.

As a further test, heating scans in Fig. 7 have been recorded with different scanning rates following cooling, with both cooling and heating being carried out at the same scanning rate. The first peak follows the trend described for the curves reported in Fig. 5, while the peak at a higher temperature is shifted and changed according to the findings of Fig. 6. This means that the heating rate does not affect the first peak.

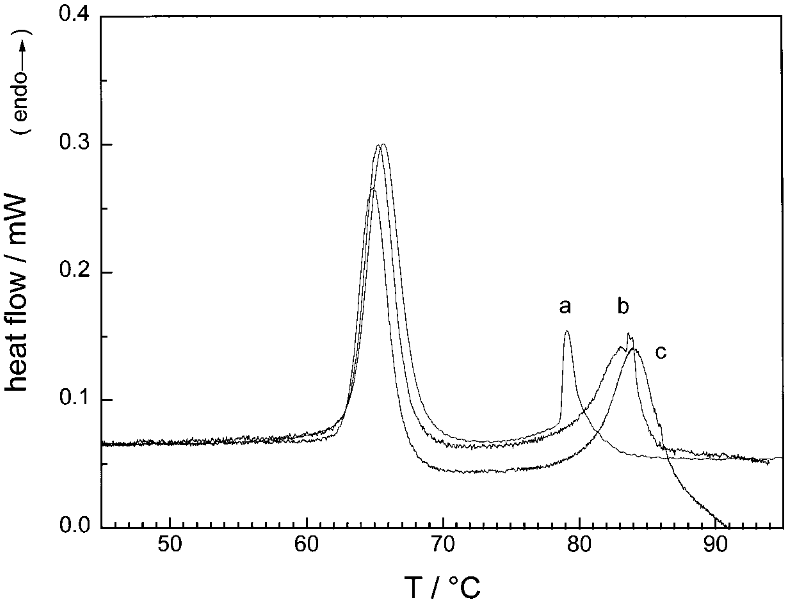

Fig. 6. Dependence of the gel melting temperatures on the heating scan rate. Note that the heating scans were recorded with a different heating scan rate of 0.5, 0.2, 0.1 K min^{-1}. PHB 1%w in DMF.

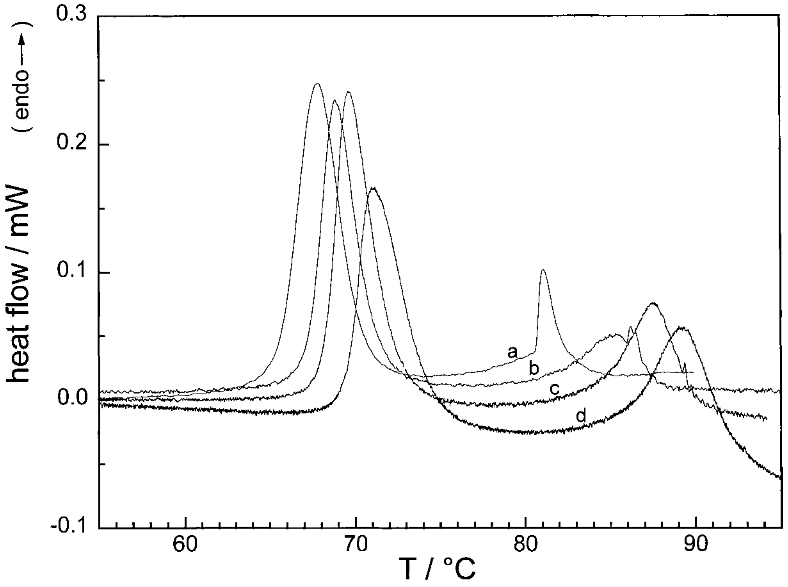

Fig. 7. Dependence of the gel melting temperatures on the cooling/heating scan rate of 0.5, 0.2, 0.1, 0.05 K min^{-1} (curves a–d, respectively). Note that the heating scans were recorded with a scan rate equal to that used for cooling. PHB 1%w in DMF.

4.5. Isothermal crystallisation

It is, therefore, clear that the shape and the position of the melting endotherms are a function of the cooling procedure followed in the preparation of the gel phase. Scans with other different profiles were obtained when isothermal conditions were chosen to form the gel after its melting at high temperatures. The

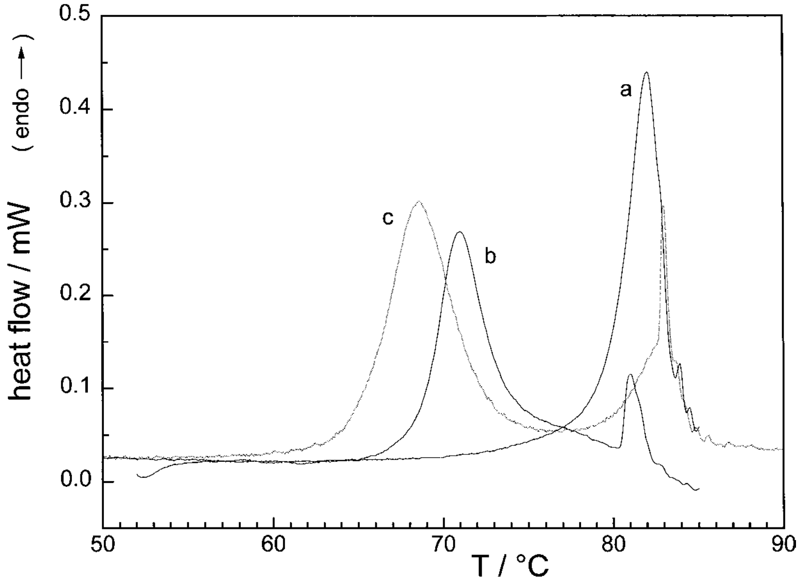

Fig. 8. Melting scans of 1% PHB gel formed: (a) upon isothermal crystallisation at 60°C for 21 h; (b) upon isothermal crystallisation at 40°C for 2 h; (c) upon isothermal crystallisation at 60°C (19 h) and cooling to 25°C.

temperature programme consisted of a heating scan followed by a cooling scan, at $\beta=-0.5\ \mathrm{K\,min^{-1}}$, to reach the isothermal temperature, at which the sample was allowed to gel for a suitable time. The different shapes of the melting curves reported in Fig. 8 for the 1% gel in DMF are strictly related to the different temperature ranges at which the gel phase was formed, provided that the scans are each obtained at the same heating rate ($0.5\ \mathrm{K\,min^{-1}}$). Curve (a) refers to a scan obtained immediately after isothermal crystallisation at 60°C; only the high melting peak (T_{m2}) is observed. Curve (b) shows the scans of a gel obtained isothermally at 40°C; the second peak is superimposed on the long-tailed curve of the first peak. Curve (c) shows the scans of a gel obtained at 60°C and then cooled to 25°C. This cooling further reduces the areas of the scans obtained under the controlled cooling rate of $0.5\ \mathrm{K\,min^{-1}}$.

Finally, annealing effects were also studied by programming a 2 h isotherm after the first melting endotherm. After the 2 h isotherm at 80°C and using the common scan rate of $0.5\ \mathrm{K\,min^{-1}}$, the second melting peak appeared at a higher temperature (~92°C) and with a larger area than that obtained in the absence of isothermal annealing, but similar to that shown in curve (a) for the sample cured at 60°C.

5. Discussion

5.1. The conformational pre-requisites

Before attempting an interpretation of the calorimetric results and constructing some speculative explanation of the gel structure and gelation mechanism, it is pertinent to summarise some of the literature on the PHB chain conformation in solution. Original experimental values of chain dimensions were reported for several polymers with varying similarities to PHB (including atactic non-chiral homologous polymer). According to experimental data of Huglin and Radwan [13], the values of the unperturbed dimensions of the stereo-regular PHB in two binary solvents also lie within a reasonable range, the characteristic ratio being in the order of 6. It is also claimed that the small negative value of the temperature coefficient is an "effect purely of chain flexibility rather than being thermodynamic in origin, and it is similar to that observed for other polymers within a temperature region displaced from the critical solution temperature". In conclusion, all the solution properties of PHB are in line with the belief that the chain is a true random coil with a moderate tendency to have some local (and statistical) pseudo-ordered sequences which originate mainly from the stereoregularity of its configuration.

The above facts lead to the conclusion that, under none of these circumstances of solvent, composition and temperature, does it seem likely that the PHB random coil chains can be easily stretched to form long fibrillar-like aggregates in the gel phase. Models for fibrillar aggregation or fringed-micelles arise especially from the polysaccharide field and have also been picturised by rotatory shadowing electron microscopy in the case of gellan, agarose or carrageenan. Even in solution, in the same concentration regime and under conditions of an ordered conformation, many of these biopolymers with low flexibility (e.g., worm-like polysaccharides with persistence length Lp>60 nm) show a remarkable deviation from the solution behaviour described for flexible molecules. They often undergo gelation in a cooperative process triggered by small changes in temperature, ionic strength. pH, etc.

A similar suggestion has also been put forward by Guenet [14] for polystyrene (PS) gels, in which the complexation with solvent is the crucial factor for chain stiffening. However, the conclusion of this work leads to a misleading message that the alternative for a polymer solution under cooling is either the formation of a fibrillar gel or crystalline phase separation. Neither the solution features nor the gel morphology and behaviour exhibited by PHB can support the molecular mechanism or the conformational motifs as those ascribed to stiff polysaccharides and solvent-complexed polystyrene.

5.2. *The description of a physical gel: some current views and speculations*

Some of the ideas developed while working on several gel systems (mainly polysaccharides) were founded on the conviction that the chain association (such as that extrapolated from the pectin or alginate world) was applicable to most of the other systems. Some complex differences among the several polymeric gel systems have already been discussed and this complexity is increased due to the difficulty of also unifying the theoretical approaches [15]. However, it is clear enough that, at least in the physical gelation processes, the important driving force is the conformational ordering of segments of chains which reach a critical size for Gibbs free energy stability. The dimensionality of the gel nucleus is governed by the stiffness of the chain. In the field of synthetic polymers there were no examples in which the presence of a mechanism like that accepted for gellan could be clearly demonstrated until Guenet reported in detail the morphology of PS gels [14]. However, the co-crystallisation of a solvent into the ordered chain cells does not seem to be a rule but rather an exception. In the PS, the toluene molecules are heavily involved in the stiffening of the chain molecules which, therefore, can be arranged in a parallel packing with a small negative entropy change. The local stiffening of the chain segments is also a pre-requisite for many other polysaccharidic chains: local conformational transitions induced by ions and sometimes mediated by water molecules are the nucleation step for the further growth of the gel junction zones. It is less reasonable to assume that other very flexible chains (e.g., amylose) which are known to form gels in dilute solutions are organised with the same mechanism. Both in the polysaccharidic and synthetic structures, crystallisation and gelation can also occur in the lamella-like organisation [15].

In order to provide a schematic model for the structural and kinetic aspects of the gelation, let us simply start from the basic assumption that a gel is formed when “at least three different chains converge in the junctions” [1]. It turns out that no limitations have to be assumed a priori on the length of the flexible spacers between junctions, nor on the size or shape of the segments entering in the junctions. Although the quantitative answer to this structural problem is still elusive, a scheme based on the structural parameters and physical variables mentioned above is drawn in Fig. 9.

The difference between the extremely idealised model (a) and model (b) resides in the fact that studies can in principle be carried out on very dilute solution of chains of model (a), disclosing a molecular conformational transition (i.e., a cooperative character is intrinsic in the chain structure, favouring the disorder to order conformation even in the absence of junctions). On the other hand, stability of the ordered chains in the lamella-like crystals is assured mainly by the energy of packing, since the folded chains (being intrinsically flexible) require segments of a large size before the free energy of the nucleus

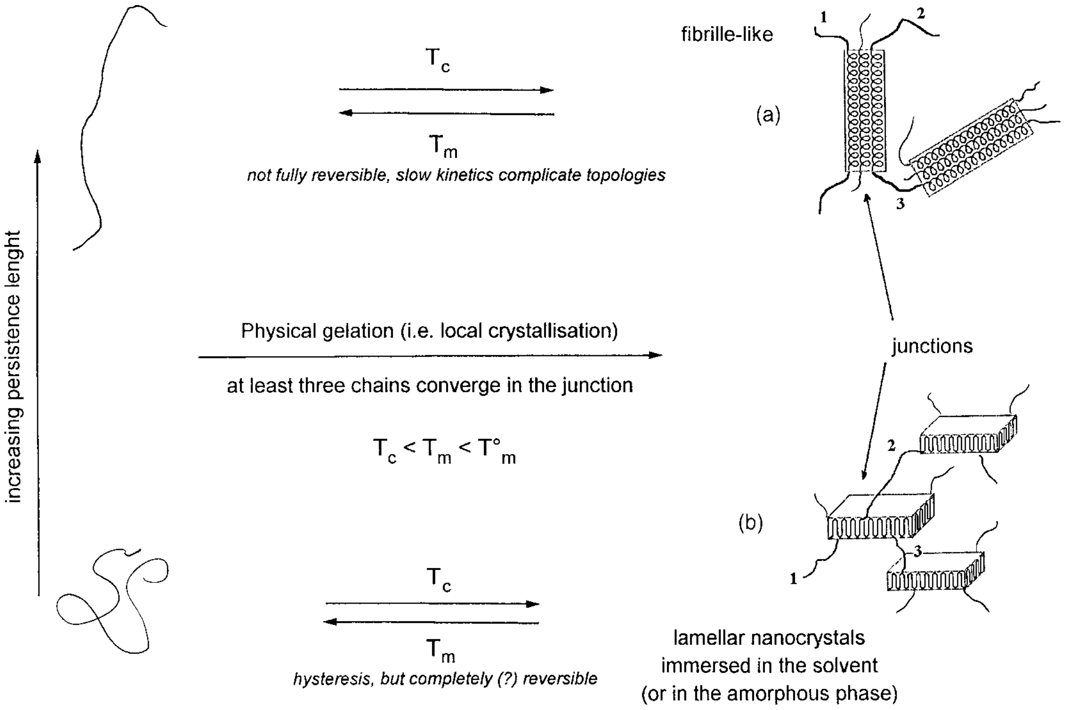

Fig. 9. A general scheme for the static chain conformation features entering into the morphology of the gel phase.

becomes negative with respect to the free-soluble chains.

5.3. A general interpretation of the gel phase diagram

Generally speaking, in most cases the decrease of the T_m of a crystalline phase occurs as a consequence of the addition of a component (solvent or plastifier) which, therefore, acts as a solvent for the amorphous/liquid phase in equilibrium at T_m with the solid crystalline phase. The first development of a theoretical treatment was due to Flory and Mandelkern [16] and used the Flory–Huggins model for the thermodynamic properties of the polymer solution. However, this is not sufficient for many cases, where the crystalline phase is formed by very small crystals (nanosize), whose dimensions are dependent upon polymer concentration (below 10%) and upon the temperature of gelation.

The complete Gibbs free-energy diagram versus temperature (Fig. 10) contains a family of curves for the liquid phase (i.e., the Gibbs free-energy curve of the liquid polymer and those of the liquid polymer with increasing concentration of the other component) and also a family of curves for the solid phase (i.e., the Gibbs free-energy curve of the perfect crystal of infinite size and those of crystals with an increasing number of defects and decreasing dimensions). A completely new surface is then generated and, for each composition, a line of melting temperatures is defined as a function of the crystal size and perfection. It should be clear at this point that the melting temperatures (see Fig. 3) can be a function of both the composition of the system and the intrinsic features of crystal size.

The fact that a 1% PHB system can form a self-standing gel provides an intuitive but compelling explanation concerning the nano-dimension of crystalline junctions of the gel phase. However, more direct proof of this should be obtained by morphological studies with electron microscopy. These studies can add evidence to the observation that the X-ray powder diffraction pattern of the PHB in DMF gives the same diffraction lines of the solid partially crystalline PHB. The broadness of the diffraction lines gives

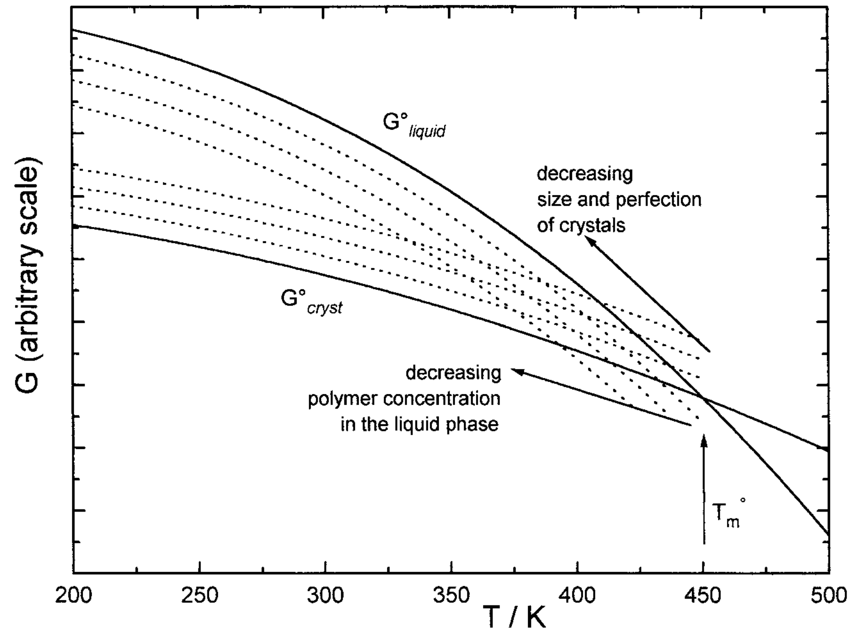

Fig. 10. Schematic diagram of the Gibbs free-energy (G) surface for solid–liquid phases. The modifications, introduced in the $G^{\circ}_{\mathrm{cryst}}$ line by the changes in dimensions and regularity of crystals and in the $G^{\circ}_{\mathrm{liquid}}$ by solution composition, are shown by the two sets of dotted lines, respectively.

a rough estimation of the size of the crystals in the order of about 5 nm.

The discussion would be incomplete if the original observation of the phase demixing upon cooling dilute solutions was not taken into consideration at this point. Segregation of the polymer chains in the two domains with different concentrations is the selective rule for the occurrence of two ordered microphases which grow at the same temperature but at different concentrations. This explanation only implies that the binodal–spinodal curves are highly asymmetric with a very steep curvature at high PHB composition, giving rise to an almost constant composition of the polymer-rich phase. In this situation the gelation line (which is, however, a non-equilibrium line!) corresponds to the de-mixing line.

5.4. The bimodal melting profile

Most of the bimodal melting profiles are susceptible to the common interpretation of the concurrent phenomena of melting and recrystallisation followed by final melting at higher temperatures. Many of these complex profiles have been observed over the last 20 years [17]. This process is the consequence of the size distribution of crystals which undergo a progressive melting following the dimension of the crystals. The melted fractions are 'undercooled liquidus' below the thermodynamic equilibrium and can crystallise mostly on the surfaces of the large crystals already present. Given the high temperature, the formation of new nuclei and further growth of these nuclei are unfeasible. Therefore, if re-crystallisation can occur rapidly, the broad melting endotherm presents a sharp exothermic peak, and under most circumstances two melting peaks are apparent.

The other current interpretation of the multiple meltings has been developed on the basis of a large body of evidence for the existence of a bimodal distribution of crystal size. A discussion of the occurrence of multiple melting peaks due to different fractions of crystallinity has been given by Mathot [18].

The origin of the double melting peaks of PHB gels reported here has been clearly ascribed to the formation of different crystalline microphases, although other experiments should be carried out to definitively confirm this interpretation.

Apparently, there is not a single method which could clarify unambiguously the origin of these multiple endotherms. Recently, evidence of in situ small recrystallisation processes underlying the melting of pure PHB (one endotherm) has been claimed through the use of temperature modulated DSC, where reversible and irreversible transformations can be discriminated within the frequency response of the experiment [19]. Although preliminary experiments with a new temperature modulated DSC are in progress in our laboratory on the PHB gel systems, it is worth mentioning that the greatest difficulty for suitable experimental conditions resides in the metastable nature of the gel within the available frequency domain.

6. Conclusions

The set of data presented in this paper and the interpretation constructed on other independent literature works suggest that the PHB gel is a suitable system for modelling solution thermodynamics and dynamic properties of segregated crystalline microphases. Much more work is being reported or is under progress to remove some of the current speculation and to quantitatively define the applicability and the constraints of this model.

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