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Structural and Thermodynamic Features of the Polyhydroxybutyrate Physical Gels

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SUMMARY: The structural and thermal properties of polyhydroxybutyrate (PHB) gels in dimethylformamide (DMF) are presented and discussed. The dilute and semi-dilute mixed PHB/DMF system undergoes a phase separation on cooling at a temperature T_{gel}, producing a gel in which the crystalline fraction is of the order of 50-60% and the diffraction pattern is that of disperse nano-crystals, with the same unit cell of the pure crystalline PHB. Upon heating, two melting peaks are normally observed at T_{m1} and T_{m2} (always higher than T_{gel}). Shapes of the peaks, temperatures and enthalpies of melting depend slightly on scanning rate on heating but on the procedure of gel preparation. Reproducibility and reversibility have always been found with repeated thermal cycles. A thermodynamic phase diagram is therefore constructed and discussed, although the morphology of the system may be subject to the experimental conditions under which the gel is prepared.

Introduction

The formation of thermoreversible (physical) gels has been observed in many dilute polymer solutions and has provoked an increasing interest in both the fundamental aspects of the morphology and of the gelation kinetics of these systems1). Gels are formed by crystallisation from quiescent solutions, while for a time the belief persisted that, apart from the biopolymer gel networks2), shearing was required. In the eighties, Mandelkern showed that the crystals formed during gelation of polyethylene are of lamella type3), like those formed from dilute solution. According to these findings, many variables are involved in the control of the resulting crystalline structures, in particular, the cooling rate, crystallisation temperature, and solvent properties. As the solution is cooled, chains form crystalline lamellae that serve as reversible cross-links if the chain entanglement in the solution is sufficiently developed. It has been suggested that the gel formation occurs at a critical concentration, defined by a proper set of values of viscosity-molecular weight and solvent-temperature. Chain entanglement is sufficient if for each crystallite, at least three chain ends leave and make up part of the formation of another lamella. This scheme is in line with Flory's concept of the minimum functionality which can generate cross-linking gelation4) and with the statistical model of Tanaka and Stochmayer5).

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In this paper the results of a study of the properties of a gel formed by a natural polyester polyhydroxybutyrate (PHB) in dimethylformamide (DMF) provide a preliminary understanding of the molecular processes at the basis of the gelation phenomenon of this polymer. Poly(D-(-)-β-hydroxyalkanoates) homopolymers and copolymers (PHA) have been isolated from different environmental microbial and non-microbial sources6) and the co-polyesters, made by β-hydroxybutyrate and β-hydroxyvalerate co(HB,HV), have been formerly produced by *Alcaligenes eutrophus* from propionic acid and glucose by I.C.I. (U.K.) under the trade name Biopol7).

Both the microbiological aspects and the solid state properties of these biopolymers have been studied in some detail. The highly crystallinity has soon been recognised for the homopolymer in its purified form8) and it has been possible to deduce the molecular features from powder diffraction study of the crystalline form9), while the experimental data on the PHB in the native granules have unambiguously provided a highly mobility of the polymeric chains10). The physico-chemical properties of the amorphous polymer (in solution) have been studied to a relatively lesser extent11), however sufficient to deduce a random coil behaviour with a considerable excluded volume effect12).

Given both the practical and scientific interests, the thermal behaviour of amorphous and crystalline morphologies of PHB under different conditions (e.g. , quenched and spherulitic habit in blends with diluents) has been studied to a great extent7,13). The structural changes and the evaluation of the polymer-solvent interactions, induced in these systems by temperature, have attracted interest in the attempt to understand the physical properties of these polymers, either in the solution state and in the gel state. In addition, owing to the biocompatibility and to biodegradability of PHB, possible applications of nano-crystalline matrices imbibed with a proper solvent may result in the field of biomedical and drug release technologies as well as in the alternative wet-spinning processing for packaging.

Results

Gel structural and morphological aspects. Upon cooling dilute and moderately concentrated (0.1%-20%) solutions of PHB in DMF from temperatures above 100°C to room temperature, the phenomenological observation of a turbidity is accomplished by the formation of a self-standing gel phase for concentrations larger than 1%. After quenching the very dilute solutions (< 0.1%), the optical activity deviates toward negative values with a sigmoidal shape, monitoring the ordering of the stereoregular chains (Figure 1). The formation of very disperse aggregated structures is also shown by the change in the viscosity as a function of time measured on the rapidly cooled solutions. Both phenomena are reversible, giving two-step sigmoidal melting profiles of the aggregated structure as a function of temperature (not shown).

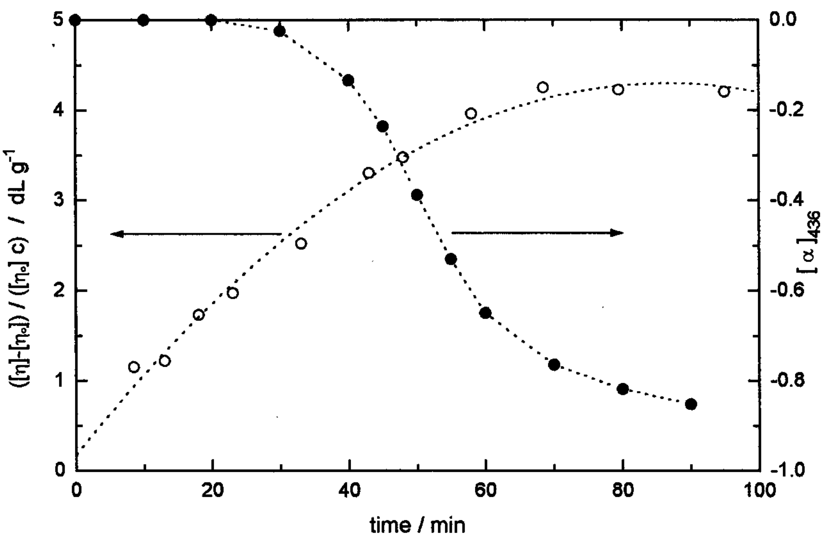

Fig. 1. Changes of reduced viscosity (O) and specific optical rotation (●) for the incipient gel formation in a 0.075% PHB/DMF solution quenched at 25°C.

Following this evidence, the formation of the gel phase and the occurrence of the thermal transitions of PHB in dilute DMF solution have been studied by differential scanning calorimetry and by a number of techniques, such as small and wide angle X-ray scattering (SAXS and WAXS), hot-stage microscopy, electron microscopy, rheology, dielectric spectroscopy, some preliminary results being reported elsewhere[14-17]. All these results are interpreted on the basis that the transitions observed in the range from 60 to 90 °C are attributed to the fusion of small crystallites. The aim is to characterise the gel phase and its phase transition by means of the different experimental techniques in order to gain information on the structural changes occurring in the polymeric phase. Summarising the new results, time-resolved small angle scattering data collected at the ELETTRA Synchrotron Radiation on mixtures at different polymer concentrations show the persistence of nano-sized particles up to the final melting temperature. The overall dimension of these particles (about 5 nm) is consistent with the value approximately evaluated from the peak broadness of our diffraction data in the wide angle range. The importance of these results lies also in the findings that the polymer organisation in the gel phase is crystalline in nature and that the dimensions of the crystallites are rather small. Furthermore, once the contribution of the amorphous has been eliminated, the WAXS diffraction peaks obtained from the gel system congruently overlap those obtained from the pure crystalline PHB. More deep knowledge about the possible bimodal distribution of the crystalline nano-particles and their kinetic formation is being obtained from the analysis of the SAXS data on the nucleation and growth of the crystallites under controlled conditions and their subsequent melting upon temperature scanning$^{18)}$.

Hot-stage microscopic observation of the gel phase gives a continuous birefringent network, which is also seen in the pictures of the solvent-dried gel by electron microscopy. These two observations are only necessary for the confirmation of the existence of a macroscopic wall-to-wall gel structure, which is responsible for the dramatic increase of the elastic component G' in the viscoelastic characterisation$^{17)}$ of the system undergoing isothermal gelation-crystallisation. Once the gel (PHB 1%) is formed, G' becomes greater that G" by more that one order of magnitude and the shear thinning behaviour is characteristic of a "weak gel", given the slope of $\log \eta^* / \log \omega \sim -1$. Finally, dielectric measurements have been undertaken to determine the changes of the electrical polarisation, which depend on the structural properties of the polymeric gel as a function of the temperature (as those evidenced below by DSC) and on the reorganisation of the solvent involved in the melting of the polymer aggregates$^{16)}$. This approach, over a frequency range from 1kHz to 1 GHz, aims to the distinct observations of the effect of the sol-gel transition on both the polymeric chain entanglements, at lower frequencies, and on the solvent, at higher frequencies.

Calorimetric results and phase diagram. The other relevant results here reported aim at elucidating the quantitative aspects of the thermodynamics of the gel phase formation and melting. These data have been obtained by using a high sensitivity micro-DSC and mainly concern the evaluation of the temperatures and the heats associated with the formation and the disruption of the gel phase under different experimental conditions. Several scanning cycles have been always recorded to check the reproducibility of the response for both the melting endotherm on heating and the gelation exotherm on cooling, which are, however, separated by a marked hysteresis. Figure 2 reports the typical thermograms obtained for the cooling (a) and the melting (b) of a PHB/DMF system (1%) at scan rate of 0.5 K min^{-1}. Two peculiarities appear in the thermograms: the anomalous endotherm preceding the gelation exotherm centred at 28°C (T_{peak}) and the splitting of the melting exotherms displaced by 10-15°C. An analysis of the possible reasons and an interpretation of the bimodal melting is given elsewhere$^{17)}$ and will not be discussed here.

Taking for granted these phenomenological results on the thermal behaviour of the PHB/DMF system, the dependence of the melting temperatures as a function of the composition (phase diagram) is given in the Figure 3. The temperature values for PHB concentrations less than 15% refer to transformations recorded at the scan rate of 0.5 K min^{-1} by using a high-sensitivity micro-DSC operating with cells of about 1 mL of volume. These conditions are suitable to give "quasi-equilibrium" values without excessive reorganisation of the mobile phase. However, this type of micro-DSC suffers from the limitation that the highest

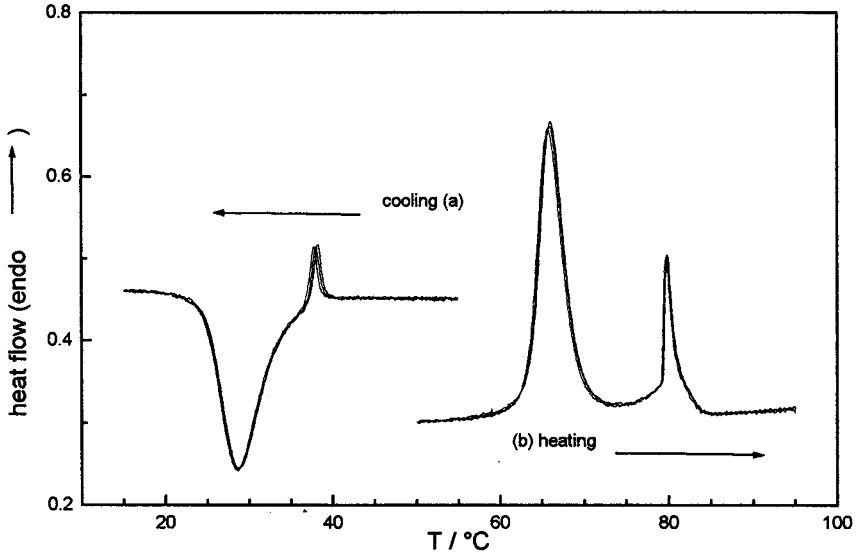

Fig. 2. Example of thermograms showing the reversibility and reproducibility upon successive cooling (a) and heating (b) scans (PHB 1%, scan rate 0.5 K min^{-1}).

temperature reachable is that of 100°C. In order to get a broader description of the phase diagram of the PHB/DMF system (for PHB concentration higher than 20%), experiments had to be performed with a conventional DSC. Thermograms have been therefore recorded in a range of temperatures from -150 °C up to 200 °C and on quenched mixtures. As an example of these results, the thermogram shown in Figure 4 reports the relevant transition encountered on the 5% PHB/DMF. The heat flow curve shows a glass transition step (at about -130 °C), followed by a cold crystallisation of the undercooled solvent (at about -107 °C). Then, the slow continuous melting of the crystallised solvent takes place from about -64 °C to -45 °C.

During all these processes the baseline constantly shows a shift each time the system changes physical state (glassy or crystalline to amorphous or liquid, respectively). It is only after all the transitions of the solvent are out of mind that the less intense endotherm due to the gel melting occurs at about 70 °C, showing clearly that the study of the enthalpy of the transitions at low PHB concentration is better resolved only by using the high sensitivity micro-DSC.

The data of the glass transition and of solvent melting temperatures have also been included in the Figure 3, with the aim of describing the supplemented phase diagram of the PHB/DMF system. The diagram clearly show some gap between the temperature values obtained on the mixed system and those obtained for the pure PHB sample.

Thermodynamics of the gel formation and melting and enthalpy of gel melting

All the low PHB concentration mixtures in the experimental conditions illustrated above show thermograms with two melting endotherms on heating. However, peak size and shape vary significantly with composition, as results especially from the experiments carried out in the composition range between 0.025% and 15% w/w with scanning rate of 0.5 deg min^{-1}.

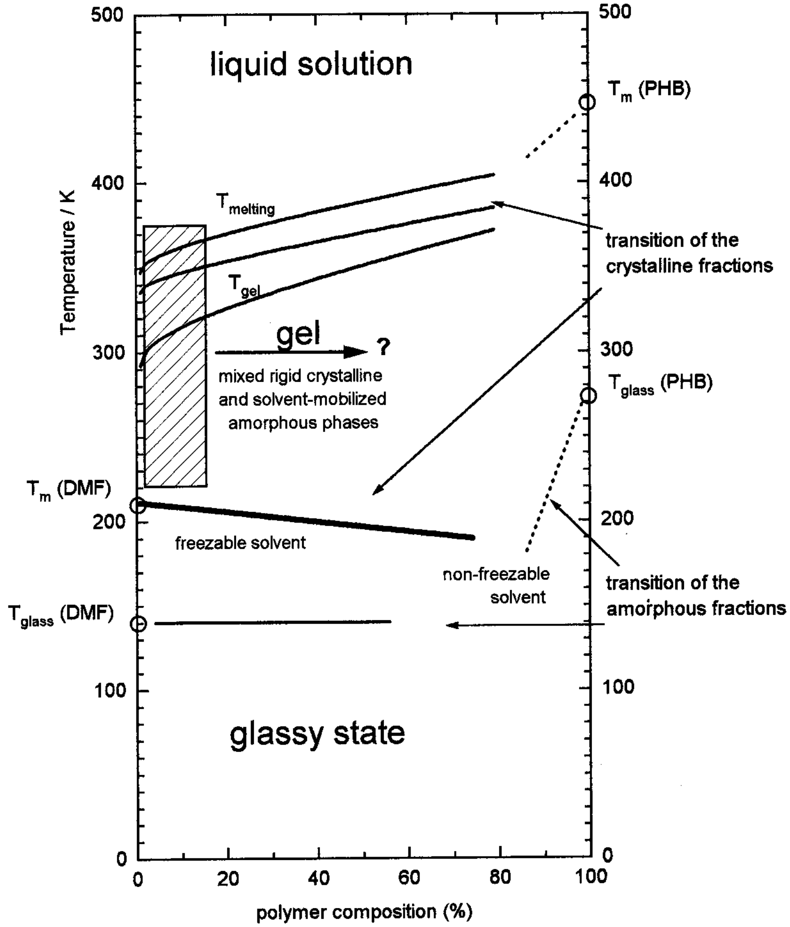

Fig. 3. Phase diagram of the PHB/DMF mixture. The data for the melting and glass transition of the pure components are reported (O). The full lines are drawn through the experimental points, the dotted lines represent the conceivable extrapolation of the experimental behaviour.

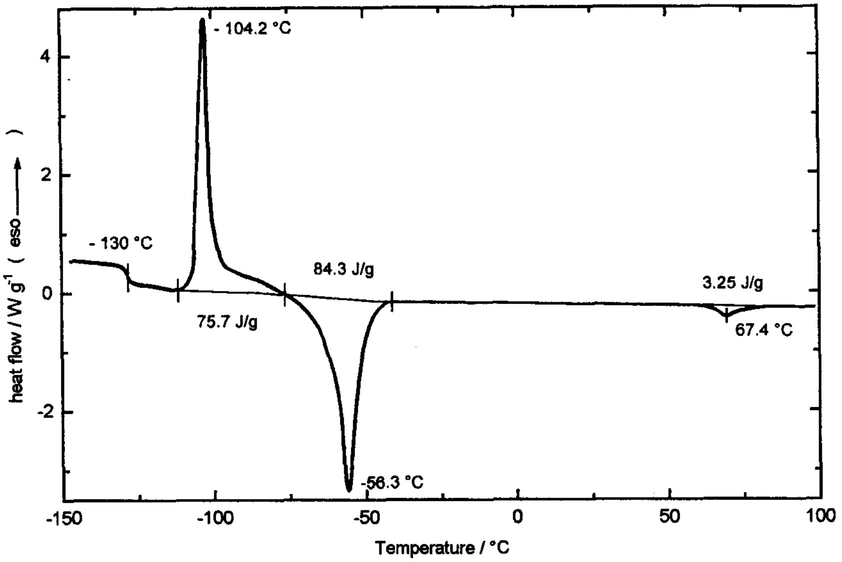

Fig. 4. Thermal behaviour of a quenched PHB/DMF mixture to -150 °C (scan rate 5 K min^{-1}). The relevant transitions are discussed in the text.

A scrutiny of the two melting peaks (see Figure 2) shows that the endotherm at T_{m2} is sharper and larger than that at T_{m1} at low concentration, while sharpness and relative size is progressively lost as the polymer concentration increases. The endotherm at T_{m1} seems to maintain a bell-shaped curve which is characteristic of the transition of independent (non-interacting) macro-domains like those occurring in the denaturation of globular proteins[19].

Given the good separation of the two endotherms it is possible to evaluate accurately the enthalpy of transition for the two melting processes. In fact, a difference of about 10-15°C is found for the two peak temperatures and the baseline is attained in between them. The data of the $\Delta_m H_1$ and $\Delta_m H_2$ against the PHB concentration are reported in Figure 5 showing some discontinuity of the melting behaviour below 1%. More interesting is the value of the total enthalpy of gel melting that progressively increases at very low polymer concentrations.

Therefore, below a given concentration the trend of both the enthalpy and the melting temperature provide an indication of some different structural organisation. The lower thermal stability but higher enthalpy of melting seems somehow in contrast with the simple hypothesis of small crystals being relevant at low polymer concentrations. These results may indicate that at low concentration either the fraction of amorphous polymer interdispersed in the system decreases and eventually only small crystals containing all the PHB are present, or that the surface interactions polymer solvent become important. To validate this hypothesis additional information is required on the structural organisation of the polymer in the crystalline phase.

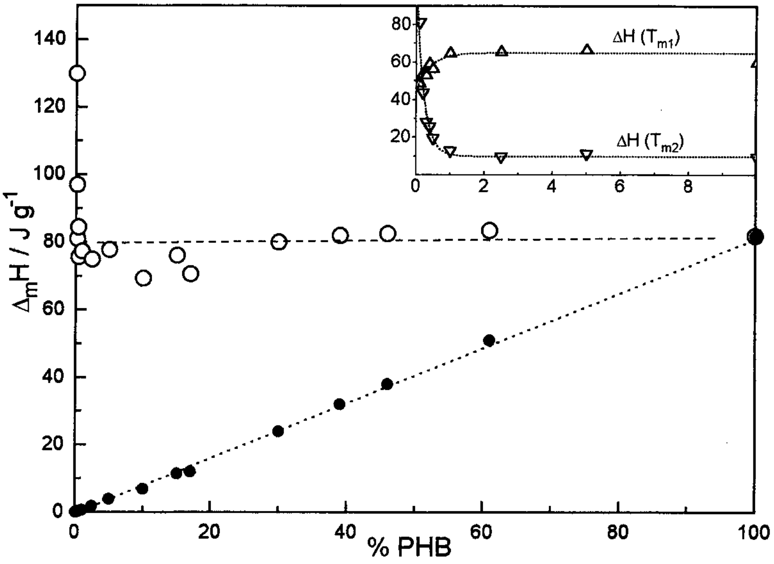

Fig. 5. Enthalpies of melting of the PHB/DMF mixture as a function of PHB concentration. Both the linear change of the enthalpy change per gram of mixture (●) and that per gram of PHB in the mixture (O) are reported. The dotted lines in the insert give the two components of the $\Delta_m H$ at T_{m1} and T_{m2} in the dilute concentration regime.

Discussion

The results here reported may provide the basis for a better understanding of the gel structure and gelation mechanism. Models for fibrillar aggregation arise especially from the polysaccharide field and have also been pictured by several microscopy methods in the case of gellan, agarose, carrageenan, and others. Many of these biopolymers have low flexibility (e.g. worm-like polysaccharides with persistence length Lp > 60 nm) and 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[2,20]. A similar suggestion has also been put forward by Guenet[21] for the polystyrene (PS) gels, in which the complexation with the solvent is the crucial factor affecting the chain stiffening. However, 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.

In fact, given the formation of the physical gel of PHB from the solution state, it is worth recalling that in dilute solution the values of the unperturbed dimensions of the stereoregular PHB lie within a reasonable range for flexible polymers, being the characteristic ratio of the order of 6. According to Huglin and Radwan[22], 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 very moderate tendency to have some local (and statistical) pseudo-ordered sequences which take origin mainly from the stereoregularity of its configuration. Therefore, under none of these circumstances of solvent, composition and temperature, does it seem likely that the PHB random coil chains can spontaneously stretch to long fibrillar-like aggregates. In agreement with these evidences obtained from the solution properties, the structural and morphological data suggest that the nanocrystals have a rather compact and regular shape with an overall dimension of about 5 nm. The size and the crystallinity of the polymeric gel are therefore compatible with the occurrence of lamella-like regular aggregates.

The other point to discuss is the more general thermodynamic aspect of the gel formation and stability. In the physical gelation processes, the important driving force is the conformational ordering of segments of chains which reach a critical size for the Gibbs free energy stability. The dimensionality of the gel nucleus is again governed by the stiffness of the chain. On this basis, it does not seem appropriate to assume that very flexible chains which are known to form gels in dilute solutions are organised with the same mechanism proposed for rigid polymers. Both in the polysaccharidic and synthetic structures crystallisation and gel can also occur in the lamella-like organisation, which is the morphology here proposed for PHB.

The dimensionality of the ordered gel phase is one of the relevant aspects which determines the decrease of the Tm of a crystalline phase. The other is the addition of a component (solvent or plastifier) which therefore acts as a solvent for the amorphous/liquid phase in equilibrium at Tm with the solid crystalline phase. The first development of a theoretical formulation is due to Flory and Mandelkern[23] and uses 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 crystals, whose dimension and morphology are dependent upon the experimental conditions[24]. In a schematic representation of the Gibbs free-energy diagram *vs.* temperature the energy surface is described by 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 by 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 the crystals with an increasing number of defects and decreasing dimensions). Melting temperatures, such as those of Figure 3, are then a function of both the composition of the system and the intrinsic features of the crystal size.

More work is in progress on these gels to validate the present results and their interpretation.

Acknowledgements

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