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Gel–sol phase transition of poly(3-hydroxybutyrate) in dimethylformamide ☆

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Abstract

The formation of thermoreversible (physical) gels in a moderately dilute solution of a low molecular weight poly(β-hydroxybutyrate), PHB, in dimethylformamide, DMF, has been studied by different techniques in order to provide a preliminary understanding of the molecular processes at the basis of the gelation phenomenon of this polymer.

The characterisation of gel phase formation has been carried out with several experimental approaches aiming at elucidating the effect of the thermal history and the kinetics of this process. Scattering data have been taken as a measure of the fraction of gelling crystalline aggregates formed as a function of time and analysed according to the Avrami equation. Characterisation of the gel–sol transition temperature(s) and energetics has been carried out by optical methods (e.g., turbidity) and by calorimetry as a function of temperature. The heat associated with the disruption and the formation of the gel has been measured by high-sensitivity differential scanning microcalorimetry.

Keywords: Differential scanning microcalorimetry; Dimethylformamide; Gel–sol phase transition; Poly(β-hydroxybutyrate); Turbidity

1. Introduction

Poly(D-(−)-β-hydroxyalkanoates) homopolymers and copolymers (PHA) have been isolated from different environment microbial and non-microbial sources [1,2].

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A family of biodegradable copolyesters, made by β-hydroxybutyrate and β-hydroxyvalerate co(HB, HV), has been produced commercially by *Alcaligenes eutrophus* from propionic acid and glucose by I.C.I. (UK) under the trade name Biopol [3].

Both the microbiological aspects [4] and the solid state properties [5] of these biopolymers have been studied in some detail, whilst the physico-chemical properties of the amorphous polymer (in solution) have been studied to a relatively lesser extent [6]. Solution studies clearly indicate that the chain dimension of PHB corresponds to that of a random coil to which, however, a considerable excluded volume effect seems to be ascribed [7]. An approach by molecular mechanics on the chain conformation in the crystalline and amorphous states has also provided the molecular characteristics which determine the morphology and other physical properties of these stereo-regular polymers [8].

While studying the solution properties of PHA, we observed some new phenomena (e.g., the presence of fluorescence bands and the formation of a gel phase) which have either not been previously reported in the literature, or have not been investigated in detail. The fluorescence properties [9] were a function of the monomer composition and related to the conformation, the solvent interactions and the molecular characteristics of the polyhydroxyalkanoate chain. In fact, from the preliminary observation that chloroform, the most common solvent for PHA, was not able to generate fluorescence properties, whereas the polymers are fluorescent in dimethylsulphoxide (DMSO), dimethylformamide (DMF) and trifluoroethanol (TFE), the conclusion was drawn that PHB is fluorescent in solvents where chain stiffness and/or aggregation is thought to increase. The spectral results of PHA in TFE were unambiguously interpreted in terms of the copolymer chemical composition [9], while in other solvents, such as DMF and DMSO, the polymer forms gelling aggregates which interfere with the fluorescence phenomenon. Here, we deal with the characterisation of the gel phase of PHB in DMF, and in particular with the thermal properties of the gel formation and dissolution.

The formation of thermoreversible (physical) gels has been observed in many moderately dilute polymer solutions and has provoked increasing interest in both the fundamental aspects of the morphology and the gelling kinetics of these systems [10]. Gels have been formed by crystallisation of polyethylene from quiescent solutions, while for a time the belief persisted that shearing was required. Manderkern and coworkers showed that the crystals formed during gelation are of lamella type, like those formed from dilute solution, and are not fringed micelles [11]. According to these findings, several chains are physically locked into the crystal, and many variables are involved in the resulting crystalline cross-linked 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 sufficient. It has been suggested that the gel formation has 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 gelation [12] and with the statistical model of Tanaka and Stochmayer [13]. Contrasting evidence exists on the effect of the molecular weight. The

critical gel concentration depends only weakly on the polymer molecular weight according to Fukui and Yamabe [14], whereas Domszy et al. [15] suggested that a high molecular weight with narrow molecular weight distribution would be expected to give the greatest entanglement.

In this paper the formation of the gel by a low molecular weight PHB in DMF has been studied by different techniques which provide a preliminary understanding of the molecular processes at the basis of the gelation phenomenon of this polymer.

2. Experimental

2.1. Polymer sample; preparation of the solutions and gels

The sample of PHB was kindly given by I.C.I. and labelled as pure PHB (stock G08). The sample was characterised after purification by following an established protocol used in our laboratory. The sample was dissolved by refluxing in chloroform for 12 h, then filtered and precipitated with acetone. The cold precipitate (stored at 4°C) was dried and stored in the amorphous solid form at −30°C. The solubilising procedure was shown to be mild enough to avoid any degradation of the polymer, while the storage conditions at −30°C were chosen to quench the amorphous morphology of the PHB, which otherwise can easily undergo physical ageing at room temperature. In fact, samples stored at room temperature always proved to be incompletely dissolved in either chloroform or trifluoroethanol by simple stirring at room temperature. The purified polymer had a weight-average molecular weight (M_w) of 3.4×10^5 as determined by light scattering and an intrinsic viscosity [η] of 2.6 dl g^{-1} (in chloroform at 30°C).

Solutions of PHB in the gel-forming solvents were prepared by dissolving the solid polymer by stirring in the appropriate amount of solvent at high temperature (about 100°C). The formation of gel occurred on cooling the solutions (either slow cooling or quenching). The gels were in all cases thermoreversible. The gel obtained from a 1% solution was self-sustaining and, although slightly opalescent, showed a clear point of solubilisation. Clearer gels were observed at lower polymer concentrations or when prepared with the addition of some co-solute. Most of the experiments reported here were carried out on 1% PHB in DMF.

2.2. Calorimetric methods

Preliminary measurements were carried out with conventional DSC instruments (both the DSC-2910 of T.A. Instruments and the Perkin–Elmer DSC7 were used). However, the fact that the transition of the gel in DMF occurred below 100°C enabled us to use the high-sensitive MicroDSC Setaram microcalorimeter. A schematic representation of the instrumental set-up and of the sequence of steps for the processing of the data has been given elsewhere [16]. The two cells (of about 1 ml capacity) were filled with the sample and with the reference solution, respectively, particular care being given to the weight balance of the two cells, in order to have both the heating and

cooling profiles within the assigned range. In many measurements a cyclic heating and cooling programme was fixed with a minimum of two scans, in order to check for the reversibility. The signal was both drawn by a conventional pen-recorder and recorded as a data file through a Burr–Brown interface on a PC. The data points were then analysed with custom-written software.

2.3. Other instrumentation

The kinetics of gel formation at a fixed temperature and gel formation and its dissolution during temperature scanning were studied by several optical methods and by measurement of viscosity. In these experiments a Jasco FP-770 spectrofluorimeter with thermostatted cell were used to obtain the scattered intensity at fixed angle (90°). A Perkin–Elmer 141M polarimeter and a Cary 220 were used for the optical rotation and turbidimetric measurements, respectively, as a function of temperature. An automatic capillary viscometer (Shott–Gerate viscometer, model AVS/T 100) was used to follow the nucleation rate at low polymer concentrations.

3. Results and discussion

3.1. Phenomenological observation of gel formation

Solutions of PHB in DMF (between 0.1 and 5%) were prepared by dispersing the polymer in the solvent at temperatures above 100°C. On cooling at room temperature, gel formation is visually observed as slight turbidity which depends on the polymer concentration and on the temperature of formation of the gel. It is important to note that a decrease in molecular weight was reproducibly found after the heat treatment for dissolution of PHB for 1 h at about 110°C, with a resulting molecular weight of 2.3×10^5 of the solubilised PHB, about 2/3 of the original molecular weight. No explanation could be given for this modest depolymerisation, which occurred also (and more effectively) for gels prepared in DMSO, other than slight hydrolysis caused by the presence of traces of water in the solvents used.

The characterisation of the gel phase was carried out with several experimental approaches aimed at elucidating the kinetics and the thermodynamics of this process. Upon cooling a dilute solution (polymer concentration $< 0.1\%$) to room temperature, major changes of molecular conformation were observed since the optical activity of the solution showed a strong deviation from the initial zero value toward negative values of rotation with a sigmoidal shape (Fig. 1a). This has been taken as an indication of a conformational process accompanying (or being a prerequisite for) the molecular ordering of the chains. A similar phenomenon was observed by Marchessault and coworkers [6] in mixed solvents and interpreted as a coil–helix transition induced by a helicogenic solvent. Formation of supramolecular ordered aggregates as a function of time is clearly shown by the change in the viscosity (increasing by about, or more than, three times) of even more dilute solutions, e.g., at concentrations of 0.025, 0.050 and 0.075% (Fig. 1b). All these observations led us to conclude that in very dilute solution

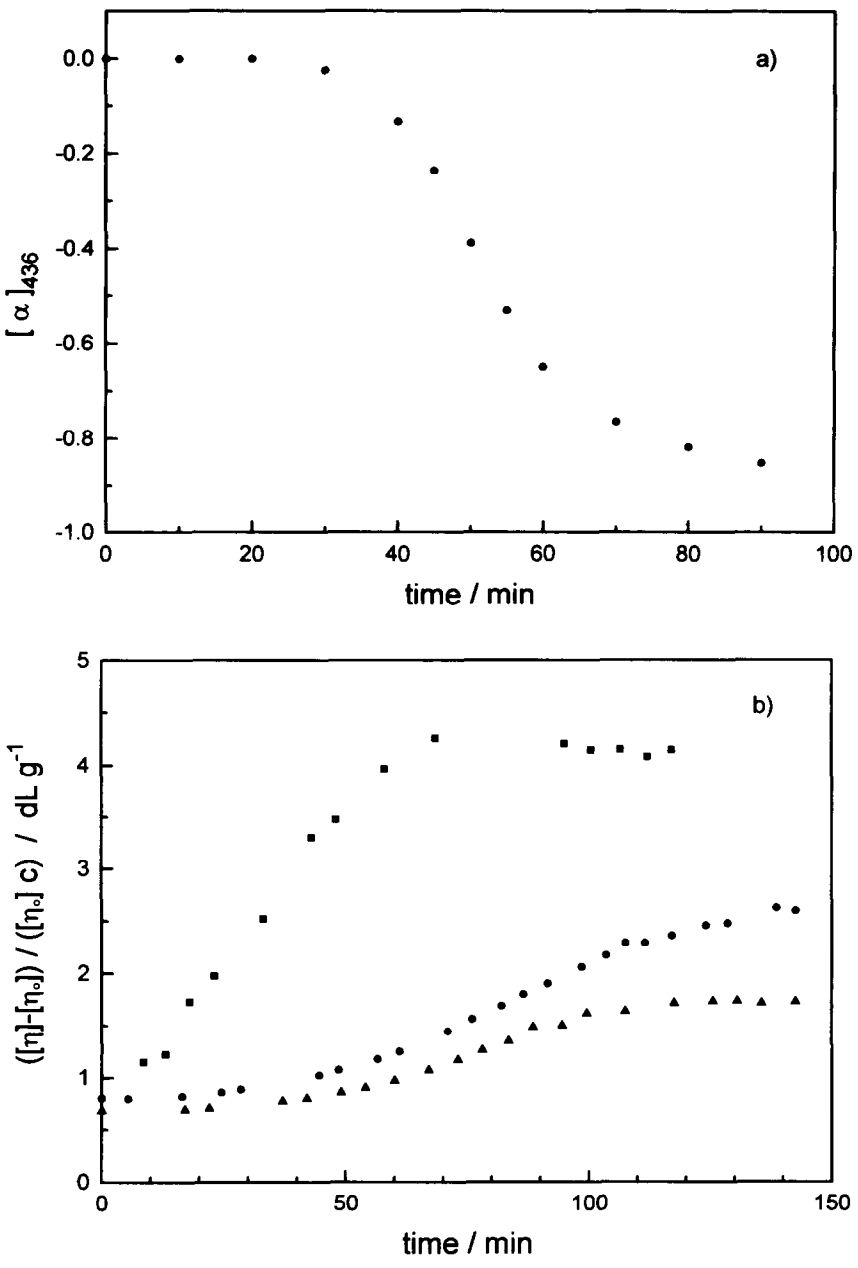

Fig. 1. Time-dependence of dilute solution properties of PHB in DMF quenched at 25°C: (a) specific optical rotation of 0.075% PHB; (b) specific viscosity of 0.025 (▲), 0.050 (●) and 0.075% (■) PHB.

PHB polymer chains form aggregates in which an ordered molecular conformation must be present.

3.2. Kinetics and thermodynamics

Quantitative analysis of the kinetics of formation of a 1% PHB gel has been carried out by measuring the increase of scattered light (fixed angle) at several temperatures (between 25 and 60°C). The values of the scattered intensity obtained at different temperatures as a function of time (logarithm scale) were approximately superposable, upon shift along the x-axis, and the kinetics of gel formation are characterised by a negative temperature coefficient.

These scattering data have been taken as a measure of the fraction of gelling aggregates formed as a function of time. Assuming that the aggregates are crystalline-like, the data can be treated according to the Avrami equation [17], by equating the relative increment of scattered intensity to the crystalline fraction $f_c = 1 - \exp[-k_T t)^n]$, to obtain the values of the exponent n and the kinetic constant k_T. The unambiguous value of 4.0 ± 0.1 has been found for the exponential coefficient of the kinetic law (Fig. 2a). This value is obtained for some kinetic crystallisation processes from dilute solutions as well as from the melt, without any implication of similarity between the morphology of the single crystals (dilute solution) and that of the spherulitic (melt). The parabolic dependence of the $\ln k_T$ curve as a function of temperature (Fig. 2b) is also typical of a crystallisation process and is defined by the limits of the glass transition temperature and the melting temperature [18], which enter in the equation $\ln(k_T) = A - B/(T - T_g)(T_m - T)$. Apparently, only one value of the glass transition temperature at about -120°C was found after quenching solutions (1–20%) from 100 to -150°C. The T_g value corresponds to the glass transition temperature of DMF. The polymer which was always found in its crystalline form (i.e., only a melting endotherm is observed at about 70–80°C, without any inflection of the glass transition nor cold-crystallisation peaks) should have maintained its individual characteristics with the glass transition around 5–10°C. The suggestion of thermodynamic behaviour with a specific mechanism for a small crystallite formation in the gel needs to be verified by other independent observations (e.g., by optical and electron microscopy and by extending the study to other systems).

Characterisation of the gel–sol transition temperature and energetics has been carried out by optical methods (e.g., turbidity) and by calorimetry as a function of temperature. The heat associated with the disruption of the gel has been measured by high-sensitivity differential scanning microcalorimetry. Several scanning cycles were shown to be reproducible, provided that a new gel phase was formed under reproducible cooling conditions after the thermal history of the solution was cancelled. Samples with different thermal history or concentration showed two endothermic peaks of different size but with a total heat constant. The average value of the total heat of transition is of $115\ J\ g^{-1}$ for the 1% gel compared with the value of $146\ J\ g^{-1}$ suggested for the heat of fusion of 100% crystalline PHB with $T_m = 186$°C [19]. The comparison is valid provided that the crystalline structure is the same, as has been confirmed by the diffraction pattern of the PHB gel. Therefore, the value of the heat of transition suggests

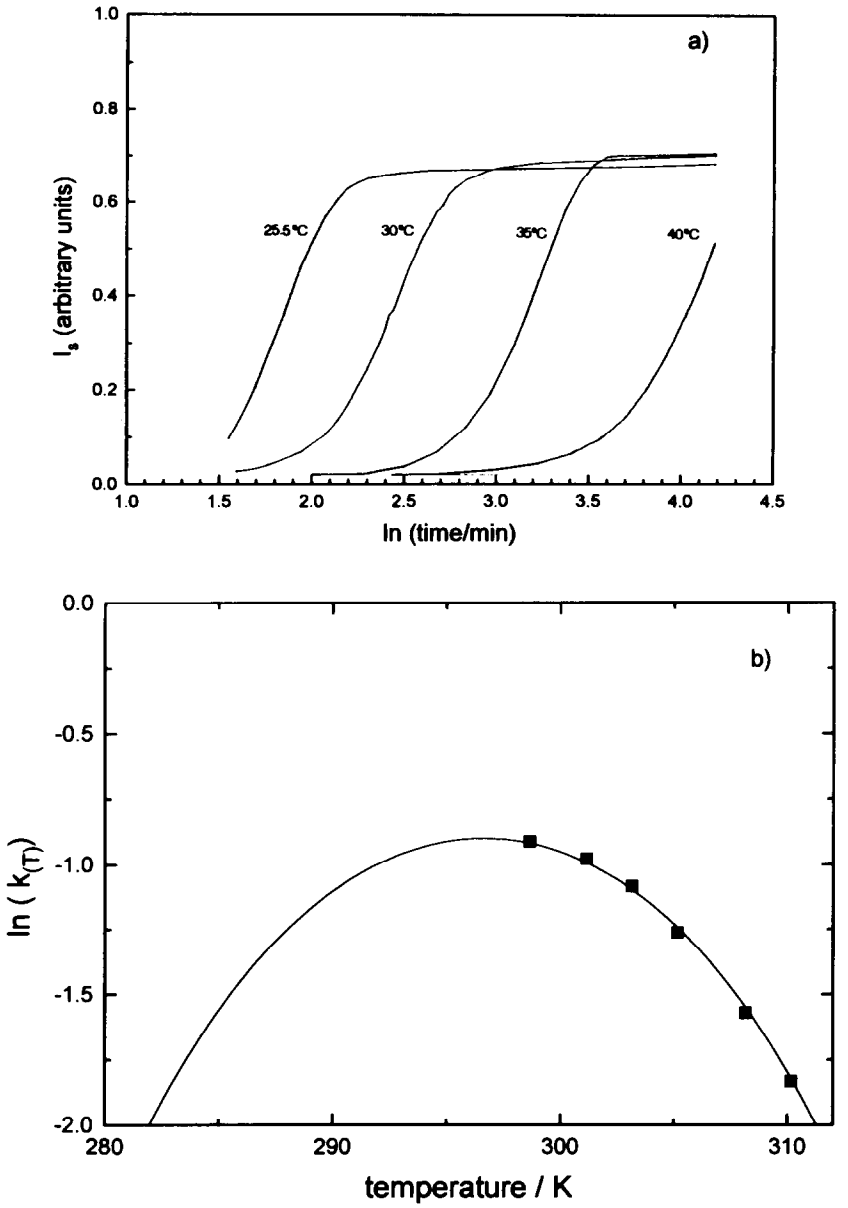

Fig. 2. Kinetics of the gel formation of 1% PHB measured by light scattering: (a) time-dependence of the scattered intensity measured at different temperatures; (b) temperature-dependence of the logarithm of the kinetic constant; the curve is obtained by fitting the equation given in the text [18] with $T_g = -20°C$ and $T_m = 70°C$.

an estimation of the crystallinity of up to 80% in the gel phase, a value similar to that obtained with PHB samples annealed at 170°C [5b] and larger than the value obtained for melt-crystallised and solvent-cast PHB. This high crystallinity value is a direct consequence of the crystallisation process occurring in a diluent, which is still fluid at the temperature of crystallisation. It is also worth noting that the enthalpy of transition decreases by about 40% when PHB concentration increases to 20%. The transition temperatures are also (partly) a function of the concentration and occur systematically in the two ranges of about 69–71 °C and about 82–84 °C.

Fig. 3 shows three different heating thermograms. Most of the thermograms were of the (a) type and were obtained either during the first heating cycle of a freshly prepared PHB gel inDMF or during subsequent heating which followed the cooling cycle in the calorimeter, when cooling conditions were fast enough for our microcalorimetric system (scan rate > 0.1 deg min^{-1}). In these conditions the area of the first peak is approximately two thirds of the total area. When the gel is formed by slow cooling (e.g., at a scan rate lower than 0.05 deg min^{-1}) the second peak decreases (about 5% of the total) or disappears and the melting curve (b) shows only one transition peak at about 72–74°C. A single melting peak (c) is observed at high temperature ($T > 82$°C) only if the gel is formed by maintaining the solution at constant temperature above 50°C. The presence of multiple melting peaks has always been ascribed to a pattern of the size distribution of the crystalline material. Pearce and Marchessault [20] have re-exam-

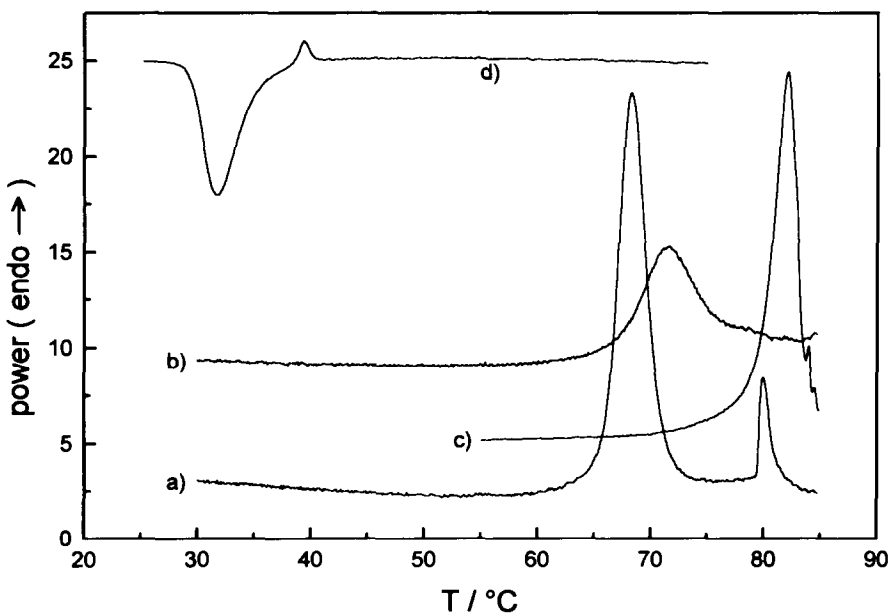

Fig. 3. Melting endotherms showing the binodal behaviour for gels obtained: (a) by isothermal crystallisation of 1% solution at $T = 30$°C; (b) by non-isothermal crystallisation (scan rate < 0.05 deg min^{-1}); (c) by isothermal crystallisation of 1% solution at $T = 60$°C; d) crystallisation exothermic peak (scan rate 0.5 deg min^{-1}).

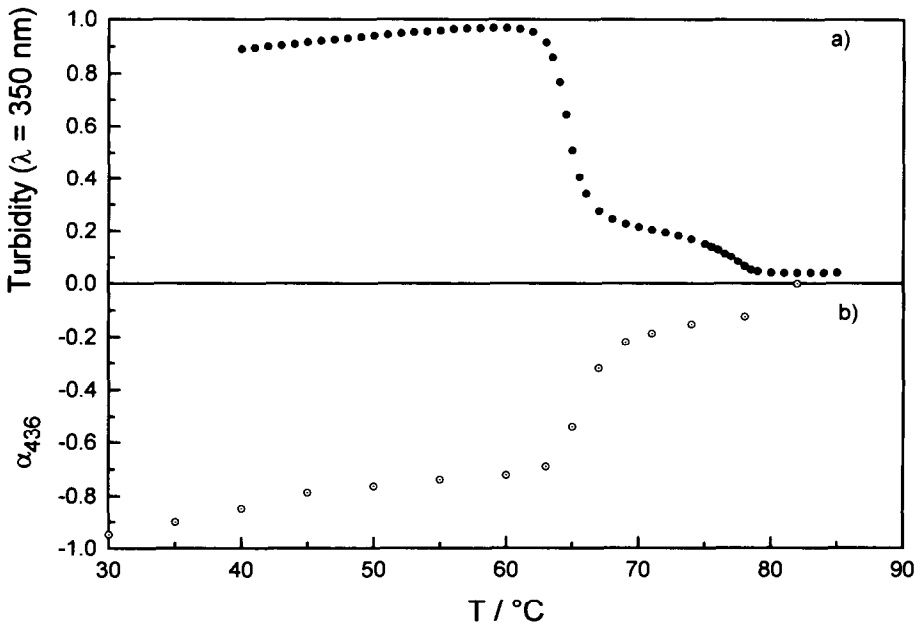

Fig. 4. Binodal "melting" transition shown by the change of turbidity (a) and by the change of optical rotation (b) of a 0.075% PHB gel prepared by quenching the gel at 30°C.

ined this phenomenon on the basis of their results on blends of isotactic and atactic poly(β-hydroxybutyrate). They suggest that the overall melt behaviour be rationalised in terms of the co-existence of annealing of "as-formed crystals" and re-crystallisation of the polymer. At their low scan rate (5 deg min^{-1}) both the melting of the pure PHB and of that in the blend seem to converge to the higher melting temperature. Furthermore, the solid formed at the low crystallisation temperature shows the higher fraction melting at the high temperature. In our results, on the other hand, the decrease in the heating rate does not affect the binodal melting nor is the peak at the higher temperature increased. The presence of a binodal shape is also supported by independent "static" results such as the temperature-dependence of both the turbidimetry at two wavelengths and the optical rotation (Fig. 4). Moreover, in our results the cooling rate was found to be determinant for the evolution of the two melting endotherms, the higher temperature peak being single only when the crystallisation temperature was above 50°C. Notwithstanding the difference in the scanning rate (from 5 to 120 deg min^{-1} in one case [20] and from 0.026 to 0.5 deg min^{-1} in the present study), an explanation for the above reported result can only imply a difference in the morphology of the crystalline material involved. In our case, given the small size of the crystallites and the high crystalline content in the PHB gel, the binodal fusion seems rather to be due to "fringed micelles" interspersed in the "lamella-like" crystallites. A morphological analysis is, however, necessary to validate this kind of gel structure.

For the non-isothermal crystallisation process the temperature of the crystallisation peak in the cooling curve also depends on the scan rate and ranges from 45°C at a scan

rate of 0.026 deg min^{-1} to 36°C at 0.5 deg min^{-1}. Furthermore, although for subsequent heating cycles the enthalpy of melting is constant, the "heat of formation" is always smaller than the "heat of melting". This apparent contradiction could be due either to a large temperature coefficient of the heat of transition (not confirmed by the difference in the heat capacity) or more reasonably to some of the heat of formation being recovered slowly after the appearance of the crystallisation peak. In addition, the cooling exotherms (scan rate > 0.05 deg min^{-1}) are characterised by a sharp but small endotherm in the initial region of the crystallisation (Fig. 3, curve d). Although not very commonly reported, this phenomenon must rely on the relaxation process of the undercooled polymer.

Finally, the formation of a gel of PHB gives a clear working hypothesis for the early findings of "apparent" conformational changes in mixed solvents. In dilute solution gel formation occurs with changes of physico-chemical properties (e.g., optical, chiroptical and viscometric properties) usually ascribed to solution disorder–order conformational changes. Conformational statistics carried out on the PHB chain with the methods of molecular mechanics and using Monte Carlo methods [21] give a reasonable interpretation for the solid state helical conformation, but also suggest an intrinsic tendency of the polymer to adopt some preferential local conformations [22] which may resemble the most stable helical one. It has already been pointed out that solvent interactions affect local conformation and may also change the polymer unperturbed dimensions [23]. The rather extended random conformation deduced from the chain dimensions measured by light scattering and viscosity [7] can therefore be ascribed to local stiffness which depends on the solvation properties of the solvent and promotes the conditions for chain aggregation (or crystallisation).

4. Conclusion

The preliminary results here reported not only show the highly structured gel phase of PHB in DMF, but also point out the conformational prerequisite for the "nano-crystallisation" of the polymer. Several methodologies have been used to characterise the kinetics of gel formation and the melting of the ordered structures in the "dilute conditions", which have not been explored at length in other similar studies. Research on the non-isothermal crystallisation and morphological studies on the gel phase are in progress.

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