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A modulated DSC analysis of microbial biocompatible polyesters [1]

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Abstract

A preliminary study of the calorimetric response of highly crystalline samples of microbial poly(hydroxybutyrate) (PHB) has been carried out using a new temperature-modulated differential scanning calorimetry (MDSC^{TM}). This instrument extends thermal analysis to a differentiation between reversible and non-reversible processes. The thermograms clearly show the reversible glass transition of PHB, even for unquenched samples. The presence of non-reversible cold crystallization, furthermore, enhances the other recrystallization phenomenon which is peculiar to the rearrangement of the crystalline regions during the melting process. It is anticipated that a thorough theoretical analysis of the thermal response using this approach will lead to new understanding of phase transfers during kinetic processes.

INTRODUCTION

Naturally occurring, biodegradable polymers that are present in the environment have been known for many years. However, only more recently have several articles appeared in the literature [1–5] on the properties of some of these polyesters, often referred to as bacterial thermoplastics. It is now appreciated that all these polyesters produced by microorganisms are aliphatic polyhydroxyalkanoates with the same basic type of unit. Interest raised by these polyesters has promoted research in several fields, from genetic engineering to applied polymer research. Because general knowledge on this subject is rapidly expanding, the authors of this article felt it necessary to produce this research note on the calorimetric properties of these polyesters, measured by innovative methods.

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Polyhydroxyalkanoates (PHA) and, in particular, the simplest homologue polyhydroxybutyrate (PHB), are a storage material and are produced by microorganisms that can depolymerize and recycle biologically the polymeric mass accumulated. In the absence of the microorganisms which can depolymerize it, PHB is considered as a stable, inert biomaterial. With respect to the biodegradability of this material in the environment, assessment is very difficult because a quantitative definition of all the external conditions is required, and the polymer characteristics and morphology [6] need to be known. Among the latter aspects, the molecular weight, degree of crystallinity, orientation degree and the surface morphology are by far the most important. All these variables (including the surface morphology) may change during the degradation, thereby making it difficult to classify quantitative standards and define degradative tests.

Many of the problems raised above may first be addressed in the thermoanalytical analysis of these semi-crystalline polymers, a technique which is routinely used in the field of polymer science.

The crystalline structure of PHB, previously determined by X-ray diffraction spectra from oriented fibres, has been refined through powder diffraction spectra of a highly crystalline sample [7]. PHB samples usually have a degree of crystallinity of about 60% and crystallize from the melt, thereby forming spherulites of large dimensions. With regard to the crystalline structure of HB–HV copolymers, the most important aspect is the high degree of crystallinity reached in all compositions, a characteristic of isodimorphic materials [8]. However, the most important transitions for the definition of thermal and mechanical properties of partially crystalline polymers are the glass-to-rubber transition of the amorphous phase and the melting of the crystalline phase, which occurs at about 5°C and 180°C, respectively [9–11]. As far as mechanical properties are concerned, it has recently been shown [12] that, given the value of the glass transition temperature, PHB undergoes physical ageing during storage at room temperature, which results in a change of mechanical properties with time.

The overall picture given by the studies on PHA is that of a completely new, widely expanding field of novel biomaterials of great potential. The fact that these polymers are thermoplastic materials and therefore can be filmed, moulded or spun by means of common melt-processing machinery has always been considered as extremely favourable for their application.

EXPERIMENTAL

Polymer samples

All PHB samples are produced from a variety of microorganisms with the same chemical structure, but often with different molecular weights.

Among microorganisms able to produce PHB, the best known is *Alcaligenes eutrophus,* used industrially by I.C.I. Ltd. in the production of PHB and butyric–valeric copolyesters marketed under the trade name Biopol. The samples of Biopol (the homopolymeric PHB, G08, and the copolymer of hydroxybutyric acid and hydroxyvaleric acid, P029) used in the present work were kindly supplied by Dr. J.F. Stageman (I.C.I., Biopolymer Division). The molar percentage of HV in the co(HB, HV) was 18%, while the molecular weight M_w of the two samples was about 5×10^5. The other PHB sample with a M_w of about 1.8×10^6 was produced by a different microrganism and was obtained through the Environmental Polytech Consortium (CO.E.PO.). It must be mentioned here that PHB production has been found in many microorganisms, some of which have already been explored by us. The main interest in these new microorganisms lies in the possibility of obtaining cellular machines that can transform cheap substrates into substantial amounts of polyesters and copolyesters of predetermined structure and composition.

Methods

The schematic operation of the temperature-modulated DSC (MDSC™, TA Instruments Inc.) is that of a typical heat flux DSC [13]. The sample and the reference pans are located on platforms formed in a thermoelectric constantan disc, which serves as the primary vector for the heat transfer. Conventionally, the temperature of the heating block surrounding the disc is programmed to change (increase or decrease) steadily in a linear fashion in time. The differential heat flow between the sample and the reference is monitored through thermocouples fixed under the disc platforms. The MDSC operates essentially in the same way, but with a remarkable option: in addition to the linear gradient of temperature applied to the heating block (defined by the scanning rate $\mathrm{d}T/\mathrm{d}t$), a sinusoidal ripple (modulation) is superimposed resulting in a heating profile which is therefore characterized by an oscillatory increase with a defined amplitude and frequency. Figure 1 shows the heating profile obtained by using a scan rate of $4\,\mathrm{K\,min^{-1}}$ and a modulation of 0.5 K over a period of 40 s.

Pulse methods are not a novelty in thermal analysis [14]; however the present apparatus is the first commercial DSC instrumentation equipped with the option of a sinusoidal modulation. The general equation describing the calorimetric response should be examined in order to appreciate the impact these frequency perturbation variables can have on the heat flow results $\mathrm{d}Q/\mathrm{d}t$

$$\mathrm{d}Q/\mathrm{d}t = C_p\,\mathrm{d}T/\mathrm{d}t + f(t, T)$$

where C_p is the heat capacity, $\mathrm{d}T/\mathrm{d}t$ the scan rate and $f(t, T)$ is a function

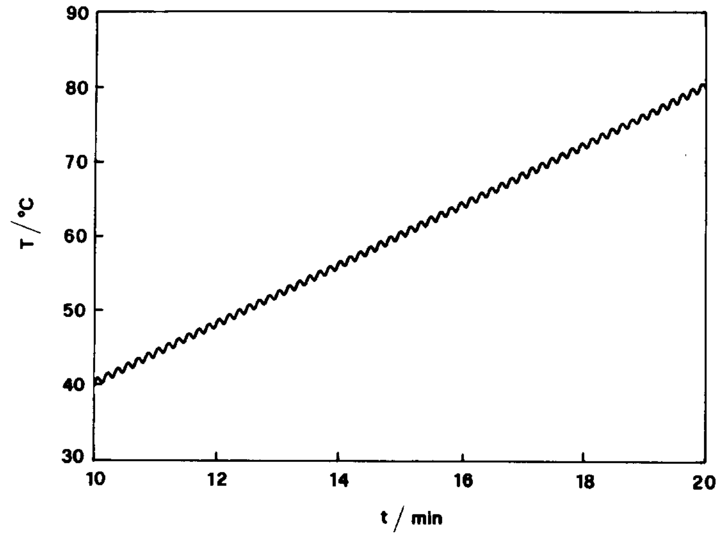

Fig. 1. Example of the temperature profile obtained by using a scan rate of 4 K min^{-1} and a superimposed modulation of 0.5 K with a period of 40 s.

of time and temperature related to the kinetic response of any transformation.

The actual thermal response can in principle be deconvoluted by using different mathematical algorithms; it still has to be fully analysed, with respect to both its practical use and its theoretical potential. However, it is more important at this stage to verify phenomenologically the instrumental results, assuming that, under some experimental conditions, the temperature excursions are small enough to allow the complete separation of the heat transfer associated with reversible processes which follow the temperature excursion, from those in which a kinetically controlled (irreversible) process is measured.

These conditions lead to the approximate equation

$$dQ/dt = C_p\, dT/dt + f'(t, T) + C \sin(\omega t)$$

where $dT/dt = a + A\omega \cos(\omega t)$, having imposed the condition $T = T_o + at + A \sin(\omega t)$.

Analysis of this equation shows that the total heat flow is the sum of two components: one component is heating-rate dependent and the other depends exclusively on absolute temperature [15]. In other words, there is one component which directly follows the modulated heating rate, and, therefore, is in-phase with the scan-rate signal, while the other is out-of-phase. The deconvolution of the raw signal is performed in real time by discrete FT, using software implemented in the TA Instruments DSC 2910 module.

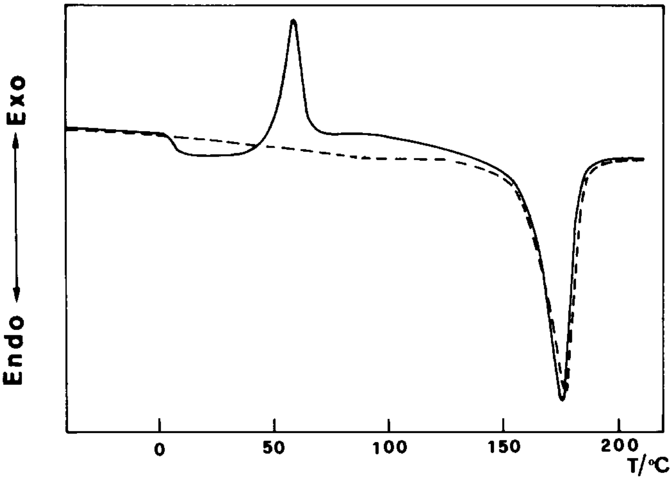

Fig. 2. Conventional calorimetric curve showing the first run (broken line) of a room-stored sample of PHB and the run of the same sample quenched from the melt (full line).

RESULTS AND DISCUSSION

The large endothermic peak in the calorimetric curve illustrated in Fig. 2 at around 175°C is peculiar to the high crystallization tendency exhibited by PHB samples of high molecular weight. The thermogram of a room-stored PHB sample (as shown by the dotted line in Fig. 2) does not present other relevant phenomena, only a slight baseline drift. Two other thermal responses are detected for samples quenched from the melt: the well-defined heat capacity jump at temperatures slightly above 0°C and the subsequent exothermic peak due to the cold crystallization upon heating at temperatures ranging between the glass transition and the fusion. Furthermore, it has been noticed that room-stored samples of PHB are subject to physical ageing, a phenomenon which increases the compactness of the polymer material by modifying the local organization of the sparse amorphous phase. In addition, keeping the material at a temperature which is slightly lower than fusion, i.e. annealing, gives not only a higher degree of crystallinity, but above all increases the size of the existing crystallites.

The calorimetric characterization of some PHB samples was carried out after the position of the glass transition and melting temperatures had been confirmed. All our previous calorimetric experiments, as well as those from other laboratories, were carried out with conventional calorimetric apparatus (Fig. 2). Figure 3 shows a comparison of the calorimetric curves of a commercial PHB sample, unquenched, obtained with the temperature-modulated MDSC. The most striking difference is that while both the raw data and the conventional data (Fig. 2) do not show any evidence of a glass transition, the deconvoluted MDSC data of the reversible component does

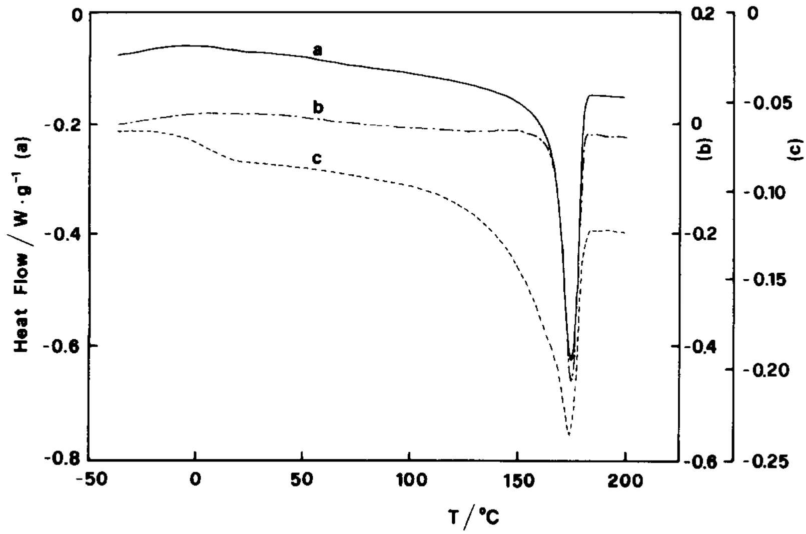

Fig. 3. Calorimetric curves of the unquenched sample of PHB-ICI deconvoluted from the signal recorded with the MDSC: a, total curve; b, non-reversible out-of-phase component; c, reversible in-phase component.

indeed present the heat capacity step, with $T = 5°C$ as mid-point. The other relevant observation, to which we will return later, is that the heats of fusion calculated for the different deconvolutions are 94, 38 and 56 J g^{-1}, for the total, the reversible and the irreversible processes, respectively. Bearing in mind the complex thermodynamics underlying the melting process of a polymeric material, this partition of the enthalpic state function has to be related to both the thermodynamic stability of size-dispersed crystals and the kinetic process which, with temperature, induces the small-size crystals to melt and then to recrystallize in a form that must be stable at that temperature. It is easy to envisage that some large fraction of this crystallizable material is more involved in the growth process of existing crystals than in the formation of new crystals. In that sense, the melting process is followed by a crystallization process which can be classified as irreversible, in as much as the chains make up part of the crystalline portions that can be melted only at a higher temperature.

Confirmation of this hypothesis is only partially shown by the small exothermic curvature appearing in the non-reversible component of Fig. 3. On this basis, it appears merely speculative. However, the thermograms for the quenched sample of PHB (Fig. 4) give more proof of the exothermic melting preceding the fusion in the non-reversible component. The presence of exothermic peaks has been previously reported for different polymers, the novelty of the present results being that the peak is ascribable to a non-reversible process only.

The information contained in the low-temperature region of the

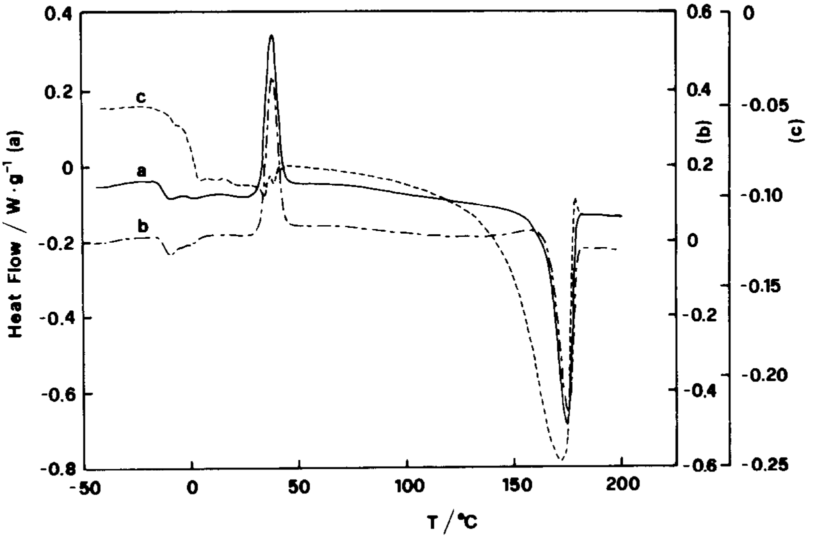

Fig. 4. Calorimetric curves of the quenched sample of PHB-ICI deconvoluted from the signal recorded with the MDSC: a, total curve; b, non-reversible out-of-phase component; c, reversible in-phase component.

thermograms is more as expected. The glass transition appears to be almost confined in the reversible component, while a non-reversible enthalpic relaxation is also disclosed. At the same time the 'cold crystallization' occurring at temperatures above the glass transition, is completely confined to the non-reversible component. A step in the value of C_p is seen in the reversible component, which can be ascribed to a true change of C_p due to the crystallization, while the ripple could be a consequence of instrumental instability in the presence of too rapid a release of heat on crystallization. Evidence for a small jump in the heat capacity profile of the reversible deconvolution of the unquenched sample is relevant to the glass transition (Fig. 3); this signal could not be reproduced with similar conventional techniques.

Another PHB sample, of different origin, has been tested to confirm these results (Figs. 5 and 6). In fact, all the features observed with the commercial PHB sample are reproduced, with the further enhancement of the peculiar melting profiles. The unquenched sample shows the small, but evident, exothermic shoulder preceding the true melting. The exothermic peak is very pronounced in the thermogram of the quenched sample, where a large amount of substance has been allowed to undergo a rapid cold crystallization, without further assessment of the crystalline phase. This PHB sample is characterized by a much higher molecular weight, which is very important for both the crystallinity reached by the sample and for the higher stability, as revealed by the higher value of the degradation temperature [11].

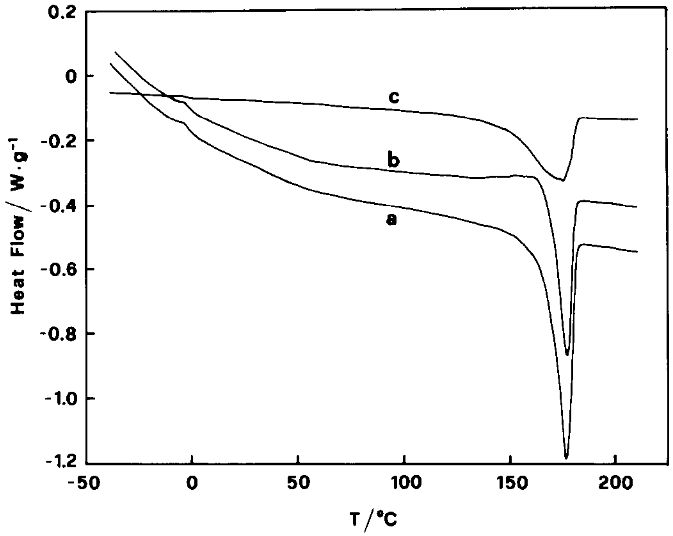

Fig. 5. Calorimetric curves of the unquenched sample of PHB-DNT deconvoluted from the signal recorded with the MDSC: a, total curve; b, non-reversible out-of-phase component; c, reversible in-phase component.

As a more general comment on the differences between the quenched and unquenched samples, it may be worth observing the fraction of material which undergoes a reversible melting with respect to the global process. The possibility of calculating the two quantities is of primary importance for testing the thermodynamic stability of the crystalline phases. This can be done by assuming that the heat associated with each component is a true measure of the mass of melted polymer. If this is correct, then the fraction of material involved in the reversible melting increases on passing from the unquenched to the quenched sample. We will not discuss the analysis of this behaviour nor the absolute values of the melting enthalpy as they really depend on the thermal history of the sample and also on the actual temperature gradient.

However, the overimposed modulation of the experimental setup reported here may introduce a perturbation on the physics of the polymeric system which merits a more detailed theoretical analysis. In order to clarify this point, some other tests must be carried out for a better understanding of the phenomena, before attempting any future quantitative work. A preliminary experiment has been carried out by changing the scan rate to $dT/dt = 1\ \mathrm{K\,min^{-1}}$, a value which barely yields a significant signal for the glass transition with many instrumental setups. It is relevant that MDSC tuning of the reversible and the non-reversible thermal response seems to remove all the random thermal perturbations from the recorder signal of the reversible process. However, it should also be mentioned that when

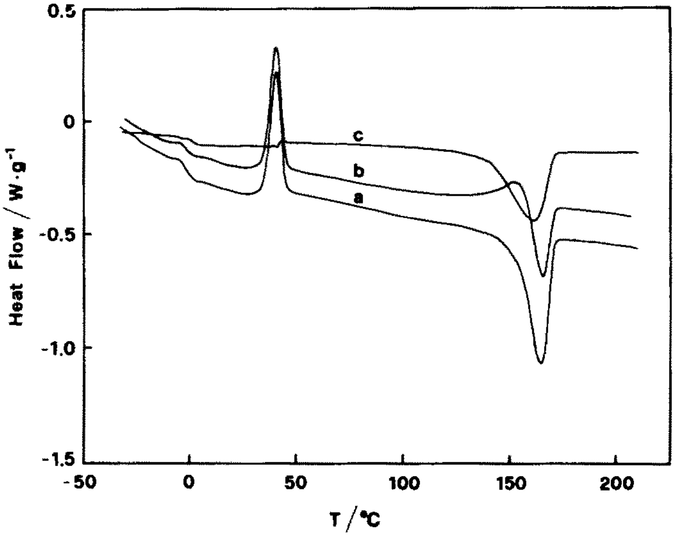

Fig. 6. Calorimetric curves of the quenched sample of PHB-DNT deconvoluted from the signal recorded with the MDSC: a, total curve; b, non-reversible out-of-phase component; c, reversible in-phase component.

large enthalpic effects are present due to sample phase transition, they may introduce a severe perturbation feedback on the modulation itself, affecting the modulated impulse and its subsequent analysis at the origin. This latter problem can also be overcome by different experimental procedures, the most simple being to decrease the scan rate.

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