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Biorelevant characterization of biopolymers

Michael Jaffe*, Zohar Ophir, Vaishali Pai

Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ 07103, USA

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Abstract

Polymeric biomaterials are synthetically derived or modified biological polymers designed for in vivo use or for use in the proximity of biological fluids. As is common in the polymer industry, these polymers are produced in bulk and then shaped for a specific end-use. Characterization of biopolymers has two purposes:

1. development of parameters for processing;
2. determination of end-use performance characteristics.

In biorelevant testing of polymers, the temperature of interest is limited to $37 \pm 3\,^{\circ}C$, and it is the time effects in aqueous environment containing biological molecules that are critical. Parameters that may serve to accelerate biopolymer response include sample surface area and the concentration of molecules present in the test environment. The development of biorelevant TA databases allows the rational correlation of biorelevant and conventional TA responses and offers an avenue for the development of accelerated aging evaluation procedures.

Polymeric biomaterials, especially new compositions, are often tested for biocompatibility, bioerosion rate or cell growth specificity without regard to the morphological structures introduced during materials processing. It will be shown that in addition to the potential influence on biological response, processing induced structure profoundly influences materials properties under biorelevant conditions. Using a combinatorial library of polymeric, bioerodable compositions, these issues will be explored with emphasis on in vivo dimensional stability and mechanical property retention.

Keywords: Biopolymers; Biorelevant analysis; Thermal analysis; Condis crystals; Mesophases; Dimensional stability

1. Introduction

Polymeric biomaterials are synthetically derived or modified polymers designed for in vivo use or for use in the proximity of biological fluids. As is common in the polymer industry, these polymers are produced in bulk and then shaped for a specific end-use. Characterization of biopolymers has two purposes:

1. development of parameters for processing;
2. determination of end-use performance characteristics.

Thermal analysis (TA) methods of polymer characterization relevant to processing are well established and require an understanding of thermal and chemical stability, phase transition temperatures and kinetics, rheology (melt or solution as appropriate) and molecular relaxation times. It is also well established that the performance characteristics of a processed polymer are as much dependent on morphology and molecular chain orientation as on backbone chemistry (see for example, the recently published book of Jaffe and

* Corresponding author.
E-mail address: jaffe@adm.njit.edu (M. Jaffe).

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Bessey [1], on orientation effects in polymers). Understanding the range of performance available through a given chemistry requires an understanding of the process–structure–property relationships of the polymer. The differences between polyethylene fiber used for ballistic protection and polyethylene film used in trash bags are mostly a function of process history.

Over the past decades, thermal analysis has emerged as the most commonly used technique for the characterization of polymers. Turi [2], has summarized polymer thermal analysis in two volumes. In chapters on the thermal analysis of fibers and the thermal analysis of films, Jaffe et al. [3] stress the importance of process history on understanding the performance of shaped polymers. He further points out that while differences in TA spectra produced in a consistent manner can be utilized to "fingerprint" a product, without an understanding of process history and use environment, the origins and value of such observed differences cannot be identified. The Turi book [2] documents the TA of biopolymers; for example, Jaffe summarizes the conventional TA of lactide polymers designed for suture applications. TA characterization of biopolymers under biorelevant conditions has not been extensively investigated. A revisiting of the literature of the past 10 years confirms that biorelevant TA techniques have been utilized in some instances (see for example, the recent work of Kajiwara and Franks [4]) but that systematic development of techniques and databases are lacking. A useful summary of the applications of TA to Biology, medicine and pharmaceuticals can be found in the biyearly reviews of Dollimore [5].

Conventional thermal analysis involves the monitoring of a material characteristic of interest as a function of temperature. In biorelevant testing of polymers the temperature of interest is limited to $37 \pm 3\,^{\circ}C$, and it is the time effects in aqueous environment containing biological molecules that are critical. While the interface chemistry between biological systems and synthetic materials at the surface is of paramount importance in determining the nature of subsequent cell growth, bulk properties and bulk property stability determine the efficacy of an in vivo device over its lifetime. Bulk properties of interest include shrinkage, mechanical properties in tension, compression and shear, creep, fatigue, environmental stability of both chemistry and microstructure, and transport phenomena of small molecules. Except for expensive and complex in vitro or in vivo conditioning followed by conventional testing methods, test methodologies for assessing such performance are largely undeveloped. Of special interest would be the ability to predict the effects of long-term in vivo aging phenomena in a reliable and accelerated manner. Utilization of TA platforms to develop data relevant to performance under the above boundary conditions defines biorelevant analysis (BA).

Selected variants of the bioerodable, desaminotyrosyl-based polyarylate combinatorial library, synthesized by Kohn and coworkers [6–8] of the Chemistry Department of Rutgers University are the model materials chosen for this study. The purpose of this work is to:

- assess the relationship of processing history to polymer structure and properties;
- develop characterization protocols relevant to in vivo use;
- produce samples of known supramolecular structure for investigation of biological response to polymer molecular orientation and phase state.

2. Experimental

Fig. 1 illustrates the chemical structure of the Kohn polyarylate library, where *Y* represents the number of methylene groups of the alkyl side chain and *R* represents the number of methylene units of the aliphatic diacid. Note that these polymers may be described as alternating poly(ester amides), with the relative amide content per mole/monomer decreasing as either *R* or *Y* increases. For simplicity, polymer compositions are referred to as poly(*Y*,*R*). Polymerization details have been described elsewhere [6]. Selected compositions were chosen for analysis, based on earlier observations of unacceptably large, in vivo shrinkage (poly(2,2), poly(2,4), poly(2,12) and unexpected preservation of process induced molecular orientation of poly(8,8), and poly(12,10).

Samples were received as powders of specified molecular weight in quantities between 20 and 500 g. Samples were characterized for moisture content and thermal stability by TGA (TA Instruments Q50 TGA, run at $10\,^{\circ}C$/min under flowing N_2 from room temperature to $250\,^{\circ}C$) and phase transition temperatures by

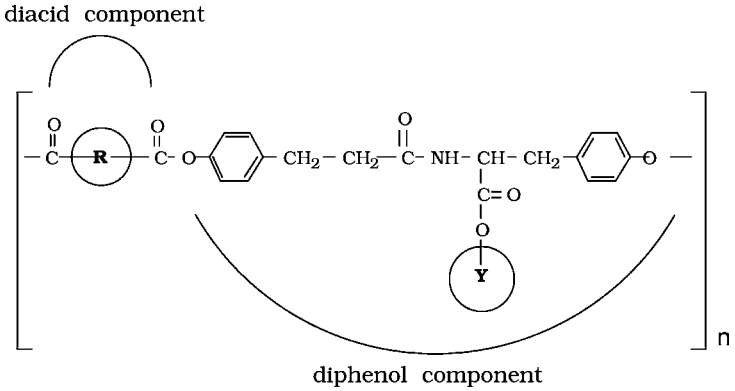

Fig. 1. Desaminotyrosyl polymers.

DSC (TA Instruments Q100 DSC, run at 20 °C/min under flowing N_2 from −50 to about 25 °C above highest transition temperature, cooled at 20 °C/min to −50 °C and reheated through the highest temperature transition).

Fiber was spun with a James plunger fed micromelt spinner, fitted with a single hole 750 μm diameter spinnerette. Films were compression molded at 175 °C in a Carver press and hand drawn to simulate fiber processing.

Dynamic mechanical properties of fiber and film samples were monitored with a Rheometrics RSAII fitted with fiber jaws and run under flowing N_2 at 10 °C/min. Fiber shrinkage was measured dry in a TA Instruments 2940 TMA fitted with fiber jaws, run at 10 °C/min. Fiber load was adjusted to be as close to zero as possible. Overall, the methods employed for the thermal analysis of the fibers, i.e. TGA, DSC, TMA and DMA, follow the techniques described by Jaffe et al. [3]. Wet shrinkage was monitored by measuring the length change of dried fiber (24 h, room temperature under vacuum) attached to small fishing floats and placed in a water bath at 37 °C for 24 h. Starting fiber lengths were about 10 cm and length changes were monitored with a ruler. This same bath was utilized to condition samples for water uptake, with 24 h being the typical conditioning time.

Wide angle X-ray scattering (WAXS) patterns were obtained using a Siemens Hi-Star X-ray area detector with Cu Kα radiation of wavelength 1.54 Å. The sample to detector distance was 60 mm.

3. Results and discussion

3.1. T_g and dimensional stability

Fig. 2 shows the T_g of a water conditioned poly(2,2) fiber as monitored by DSC (first heating) and of the same sample after drying in the DSC instrument by heating to 125 °C and cooling to −50 °C before reheating. The T_g of the dried sample is about 85 °C (start ∼70 °C) while the wet sample shows a T_g of about 50 °C with the T_g process starting below 40 °C. Note that there is no evidence of higher temperature transitions through 165 °C. Fig. 3 shows the water uptake of poly(2,2) powder after storage in 37 °C water (3.1%) and room temperature air for 24 h (1.3%), as measured by TGA. Fig. 4 shows the start of the T_g process, as monitored by DSC, as a function of water content for poly(2,2). At about 3.2% water, the temperature of initiation of the T_g process is reduced to 37 °C. Fig. 5 shows the shrinkage of poly(2,2) fibers measured after treatment in water at 37 °C, as a function of time. After about an hour and a half induction time the fiber begins to shrink, with the shrinkage reaching a value of about 35% in less than 1 day. This level of shrinkage is unacceptable for most in vivo applications. Fig. 6 shows the effect of drawdown during spinning on the maximum wet fiber shrinkage measured, indicating that the increasing molecular orientation imparted in spinning leads to increased shrinkage in the resulting as-spun fiber. Fig. 7 shows the shrinkage of a dry fiber (same process history as the fiber shown in Fig. 5), indicating

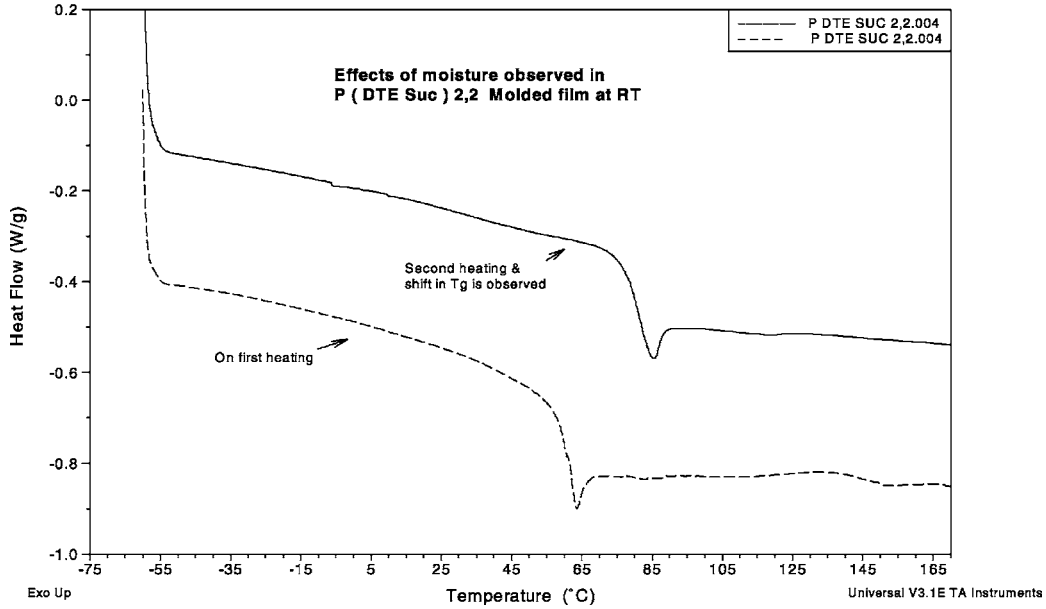

Fig. 2. Water plasticization of poly(2,2) T_g DSC.

the start of dry fiber shrinkage at 85 °C, and increasing to a maximum of 28% during the experiment, agreeing reasonably with the 37 °C wet value of 35%. At this water content, reached under in vivo conditions with poly(2,2) in less than 1 day, the fiber will be above its T_g and entropic shrinkage will occur. Fig. 8 shows the change in real modulus and tan δ as a function of water content for poly(2,2) fibers. For the water saturated

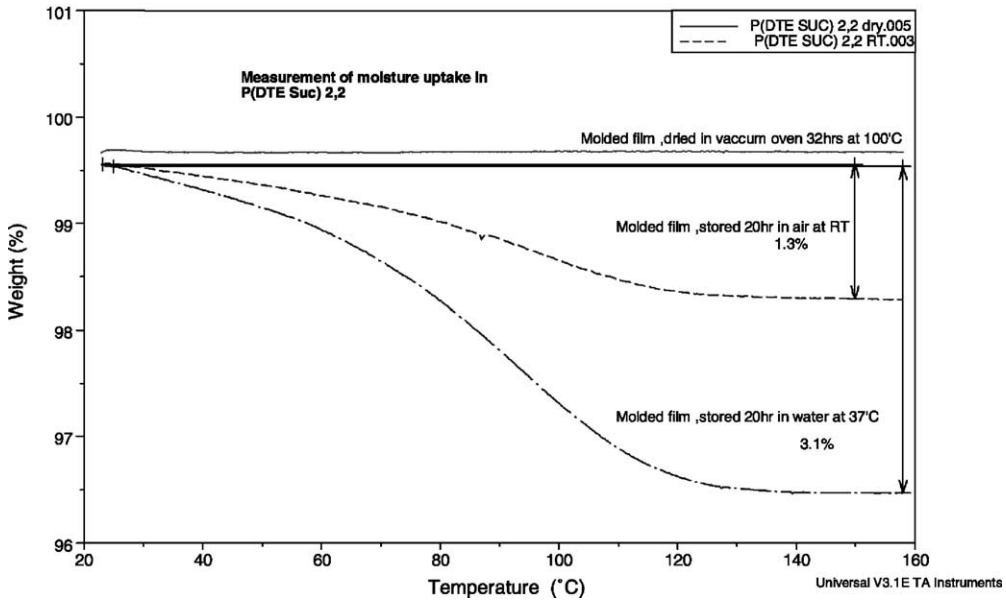

Fig. 3. Water uptake of poly(2,2) TGA.

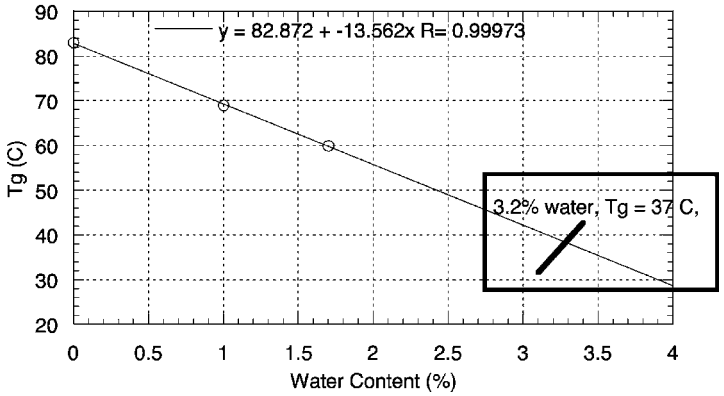

Fig. 4. Onset of T_g of poly(2,2) as *f*(water).

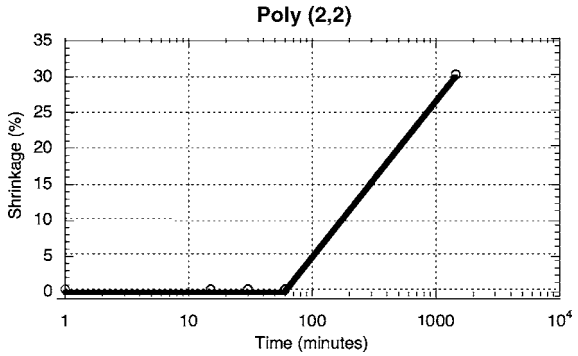

Fig. 5. 37 °C water shrinkage as a function of time.

sample, it is observed that both the drop-off in modulus and tan δ occur at significantly lower temperatures than the dry samples, confirming the plasticization effect of water on the poly(2,2) chemistry and indicating that both dimensional stability and mechanical performance are reduced under biorelevant conditions.

Water plasticizes the poly(ester amides) of the polyarylate combinatorial library, with the effect maximizing as the amide content per mole of monomer of the composition increases. The plasticization of water on aliphatic polyamides is well known [9]. Clearly, as the dry T_g of the library polymers drop with increasing backbone and side chain flexibility [6] the absolute value of the plasticization effect to bring T_g below 37 °C is also reduced. The rapid 37 °C wet shrinkage

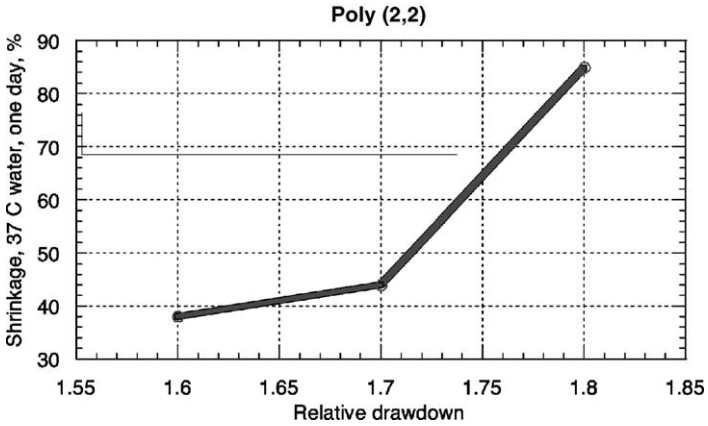

Fig. 6. One day 37 °C wet shrinkage as a function of drawdown.

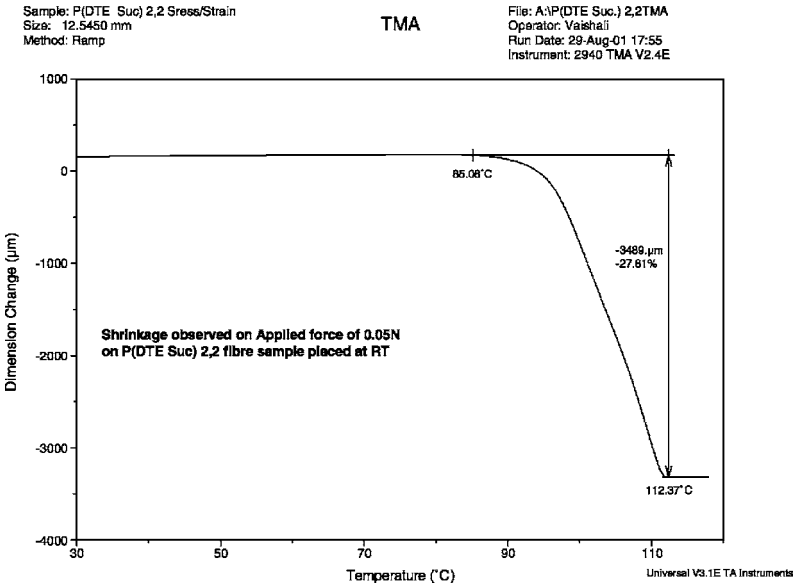

Fig. 7. 2,2 Fiber-TMA shrinkage.

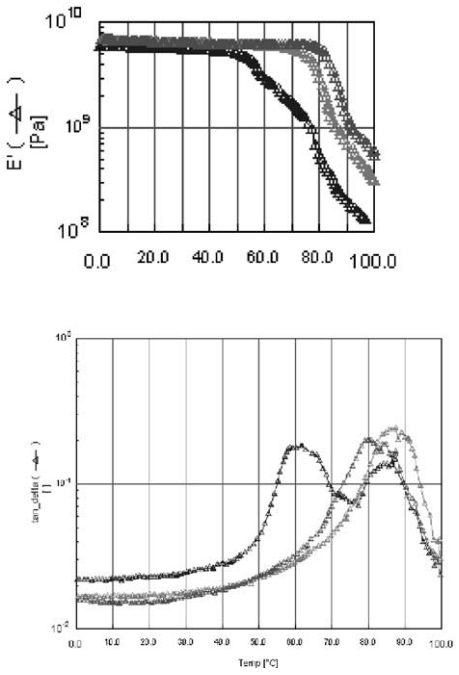

Fig. 8. Dynamic mechanical analysis as a function of water content: (1) 3.1% water; (2) 1.2% water; (3) dry.

of poly(2,4) and poly(2,10) fibers, shown in Fig. 9, illustrates the need to examine water plasticization effects across the polyarylate library. Application of known fiber processing technology to these fibers can mitigate poor dimensional stability [2], for example, annealing oriented poly(2,2) fibers at 125 °C at constant length or with relaxation up to 10% can reduce shrinkage at T_g to an acceptable less than 10% with minimal impact on mechanical properties. The polyarylate compositions with side chains and aliphatic diacids of greater than six methylene units show low moisture regain (>1%) and observed plasticization effects are minimal.

4. Long range order

The two-dimensional wide angle X-ray scattering patterns of oriented poly(2,4) and poly(12,10) fibers is shown in Fig. 10. The poly(12,10) fiber scattering pattern has two symmetrical equatorial arcs suggesting a spacing about 4 Å, and a pair of intense meridional streaks corresponding to a spacing of 29 Å, which is the length of the poly(12,10) monomeric unit. It is clear from these patterns that while the poly(2,4) is an amorphous polymer, the poly(12,10) shows

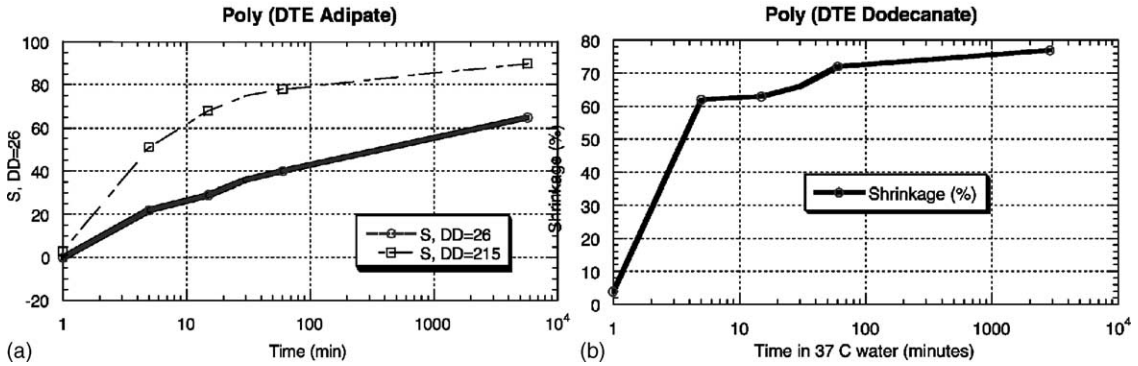

Fig. 9. 37 °C water shrinkage as a function of time: (a) poly(2,4); (b) poly(2,10). DD is fiber drawdown.

definite long range order. Fig. 11 shows a DSC trace of poly(12,10) annealed for 20 h at room temperature and heated from −50 °C at 20 °C/min to 125 °C followed by cooling at 20 °C back to the starting temperature and reheated. On first heating a pre-melting endotherm, at about 40 °C and related to the annealing conditions is noted [3] followed by melting at about 58 °C. A small and indistinct endotherm is observed at about 70 °C. Upon cooling, a sharp endotherm is observed at 50 °C. Reheating shows a single large endotherm at 58 °C, followed by indications of a smaller process at about 70 °C. Heat of fusion is about 20 J/g, which would correspond to a crystallinity of about 14% if the heat of fusion of the crystal is taken to be similar to that of poly(ethylene terephthalate), and the morphology of the fiber was assumed to be semi-crystalline. These results show unequivocally that the poly(12,10) polymer forms an ordered phase and the kinetics of the phase change are fast enough to show phase formation during 20 °C/min cooling in the DSC. The WAXS pattern, when coupled with the low heat of fusion and low temperature transitions noted in the DSC, suggests the poly(12,10) possesses a highly layered mesogenic structure, with layering similar to that of a smectic or discotic liquid crystal [10]. Fig. 12 shows the effect of annealing on the "melting" of the poly(12,10) fibers. Changes in the position and magnitude of the lower temperature endotherms are also noted to be changing in a regular fashion. Research into the origins of these transitions is in progress. Increasing the annealing temperature moves the major endothermic peak to higher temperatures, similar to the DSC response noted for annealed, semi-crystalline polymers [2]. The increase of the

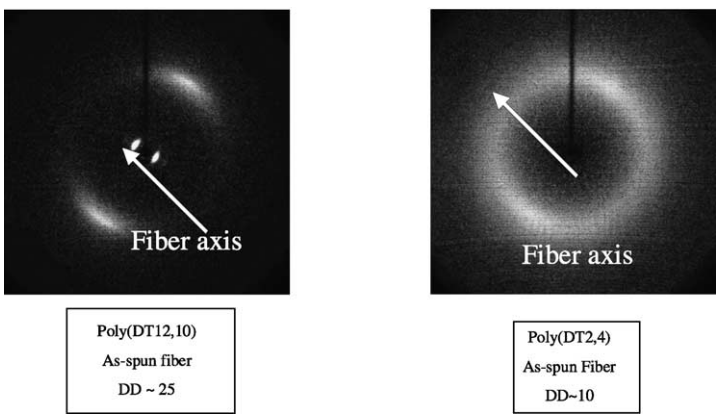

Fig. 10. Two-dimensional X-ray diffraction.

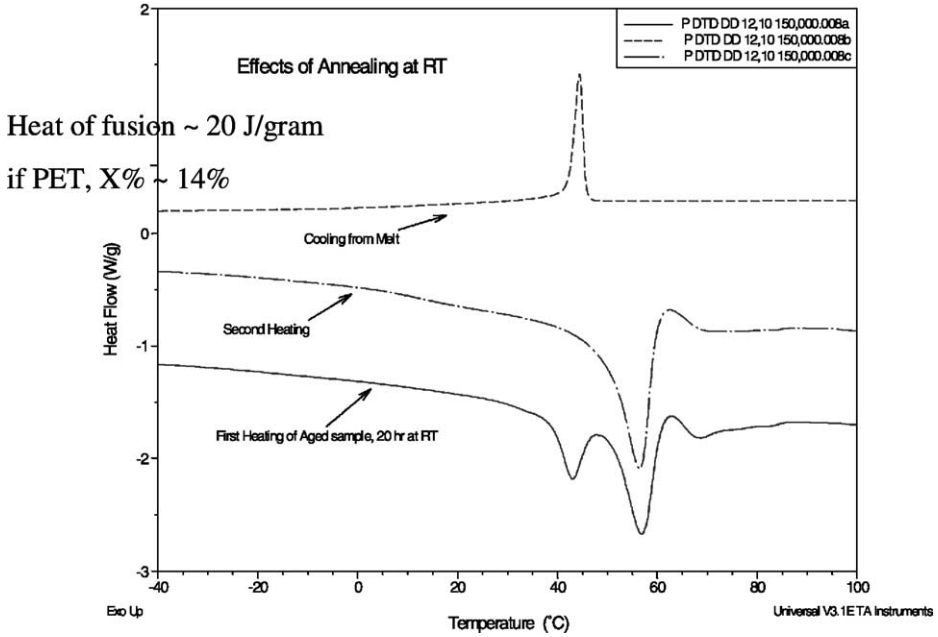

Fig. 11. DSC of poly(12,10) as a function of annealing history.

observed major peak endothermic peak temperature with the annealing temperature, suggesting a "crystal" perfection mechanism [2] is shown in Fig. 13. Similar effects have been reported by Jaffe and Warner [11] for annealed liquid crystalline polymers. Fig. 14 shows a modulated DCS trace for the same poly(12,10) material. Only a broad endotherm is observed in the neighborhood of the more distinct endotherm noted

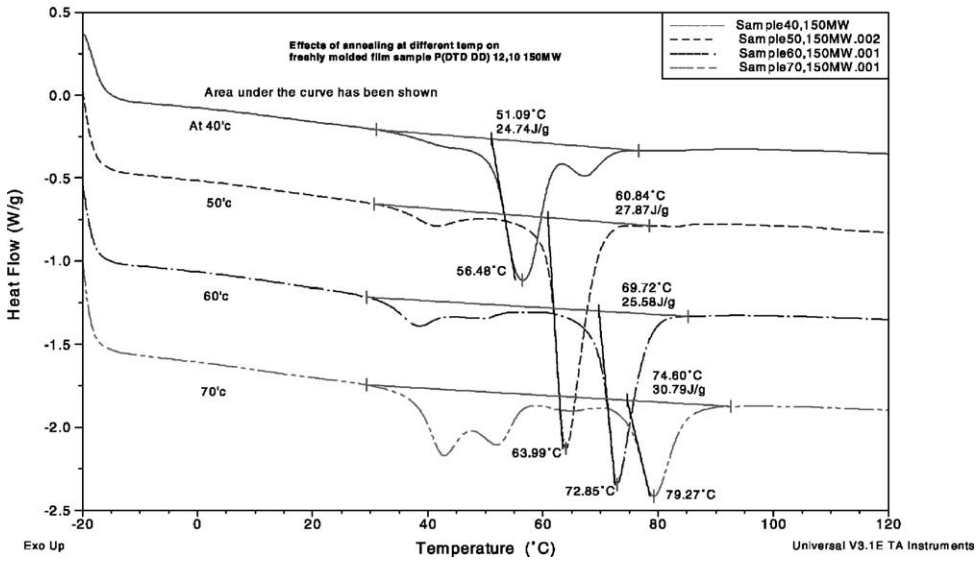

Fig. 12. Poly(12,10) effect of annealing.

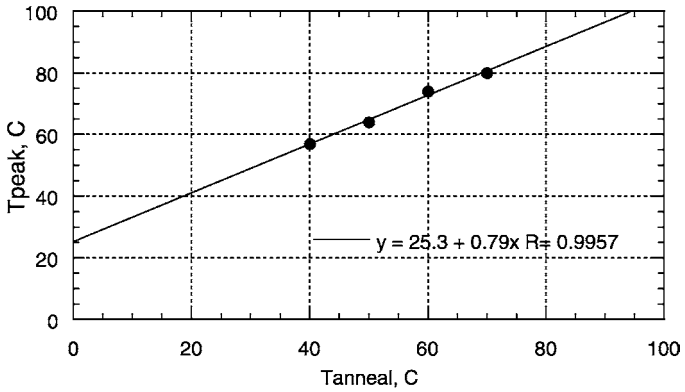

Fig. 13. Poly(12,10) major peak "melting" temperature a function of free to shrink anneal temperature, 1 h residence time.

in the conventional trace, consistent with a perfecting ordered phase or mesophase. The WAXS pattern of poly(8,8) is similar to that of poly(12,10), except that there exists multiple-order meridianol spots, the first order of which stands for a repeat of 27.6 Å, equal to the length of a monomeric unit of poly(8,8).

Fig. 15 shows a DSC trace of a poly(8,8) fiber showing "melting" behavior similar to the poly(12,10) but does not show "crystallization" on cooling at 20 °C/min. The long range ordered structure reforms in poly(8,8) after annealing at room temperature for several hours, indicating significantly slower ordered phase formation kinetics than observed for the

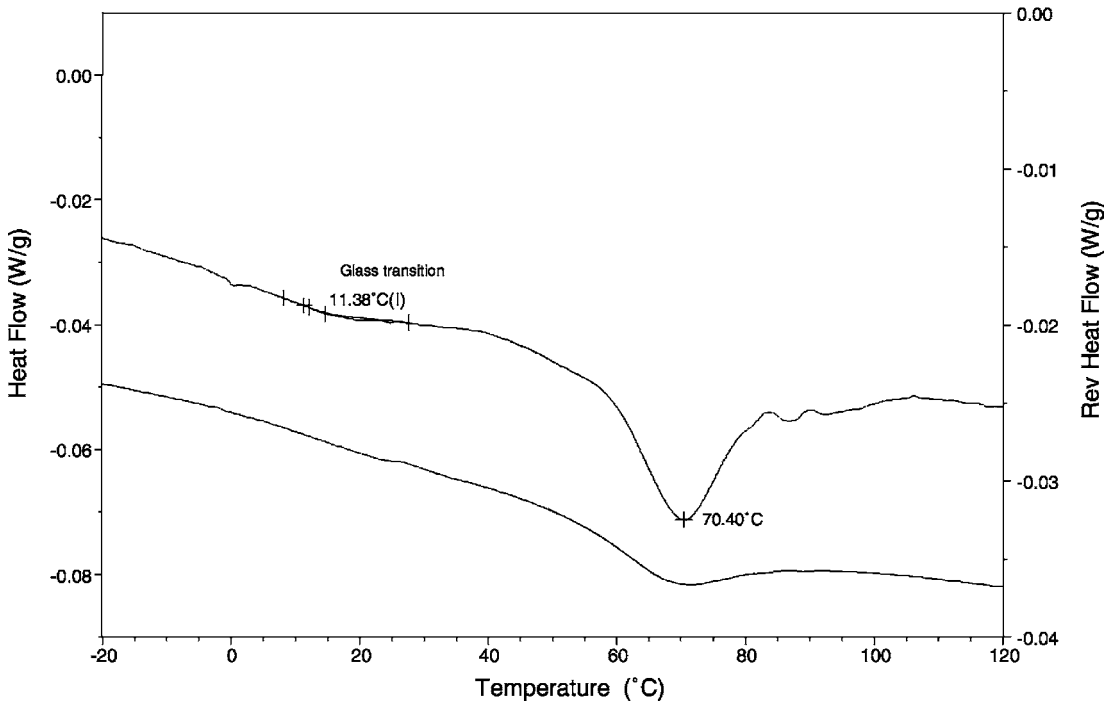

Fig. 14. Poly(8,8) modulated DSC.

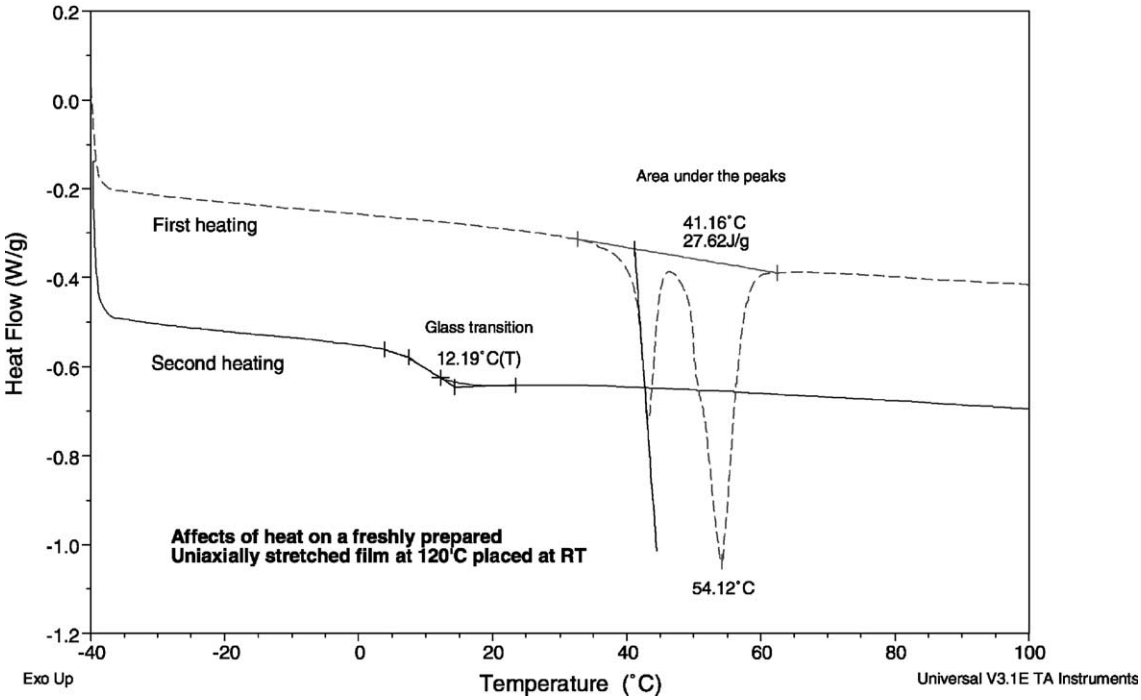

Fig. 15. Poly(8,8) uniaxially stretched film anneal at 120 °C.

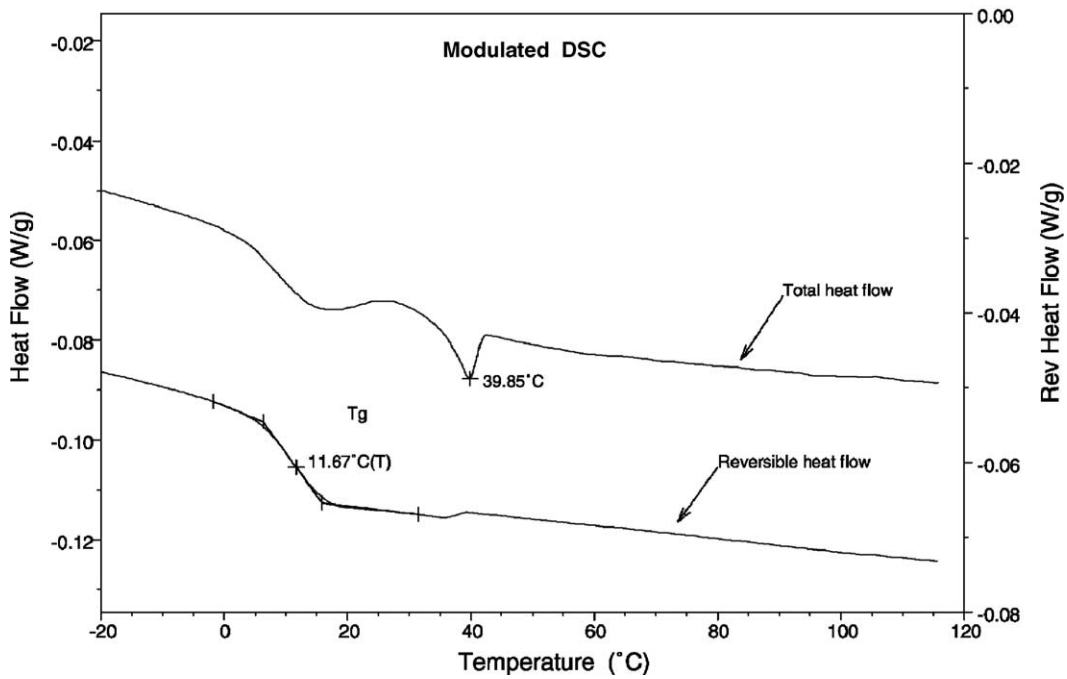

Fig. 16. Poly(8,8) annealed room temperature, 24 h.

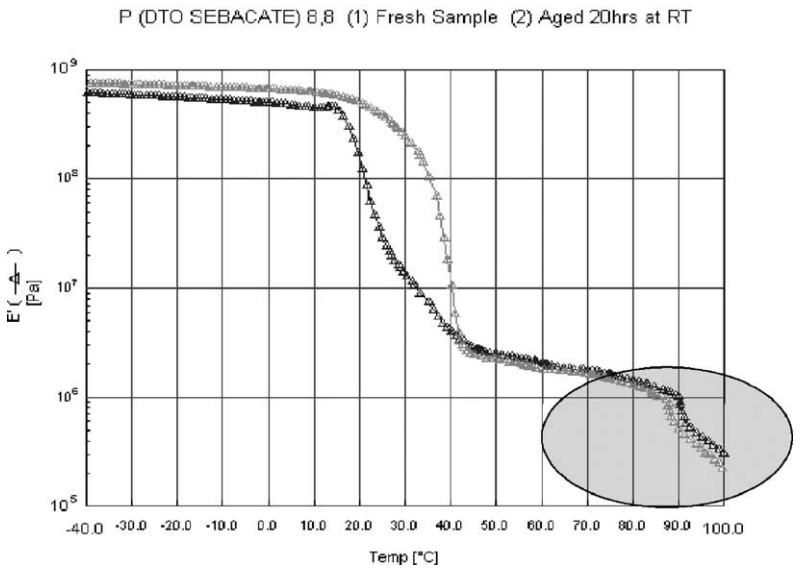

Fig. 17. DMA of poly(8,8) as a function of aging.

poly(12,10) and consistent with the lack of an endothermic transition in the modulated DSC trace shown in Fig. 16. Fig. 17 shows the real modulus response, as measured by DMTA, of an oriented poly(8,8) film, contrasting a freshly prepared sample with one crystallized by annealing for 24 h at room temperature. Note the rapid drop-off of the modulus at T_g (15 °C) for the fresh, amorphous sample, and the much slower drop-off, still beginning at a 15 °C, for the "crystallized" sample. At room temperature through 37 °C the difference of modulus is more than an order of magnitude. After "melting" at about 40 °C, the modulus levels below T_g and above "T_m", of fresh and annealed films are identical. These results illustrate the profound and unexpected performance tuning available within the polyarylate library.

Models of the poly(8,8) and poly(12,10) structure, consistent with the X-ray scattering results, suggests alignment along the molecular chain axis, perhaps stabilized by hydrogen bonding through the amide linkage, with the side chains sitting in layers normal to the chain axis. The observation of similar order in flexible, non-mesogenic macromolecules has recently been reviewed by Godovsky and Makarova [12]. While the details are not well understood, it has been observed that some flexible backbone polymers with flexible side chains can form columnar or discotic mesophases [13,14]. Although characterized by conformational disorder, these molecules form two-dimensional lattices in the plane perpendicular to the chain axis, a structure similar to that observed here. These phase transition behavior of these conformationally disordered crystals have extensively studied by Wunderlich et al. [15,16], and the DSC results of both the 12,10 and 8,8 compositions are consistent with previous observations on conformationally disordered systems. An extensive evaluation of these unexpected and provocative structures is currently underway by Wu and Jaffe [17].

5. Conclusions

These studies have indicated that there are strong plasticization effects noted for compositions with small values of *R* and *Y* and the long range order which stabilizes oriented structures in compositions with large values of *R* and *Y*. Water plasticization of T_g to below 37 °C leads to rapid loss of mechanical properties, coupled with unacceptable levels of shrinkage, for in vivo devices made from compositions of the upper left quadrant of the library matrix. This can be monitored directly and quantitatively by contrasting the thermal analysis of dry and water conditioned

samples. Given the exciting biological response observed with the desaminotyrosylethyl carbonate polymer [6], this could be a critical finding for successful biomedical application of short side chain polyarylate compositions. All of the compositions in the lower right quadrant of the library matrix have T_gs significantly below room temperature and were expected to be highly compliant materials under in vivo conditions. It has been shown that the property range of these polymers are stabilized and enhanced through long range structure formation, rendering them unexpectedly useful as candidate materials for biomedical usage. While thermal analysis under biorelevant analysis conditions for these compositions is not significantly different than the TA on dry samples in air, the significance of the TA revealed ordered structures to in vivo usage and property retention is critical. The detailed definition of the observed, apparently mesogenic structures will be treated separately (17). Work is in progress to further define the performance range of library polymers though a combination of polymer chemistry and polymer material science.

It has been shown that thermal analysis platform is an informative characterization platform for examining the biorelevant behavior of polymeric biomaterials. While the structural richness of the desaminotyrosyl-based polyarylates examined here was unexpected, TA proved a discerning tool for providing insight into the range and nature of the observed behavior. It is expected that many polymeric biomaterials with complex backbone geometries and/or stereochemical variations possible in the backbone will prove equally amenable to performance control through morphological manipulation. Experiments to define the impact of protein absorption, cell attachment, cell differentiation, cell proliferation and extra-cellular matrix expression on the TA response of these materials are in progress.

Acknowledgements

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