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Polymer 45 (2004) 3157–3162
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Cross-linked amino acid-containing polyanhydrides for controlled drug release applications

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Received 25 December 2003; received in revised form 9 March 2004; accepted 11 March 2004

Abstract

Cross-linked amino acid-containing polyanhydrides based on *N*-trimellitylimido-β-alanine (TMA-ala) or *N*-trimellitylimido-glycine (TMA-gly) and sebacic acid (SA) were synthesized by copolycondensation using 1,3,5-benzenetricarboxylic acid (BTC) prepolymer as a cross-linking agent. Differential scanning calorimetric (DSC) studies of the insoluble copolymers revealed only one sharp melting transition, indicating their cross-linked and/or randomly copolymeric structure. These cross-linked polymers can degrade completely into water-soluble molecules such as amino acids and natural fatty acid, which can be further metabolized by the body. This is an improvement over the photo-cross-linked polyanhydrides reported in the literature, whose cross-links hydrolyze to poly(methacrylic acid), a non-degradable and non-metabolizable macromolecule with limited biocompatibility. The cross-linked polymers were mixed with *p*-nitroaniline (PNA) and compressed into discs. In vitro degradation and solute release into physiological media were quantified for up to 70 h. Results showed that, relative to their linear counterparts, cross-linking has little effect on the degradation and solute release of these specific polymers.

Keywords: Polyanhydride; Cross-linking; Amino acid

1. Introduction

Polyanhydrides have been tested extensively as bioerodible supports in controlled drug release (CDR) applications [1]. Some polyanhydride-based formulations are now clinically available for treating glioblastoma multiforme, a universally fatal form of brain cancer [2]. Linear amino acid-containing polyanhydrides were first developed in 1990 [3], and later tested in orthopaedic [4–6] and CDR applications [7]. Photo-cross-linked polyanhydrides for load-bearing applications were also synthesized [8–10] by irradiating prepolymers containing methacrylate end groups. Alanine-containing cross-linked polyanhydrides were also prepared by a similar method [11]. A degradation product of these cross-linked polymers was polymethacrylic acid, a non-degradable and non-metabolizable macromolecule with limited biocompatibility [12].

In this report, cross-linked amino acid-containing poly(anhydride-*co*-imide)s based on *N*-trimellitylimido-β-alanine (TMA-ala) or *N*-trimellitylimido-glycine (TMA-gly), sebacic acid (SA), and 1,3,5-benzenetricarboxylic acid (BTC) were synthesized and characterized [13,14]. These polymers were cross-linked exclusively by anhydride linkages, and therefore can degrade completely into water-soluble molecules such as amino acid and natural fatty acid. This is an improvement over the photo-cross-linked polyanhydrides reported in the literature, which yield poly(methacrylic acid) as a by-product.

2. Experimental section

2.1. Materials

Trimellitic anhydride (97%, Aldrich), sebacic acid (99%, Aldrich), glycine (98%, Aldrich), β-alanine (99%, Sigma), dimethylformamide (99.8%, Aldrich), acetic anhydride (Certified ACS, Fisher Scientific), toluene (Certified ACS, Fisher Scientific) and ether (Certified ACS, Fisher Scientific) were used as received. The reported procedure [15]

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doi:10.1016/j.polymer.2004.03.035

was modified in order to synthesize the monomeric sebacic acid (SA) prepolymer as follows: 20 g SA were mixed with 500 ml acetic anhydride (Ac_2O) and refluxed for 30 min. Excess Ac_2O was removed by evaporation at 40 °C, followed by application of very high vacuum ($<4\times10^{-3}$ mbar) at room temperature. The crude prepolymer was purified by re-crystallization from toluene at −20 °C. Yield: 80%. Melting point: 23–25 °C. Purity: >99% (GC–MS). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.39$ (dd, $J = 5$, 10 Hz, 4H, $H_{2,9}$), 2.15 (s, 6H, $H_{2',2''}$), 1.59 (dd, $J = 10$, 5 Hz, 4H, $H_{3,8}$), 1.27 (m, 8H, $H_{4,5,6,7}$) ppm. ^{13}C NMR (500 MHz, $CDCl_3$): $\delta = 169.23$ ($C_{1,10}$), 166.52 ($C_{1',1''}$), 35.14 ($C_{2,9}$), 28.92 ($C_{3,8}$), 28.70 ($C_{4,7}$), 24.11 ($C_{5,6}$), 22.23 ($C_{2',2''}$) ppm. The TMA-ala, TMA-gly, and BTC prepolymers were prepared as previously described [16,17]. *N*-Trimellitylimido-β-alanine prepolymer (TMA-ala): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.44$ (s, 1H, $H_{3'}$), 8.41 (d, $J = 10$ Hz, 1H, $H_{5'}$), 7.95 (d, $J = 10$ Hz, 1H, $H_{6'}$), 4.04 (dd, $J = 5$, 10 Hz, 2H, H_3), 2.91 (dd, $J = 5$, 10 Hz, 2H, H_2), 2.41 (s, 3H, $H_{2''}$), 2.21 (s, 3H, $H_{2'''}$) ppm. *N*-Trimellitylimido-glycine prepolymer (TMA-gly): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.48$ (s, 1H, $H_{3'}$), 8.43 (d, $J = 6$ Hz, 1H, $H_{5'}$), 7.98 (d, $J = 6$ Hz, 1H, $H_{6'}$), 4.55 (s, 2H, H_2), 2.40 (s, 3H, $H_{2''}$), 2.27 (s, 3H, $H_{2'''}$) ppm. 1, 3, 5-Benzenetricarboxylic acid prepolymer (BTC): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.81$ (s, 3H, ArH), 2.40 (s, 9H, CH_3) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): $\delta = 165.81$ (Ar–*C*=O), 160.46 (CH_3–*C*=O), 137.01 (Ar*C*), 131.17 (Ar*C*H), 20.91 (*C*H_3–C=O) ppm.

2.2. Polymerization

The prepolymers were mixed in predetermined molar proportions (TMA-ala/SA/BTC = 20/80/5, TMA-gly/SA/BTC = 30/70/5, or SA/BTC = 100/5), and melt-polymerized at 180 °C under high vacuum ($<4\times10^{-3}$ mbar). In all cases, the mixture gelated after 30 min, and the reaction was continued for another 30 min. The polymers were then crushed and immersed in ether overnight to remove any acetic anhydride residue.

2.3. Methods

Nuclear magnetic resonance (NMR) spectra were obtained with a Bruker AC-500 spectrometer (Bruker AG, Fällanden, Switzerland) or a Varian Gemini 300 FT-NMR spectrometer (Varian Inc., Palo Alto, CA) at room temperature in $CDCl_3$. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrometry was performed on a Bio-Rad FTS 300MX spectrometer (Bio-Rad Laboratories Inc., Cambridge, MA). Differential scanning calorimetric (DSC) and thermogravimetric analyses (TGA) were conducted on a simultaneous thermal analyzer (STA1500; Rheometric Scientific, Piscataway, NJ) set at a heating rate of 10 °C/min.

2.4. In vitro degradation and drug release experiments

To formulate polymer-drug matrices, the polymer and *p*-nitroaniline (PNA, Aldrich Chemical Company Inc., Milwaukee, WI) were ground using either a mortar or a Scienceware Micro-mill Grinder (Bel-Art Products, Pequannock, NJ) and sieved to obtain microparticles (<212 μm). The polymer and PNA (10 wt% loading) were vortex-mixed and compression-molded in a benchtop press (Carver Inc., Wabash, IN) into discs (0.207 ± 0.001 g, 13.0 ± 0.1 mm diameter, 1.20 ± 0.05 mm thick), by applying 5000 psi for 10 min at room temperature. Drug-free matrices were prepared similarly. In vitro degradation and drug release studies were performed by laying the discs flat at the bottom of Pyrex® bottles containing 200 ml of 0.1 M phosphate-buffered saline (PBS, pH = 7.4). The bottles were placed in an incubator-shaker (Infors AG, Bottmingen, Switzerland) set at 37 °C and 100 rpm. At predetermined intervals, aliquots of the incubating media were withdrawn and assayed in a Beckman DU Series 7000 UV–vis spectrometer (Beckman Instruments Inc., Fullerton, CA). Every 12 h the spent buffer solution was decanted and the polymer discs were placed in fresh 200 ml 0.1 M PBS solution (pH = 7.4). The cumulative densities at 306 nm (absorption maximum for TMA-gly) and 381 nm (absorption maximum for PNA) were used to monitor the degradation and drug release processes, respectively. The experiments were stopped at about 60 wt% loss for the drug-free polymer matrices and at about 75 wt% loss for the polymer–PNA matrices because of severe cracking of the discs. The PNA release data was normalized by the theoretical absorption at 381 nm for 100% PNA release. Duplicate discs were used in all experiments.

3. Results and discussion

3.1. Polymer synthesis and characterization

By carefully selecting the reaction conditions (temperature and monomer concentration) and the purification procedure, the monomeric form of the SA prepolymer was prepared (^{1}H NMR spectrum shown in Fig. 1).

The integration ratio of the peaks at 2.39, 2.15, 1.59, and 1.27 ppm was 4/6/4/8, in exact agreement with the theoretical value. The purity of the SA prepolymer by gas chromatography (GC) was >99%, while the melting point was 23–25 °C. This is the first report of the NMR and melting point parameters for this monomer, and the procedure represents the first successful synthesis of the monomeric SA prepolymer using the reflux method. The SA prepolymer data in the literature of the past 20 years were for the oligomeric form (degree of polymerization = 3.9–9.0), with melting points between 67 and 69 °C [18]. A monomeric SA structure ensures that the final copolymer will be truly random in nature. On the other hand, the

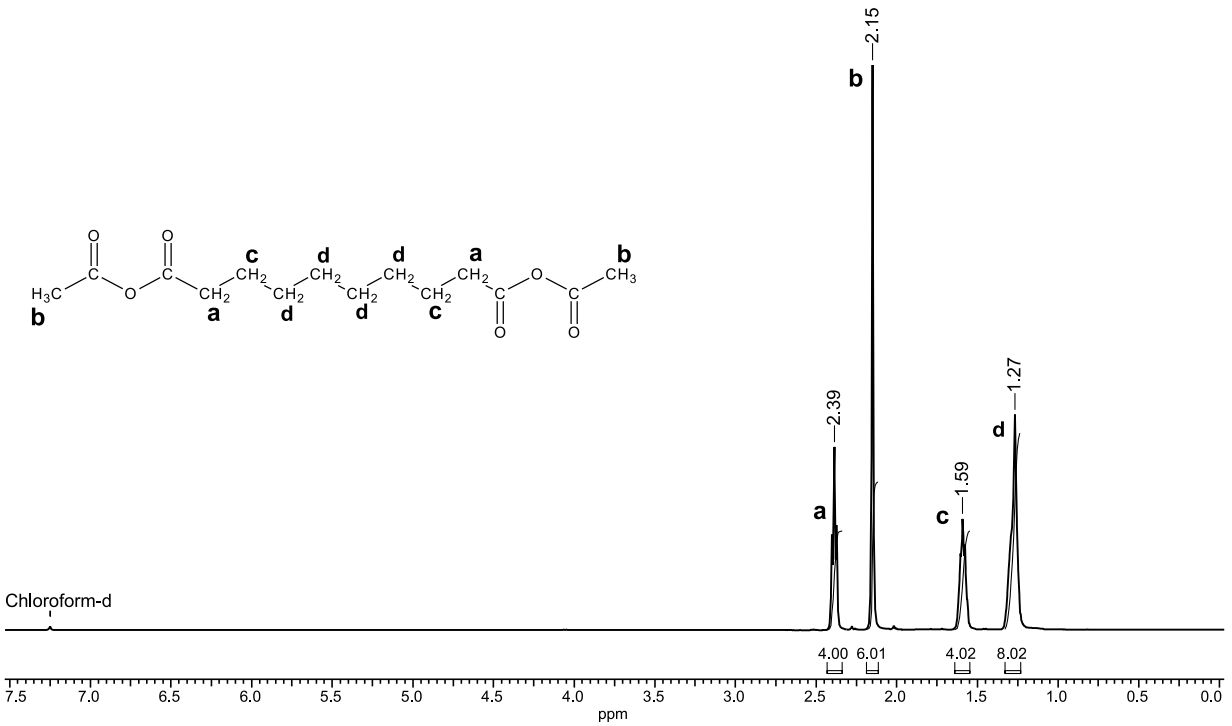
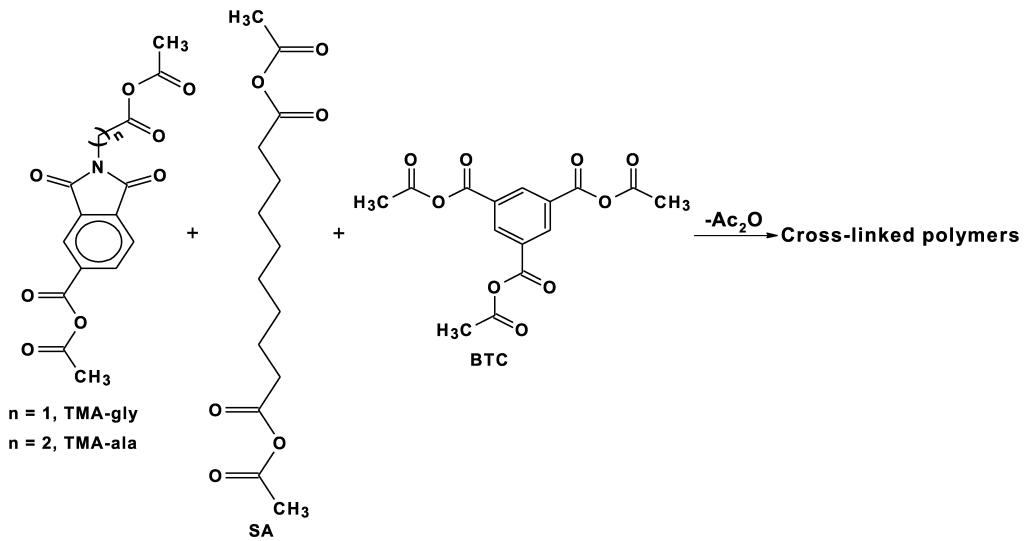


Fig. 1. ^{1}H NMR spectrum of the monomeric SA prepolymer.

oligomeric SA prepolymer may cause the final copolymer to undergo microphase separation, leading to drug partitioning between the phases, unpredictable degradation, and uncontrolled drug release [19]. The monomeric form of the SA prepolymer prepared in this study could improve the degradation and release profiles of the Gliadel® formulation, currently used to administer carmustine (BCNU) to brain cancer patients.

By using the 5% BTC prepolymer as a cross-linking agent in the copolycondensation of TMA-ala or TMA-gly and the SA prepolymers, cross-linked poly(anhydride-*co*-imide)s were prepared as shown in Scheme 1. The copolymers obtained were insoluble in most of the common solvents, but readily formed gels in chloroform, tetrahydrofuran, and dioxane, indicating their cross-linked nature. On the other hand, copolymerization of TMA-ala or TMA-gly and SA prepolymers without BTC or with 0.5–2% BTC led to highly soluble products, which had been characterized as linear or branched polymers by others [16,17]. When the BTC was above 5%, a very tough solid formed that integrated with the reaction flask and could not be isolated.

The copolymers were then characterized by ATR-FTIR spectroscopy as shown in Figs. 2 and 3 for the alanine-containing copolymer (CTAS) and the glycine-containing



Scheme 1. Synthetic route to cross-linked poly(anhydride-*co*-imide)s.

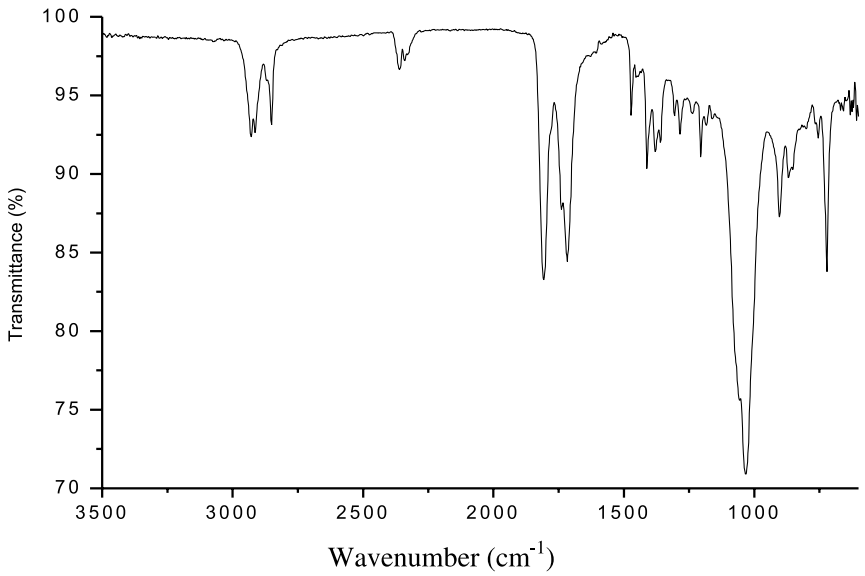


Fig. 2. ATR-FTIR spectrum of the CTAS copolymer.

copolymer (CTGS), respectively. As can be seen, both spectra showed peaks at 1811, 1724, and 1028 cm^{-1} characteristic of carboxylic anhydride groups, and peaks at 1784 and 1409 cm^{-1} typical of the imide groups. Cross-linked poly(sebacic acid) was prepared and characterized similarly.

The insoluble cross-linked copolymers synthesized were further tested using STA (DSC and TGA). Figs. 4 and 5 show the STA profiles for CTAS and CTGS, respectively. Unlike their linear counterparts which displayed two or more melting transitions [6,16,20], both CTAS and CTGS showed only one sharp melting transition, implying their cross-linked and/or randomly copolymeric structure. For example, two distinct melting transitions at 55 and 61 °C were often observed for linear TMA-gly/SA (30/70) copolymers [16], while only one melting transition at 62.2 °C was observed for the CTGS copolymer. The thermal decomposition temperature (T_d) of all cross-linked polymers agreed well with those of their linear counterparts.

3.2. In vitro degradation and drug release studies

CTGS was then further submitted to standard in vitro degradation and drug release studies by using PNA as a model drug. Fig. 6 shows the profile of TMA-gly release from CTGS matrices, as well as the TMA-gly and PNA release from PNA-formulated CTGS matrices. For polymer discs made by CTGS alone, the TMA-gly release was

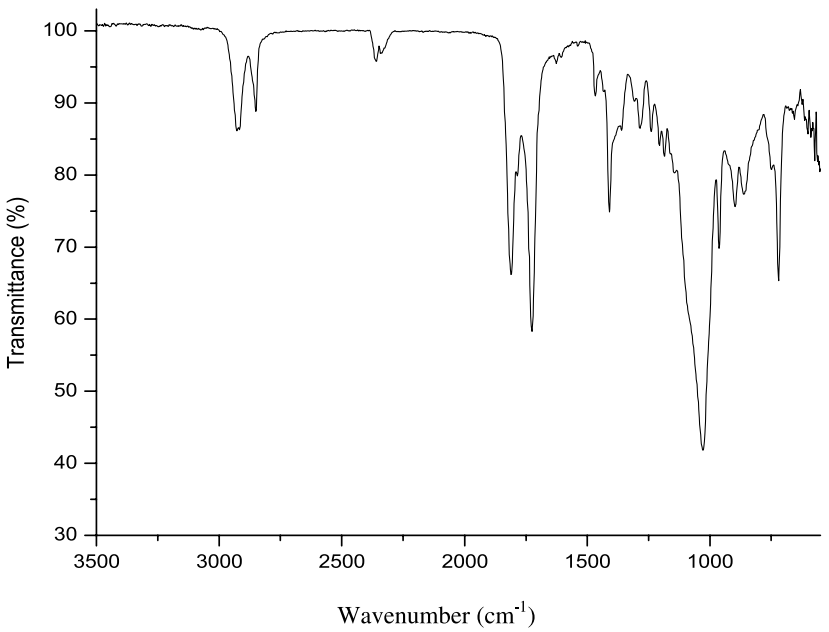


Fig. 3. ATR-FTIR spectrum of the CTGS copolymer.

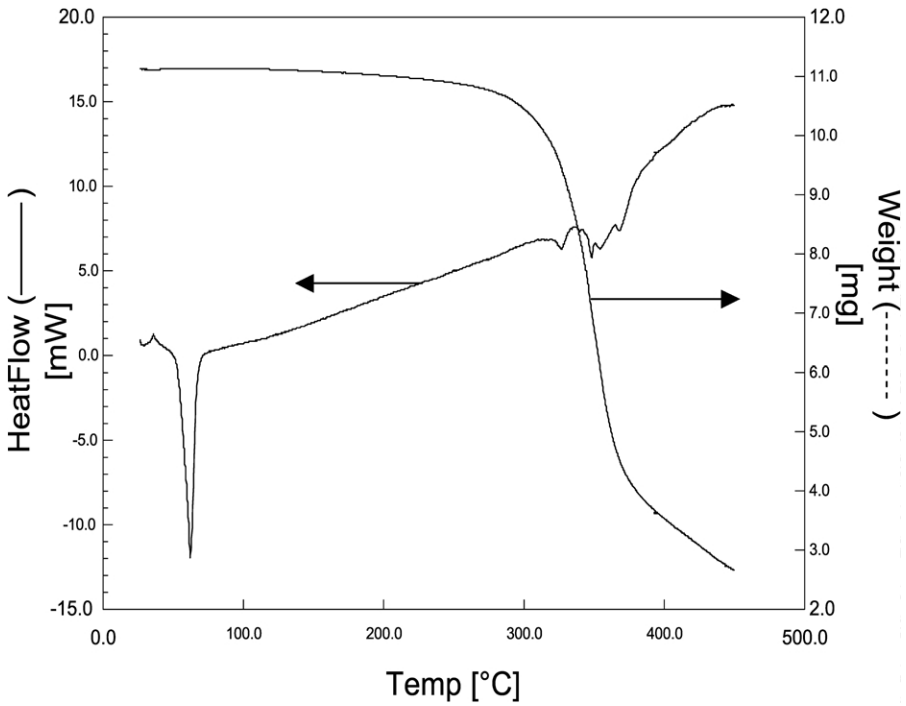


Fig. 4. DSC and TGA profiles for the CTAS copolymer.

(represented by curve A in Fig. 6) similar to its linear counterpart TMA-gly/SA (30/70) [5,6], indicating that cross-linking has little effect on the degradation behavior of this particular polymer, possibly due to its high hydrophilicity and low degree of cross-linking.

For PNA–CTGS matrices, both TMA-gly and PNA release kinetics (curves B and C in Fig. 6) followed closely that of drug-free CTGS degradation (curve A in Fig. 6). However, the degradation of the CTGS–PNA matrices was faster than that of the drug-free CTGS matrices possibly due to a more open internal structure caused by the presence of the drug.

4. Conclusions

Cross-linked amino acid-containing polyanhydrides were synthesized and characterized by FTIR, DSC, and TGA. The degradation and drug release characteristics of these polymers were found to be similar to those of their

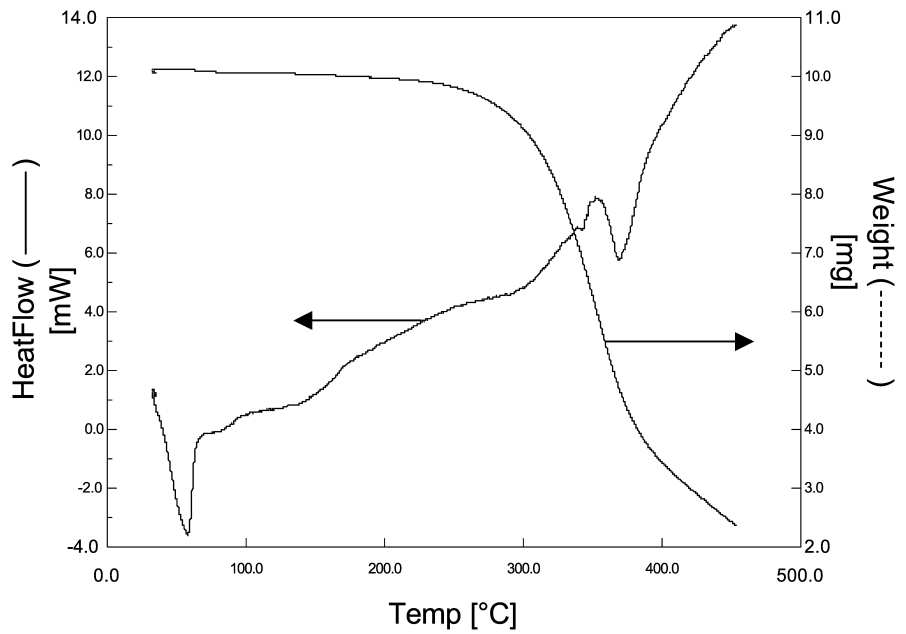


Fig. 5. DSC and TGA profiles for the CTGS copolymer.

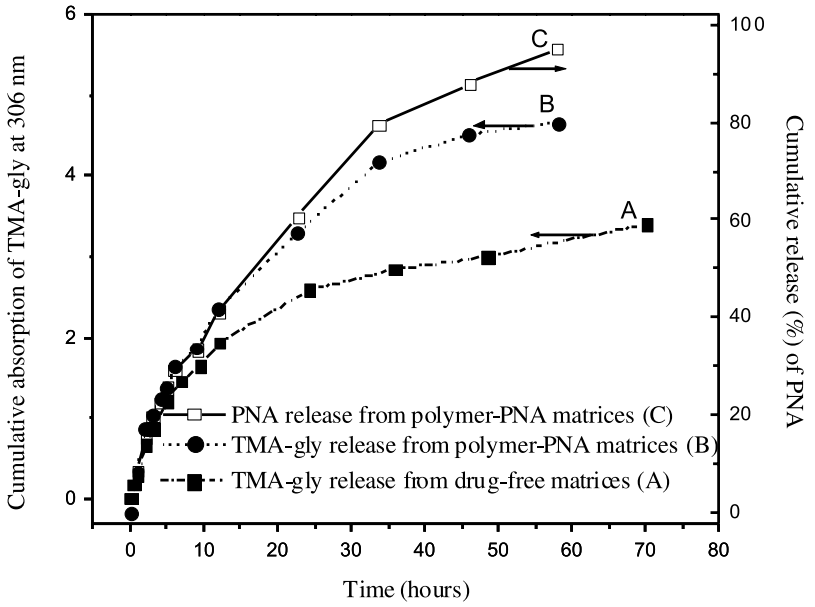


Fig. 6. TMA-gly and PNA release from CTGS matrices.

linear counterparts. Changes in their chemistry, including an increase in the degree of cross-linking and hydrophobicity, as well as in the formulation protocol, are being evaluated in order to extend their useful life and to expand their range of applications.

Acknowledgements

Funding for this study was provided by the NIH-MBRS SCORE Program (grant S06GM08103).

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