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Multifunctional Polyesters as New Candidate Materials for Biomedical Applications. Synthesis and Structural Characterization

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Summary: The present work was aimed at the development of functional polymeric materials to be used in the targeted delivery of proteic drug and tissue engineering fields. The adopted strategy was based on the design of special polymer classes whose structures and functionality could be easily modified by finely tuned synthetic procedures. Poly(ether ester)s containing H–bonding units were chosen as promising materials for the proposed applications. Commercially available precursors were successfully used for the synthesis of symmetrical diesters containing different H–bonding groups (amide, carbamate, and urea moieties). In all cases, pure products were obtained in good yields. Bulk polycondensation of the monomeric precursors with different mixtures of 1,4–butanediol and PEG1000 diol afforded a variety of high molecular weight polymeric structures. Physical–chemical characterization of the polymers indicates that their thermal, mechanical, and swelling properties can be tailored by a proper selection of the H–bonding group and of the composition of the feed mixture.

Keywords: H-bonding units; multifunctional polymers, poly(ether ester); structure-property relationship

Introduction

Recent progresses in medical, biological, pharmaceutical, and material science allow for the design and the preparation of highly engineered materials, whose final performances can be tuned to specific applications. In particular, two disciplines emerged in the last decades, that is targeted drug delivery technology and tissue engineering, which are in high demand for materials with specific and finely tailored structural and functional properties. Synthetic polymers are of great interest in this respect, because of the wide range of properties that can be attained by careful

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modulation of their chemical structure. The challenge consists in the development of materials that can combine optimized bulk properties, like thermal and mechanical properties, and hydrophilic-hydrophobic balance, which directly influence resistance and degradation pattern, with an engineered surface, to meet the fundamental requirements of biocompatibility. Indeed, the suitable design of the material surface may allow for highly specific interactions with the biological environment, implying for example selective cell recognition (*bioactive surfaces*) and for designing biomimetic surfaces (*stealth surfaces*).

The development of functional materials, which exhibit microphase separation, may represent a solution to these issues. Indeed, the presence of phase separated domains may result a valuable feature for macromolecular assembly. In this respect, the most investigated materials are amphiphilic block copolymers that can be tailored over a wide range of physical–chemical properties by varying the size and the content of the hydrophilic and hydrophobic segments. However, the realization of a blocky structure is not the only option to obtain phase separated macromolecular domains. Actually, molecular interaction and structure organization of synthetic macromolecules can be successfully modulated by the introduction of strongly interacting functional groups that promote the segregation of different phases. The role of the hydrophobic block is to create physical crosslinks in the "soft" hydrophilic matrix, thereby enhancing the self–assembly tendency. Contrarily to chemically crosslinked materials, physical crosslinks are reversible and can be disrupted at high temperature or in suitable solvents, allowing for easy material processing. Following this concept, a series of poly(ether ester) multiblock copolymers, based on hydrophilic poly(oligo(ethylene oxide) terephtalate) (PEOT) and hydrophobic poly(butylene terephthalate) (PBT) segments were prepared[1] and soon applied in textile[2] and biomedical fields[3]. Although materials containing aromatic moieties efficiently yield phase segregation, those moieties are not very much suited on biomedical applications, due to the potential toxicity of their degradation products. Therefore, the possibility of obtaining phase separated materials containing only aliphatic structures was investigated. In this connection, polymers containing chemical residues able to promote H–bonding interactions played a major role, since the control of the degree of H–bonding is a well–established and effective method for modulating the material characteristics. In this perspective, structurally regular aliphatic poly(ester amide)s, poly(ether ester amide)s and poly(ester carbamate)s were prepared and characterized[4-8], and investigated as biodegradable scaffolds for tissue engineering application[9], and as devices for protein (lysozyme) delivery[7]. Similarly, poly(ether ester amide)s containing ε–caprolactone residues were synthesized and applied as protein drug delivery systems[10,11]. In the present paper, we report the synthesis of PEG containing poly(ether ester)s carrying amide, carbamate, and urea H-bonding groups. The influence of structural features, such as type of H-bonding unit and chemical composition on the chemical-physical properties of the copolymers was also investigated.

Materials and Methods

All the solvents and products were carefully dried before use, according to standard procedures. Dimethyl 7,12-diaza-6,13-dione-1,18-octadecanedioate (M4A4), dimethyl 9,16-diaza-8,17-dione-1,24-tetracosanedioate (M6A6), 9,16-diaza-7,18-dioxa-8,17-dione-1,24-tetracosanedioate (E5C6), and diethyl 5,7,14,16-tetraaza-6,15-dione-1,20-eicosanedioate (E3U6) were prepared as reported elsewhere[12].

Polycondensation reactions were performed according to a slight modification of a reported method[5]: an equimolar amount of monomer carrying H-bonding units was added to a mixture of PEG1000 diol and 1,4-butanediol, by using 0.1% $Ti(OBu)_4$ as catalyst and 1% Irganox as antioxidant. The temperature was steadily increased up to the final value (165 or 220 °C, depending of the monomer thermal stability) while pressure was decreased to 0.1 mbar.

DSC analyses were performed under nitrogen atmosphere on 5-10 mg samples by using a Mettler TA 4000 instrument. Indium and gallium samples were used as calibration standards. TGA analyses were carried out on 10-12 mg samples in the 50-700 °C range under nitrogen atmosphere at 10 °C/min heating rate, by using a Perkin Elmer TGA 7 instrument. FT-IR spectra were recorded under dry nitrogen atmosphere on liquid films, cast polymer films, or KBr pellets by a BIORAD FTS-60 spectrophotometer. NMR spectra were recorded on 10% (w/v) solutions in perdeuterated solvents, at 25 °C by a Varian 300 instrument. Viscosity measurements were carried out in $CHCl_3$/MeOH 1:1 (v/v) at 25°C in Ubbelohde OC viscometer. Determination of equilibrium water uptake was performed in phosphate buffer saline (PBS). Equilibrium water uptake was evaluated as the weight gain of the polymer sample after conditioning at 37 °C. Static contact angles were determined by an OCA 15 Contact Angle Goniometer. Films were cast on glass slides from 0.5 % polymer solutions and carefully dried under vacuum.

Results and Discussion

Monomers

The general structure of the synthesized monomers is sketched in Figure 1. Amide, carbamate, and urea groups were chosen as H-bonding moieties.

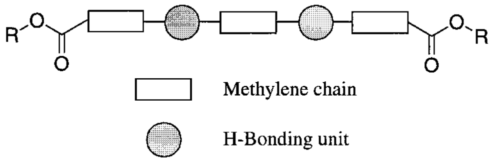
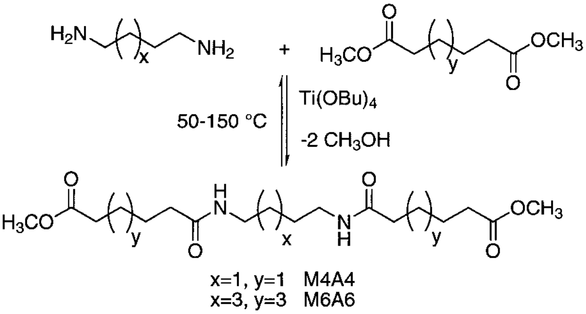


Figure 1. Schematic structure of the synthesized H-bonding monomers.

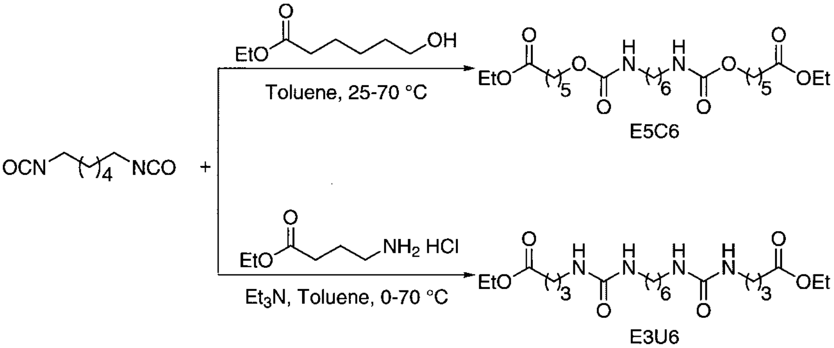
In order to isolate and investigate the role of the H-bonding units, the inner polymethylene chain was maintaned costant in length (six methylene groups) and the functional group was changed (amide in M6A6, carbamate in E5C6, urea in E3U6). The effect of the length of the polymethylene segment was then evaluated by comparing M4A4 and M6A6 samples, consisting of the same H-bonding unit (amide), and tetramethylene and hexamethylene segments respectively.

Amide containing monomers, that is dimethyl 7,12-diaza-6,13-dione-1,18-octadecanedioate (M4A4) and dimethyl 9,16-diaza-8,17-dione-1,24-tetracosanedioate (M6A6) were prepared in 62 and 88% yield, respectively, by reacting 1,4-butylenediamine with dimethyl adipate and 1,6-hexamethylenediamine with dimethyl suberate in the melt, by using $Ti(OBu)_4$ as catalyst[5] (Scheme 1). By optimization of the experimental conditions, the formation of oligomeric compounds was drastically reduced, and the crude products were furtherly purified by precipitation in selected solvents.

Carbamate and urea containing monomers, that is diethyl 9,16-diaza-7,18-dioxa-8,17-dione-1,24-tetracosanedioate [E5C6] and diethyl 5,7,14,16-tetraaza-6,15-dione-1,20-eicosanedioate [E3U6] were prepared in 80-90% yield starting from a 1:2.1 mixture of hexamethylene diisocyanate with ethyl 6-hydroxyhexanoate and ethyl 4-aminobutyrate hydrochloride, respectively (Scheme 2).



Scheme 1. Preparation of amide containing monomers.



Scheme 2. Preparation of the carbamate and urea containing monomers.

The solubility behavior and the thermal stability of the synthesized monomers resulted highly dependent upon both the type of H-bonding unit and of the length of the methylene spacer. Considering the nature of the H-bonding unit, the solubility in protic and polar aprotic solvents increased in the order carbamate > amide > urea. Moreover, amide containing monomers showed much higher thermal stability (T_d 350 °C) than the corresponding carbamate (T_d 180 °C) or urea (T_d 250 °C) containing compounds. On the other hand, an increased number of methylene units not only made the materials more hydrophobic but also favored a better molecular packing, thus determining an increase in higher melting temperature and thermal stability. It is worth noting that only E5C6 presented a single endothermic peak in the DSC trace, whereas the other monomers exhibited two endothermic transitions on heating and two exothermal peaks upon cooling (Fig.2). This behavior was attributed[12] to the presence of different crystal structures or to a conformational rearrangement during the first endothermic transition.

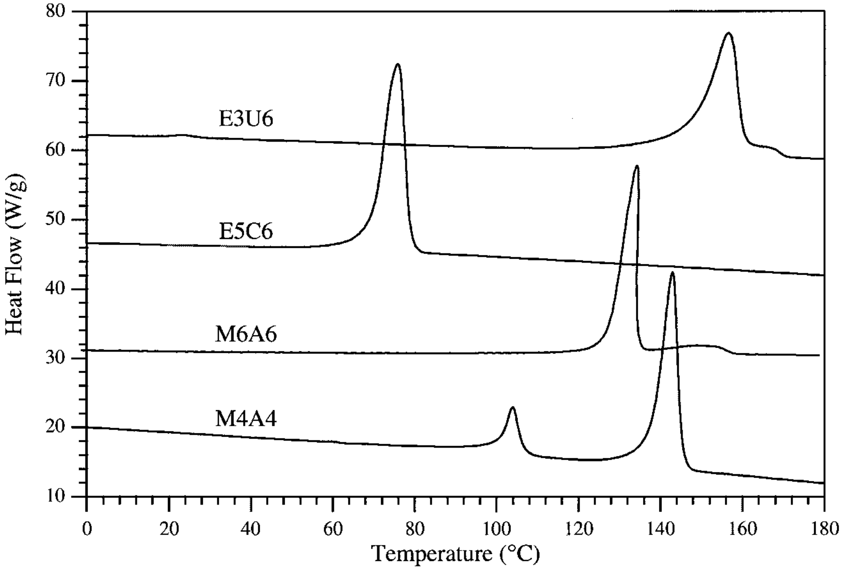
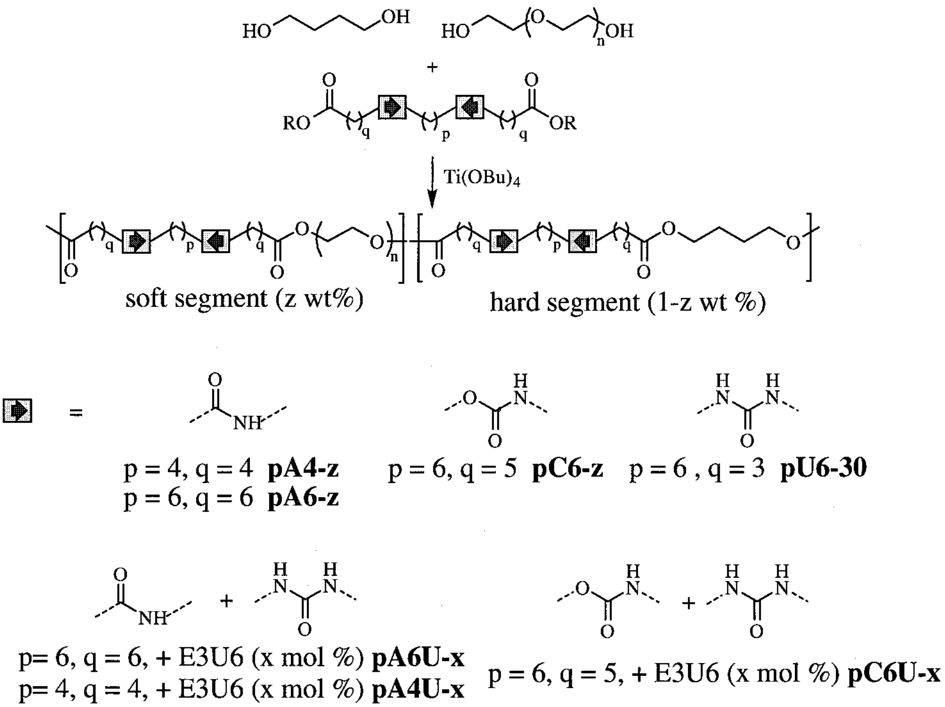


Figure 2. DSC heating curves of the synthesized monomers.

Polymers

Polycondensation of the synthesized diester monomers containing H–bonding groups (M4A4, M6A6, E5C6, and E3U6) with PEG1000/1,4–butandiol mixtures has been applied to synthesize a series of polymeric materials (Scheme 3), that are poly(ether ester amide)s, poly(ether ester carbamate)s, poly(ether ester urea)s and copolymers polyesteramide/urea and polycarbamate/urea.

The structure of the resulting polymers can be schematically represented by an alternating sequence of diol units and H–bonding monomeric residues. Following an interpretative paradigm which appears consistent with the experimental results, it is convenient to consider the macromolecular chain constituted of "soft" and "hard" segments, whose constitutional units can be identified by considering their degree of conformational freedom. In such a view, the segment constituted by an H–bonding unit and a 1,4–butanediol residue is identified as the hard segment, whereas that consisting of the H–bonding unit and PEG1000 is the soft one. It is therefore clear that the final hard/soft composition of each material can be tailored by simply changing the composition of the diol mixture.



Scheme 3. Polycondensation of H-bonding monomers with 1,4-butanediol and PEG1000.

Polycondensation reactions were performed in bulk by using $Ti(OBu)_4$ as catalyst. After purification, copolymer composition was evaluated by ^{1}H-NMR analysis, by comparison of diagnostic peaks belonging to the three different monomer units (Table 1). In all cases, a slightly higher PEG content was found in the polymer than in the feed, suggesting that a small amount of 1,4-butanediol distilled off from the reaction mixture during the polymerization process.

Table 1. Polycondensation reaction of H-bonding monomers with 1,4-butanediol and PEG1000

Run	Polymerization conditions					Yield	Polymer	
	M_1	E3U6 (mol %)[c]	PEG1000[a] (% mol)	Final T (°C)	Duration (h)	(%)	PEG1000[a,b] (% mol)	[η] (dl/g)
pA4-30a	M4A4	0	8	220	10	67	13	0.53
pA4-30b	M4A4	0	10	220	24	68	12	0.86
pA4-40	M4A4	0	11	165	35	84	22	0.36
pA4-50	M4A4	0	17	165	35	65	21	0.32
pA4-70	M4A4	0	35	165	35	79	39	0.33
pA4U-5	M4A4	5	14	165	35	60	14	0.29
pA4U-10	M4A4	10	14	165	35	55	14	0.21
pA6-30	M6A6	0	10	220	24	30	15	1.47
pA6-40	M6A6	0	13	165	35	89	19	0.40
pA6-50	M6A6	0	20	165	35	81	25	0.32
pA6-70	M6A6	0	36	165	35	53	50	0.42
pA6U-5	M6A6	5	17	165	35	56	18	0.41
pA6U-10	M6A6	10	14	165	35	55	13	0.42
pC-30	E5C6	0	10	165	35	66	13	0.55
pC-40	E5C6	0	14	165	35	35	19	0.49
pC-50	E5C6	0	21	165	35	63	24	0.56
pC-70	E5C6	0	38	165	35	79	45	0.61
pCU-5	E5C6	5	9	165	35	53	11	0.39
pCU-10	E5C6	10	9	165	35	45	11	0.53
pU-30	E3U6	100	12	165	35	63	n.d.	n.d.

[a] Evaluated as 100·mol PEG/(mol PEG + mol 1,4-butanediol). [b] Evaluated by ^{1}H–NMR analysis. [c] Referred to M_1.

In order to establish a correlation between the polymeric material properties and structural features, such as the nature of the H-bonding units and PEG content, the synthesized polymers were characterized by TGA and DSC analysis, contact angle measurements and by determining the swelling in PBS. Both bulk and surface properties of the polymeric materials were found strongly dependent upon, and hence finely tunable by the polymer structural characteristics. Indeed, the nature of the H-bonding units largely influences the polymer thermal properties (Figs. 3 and 4).

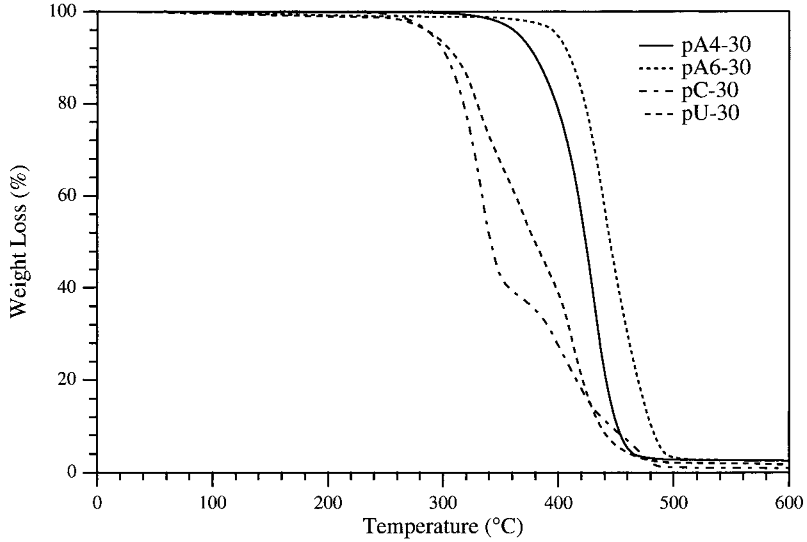


Figure 3. TGA analysis of poly(ether ester)s of the same composition containing different H-bonding units.

The water contact angles and the equilibrium swelling behavior (performed in phosphate buffer solution) of the investigated materials was also influenced by the nature of the H-bonding unit. This influence could be rationalized in terms of both the hydrophilic characteristics of the H-bonding moiety (carbamate>urea>amide) and the flexibility of the resulting macromolecular chain. Chain mobility and flexibility seem to play a major role also in determining tensile behavior of the prepared samples[12].

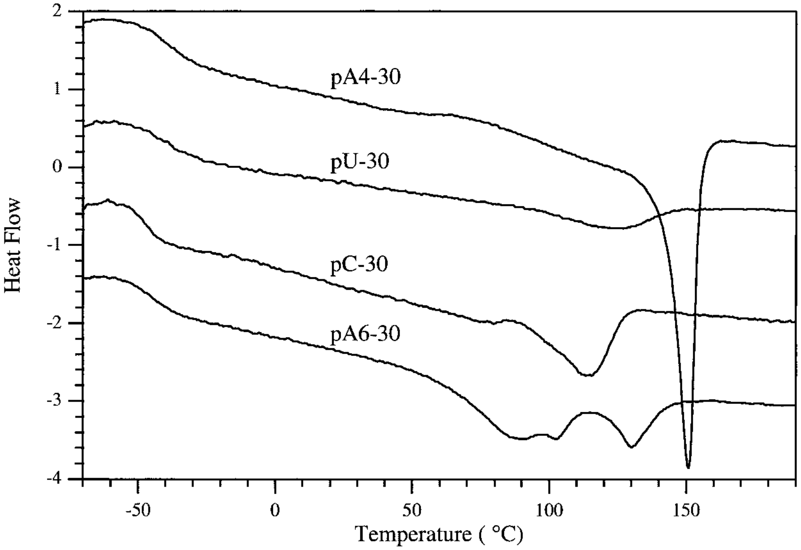


Figure 4. DSC analysis of poly(ether ester)s containing H-bonding units of different nature.

As expected, the varying PEG content changed the hydrophilic-hydrophobic balance, resulting in a large influence on the swelling behavior, surface hydrophilicity and therefore surface free energy of the material. Moreover, the PEG content was found to play a major role also in affecting material thermal properties, both in terms of Tg value and melting behavior. Indeed, the Tg values decreased on increasing the PEG content, because of the increased chain flexibility (Fig. 5).

Generally, the complexity of DSC traces increased with increasing the PEG content. This behavior can be attributed either to the higher flexibility of the macromolecular chain, which gives rise to less organized or imperfect crystalline domains of the H-bonding interacting units, or to the propensity of the PEG segments to crystallize. Accordingly, both the first-order transition relevant to the melting temperature and the associated enthalpy in the carbamate copolymer series decreases as PEG content increases (Fig. 6). This can be taken as an indication that the H-bonding units tend to form less ordered crystals on increasing PEG content.

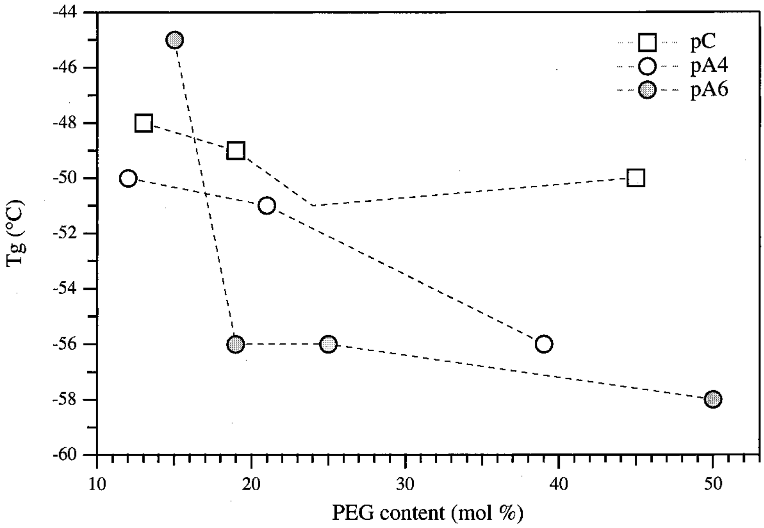


Figure 5. Dependence of the glass transition temperature (Tg) on the PEG content of functional poly(ether ester)s containing H-bonding units.

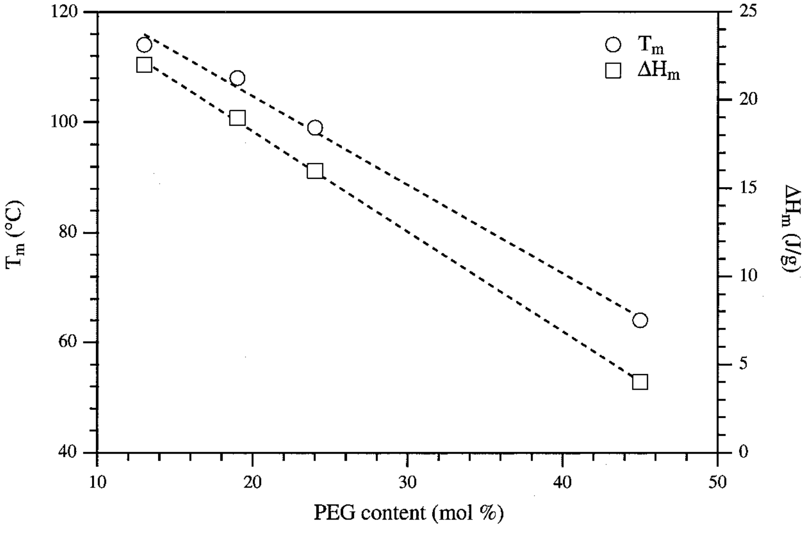


Figure 6. Dependence of the melting peak temperature (T_m) and associated ΔH_m on the PEG content of carbamate copolymer series (pC-x).

Conclusions

The preparation of symmetric aliphatic diesters containing H-bonding moieties (amide, carbamate and urea) can be performed by diester aminolysis, and diisocyanate coupling with diols and diamine to yield diester diamides, diester dicarbamates, and diester diureas, respectively. The adopted procedures allow the synthesis of the functional diesters in good yields (62-95%) and high chemical purity. The hydrophilic–hydrophobic balance and the thermal properties of the investigated monomers can be easily modulated by incorporation of different H-bonding units and by suitable choice of the structure of the precursors. In all cases, the diester containing H–bonding units are stable at least up to 150 °C. This aspect represents an important feature for their use in polycondensation reactions.

Poly(ether ester)s containing H-bonding units can be obtained by $Ti(OBu)_4$ catalyzed, polycondensation in the bulk of the synthesized monomers with different mixtures of 1,4–butanediol and PEG 1000 diol. The applied synthetic strategy is indeed effective for the attainment of high molecular weight products in fairly good yields. The kind of the H-bonding unit and the molar ratio between the two diols is of paramount importance in determining the thermal properties and the hydrophilic-hydrophobic balance of the prepared polymeric materials. Moreover, the presence of strongly interacting H-bonding units affords materials tending to give phase segregation. However, due to the limited number of investigated samples, no simple and direct structure-property correlation can be drawn based on the reported results. The synthesized materials appear to be good candidates for biomedical applications, particularly by taking into account their inherent hydrophilicity and the presence of PEG segments.

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