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A New Chain Extension Reaction on Poly(lactic–glycolic acid) (PLGA) Thermal Oligomers Leading to High Molecular Weight PLGA-Based Polymeric Products

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Abstract: A novel and effective chain-extension procedure for obtaining high-molecular-weight PLGA-based bioerodible polymers is described. This procedure consists in a polycondensation reaction between PLGA thermal oligomers of average molecular weight 1000–3000 and bischlorofomates of either simple diols or α,ω-bis-hydroxylated oligomers, such as polyethyleneglycols or bis-hydroxy terminated poly-ε-caprolactones. In the latter case, multiblock copolymers are obtained. By varying the nature and the length of the starting diol, as well as the lactic/glycolic acid molar ratio in the starting PLGA oligomer, products with widely different physicochemical properties can be synthesized. In particular, dissolution times in aqueous media can be tuned within an ample range, from a few days to several months. This provides exceptional opportunities for designing products whose properties are tailored in view of specific applications in the biomedical field. © 1998 SCI.

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Key words: poly(lactic-glycolic acid); bioerodible polymers; multiblock copolymers; bischlorofomates; polycondensation; polyethyleneglycol; poly-ε-caprolactone

INTRODUCTION

In recent years, the polycondensation products of lactic and glycolic acids, commonly referred to as poly(lactic–glycolic acid) or PLGA, have acquired paramount importance as a family of biodegradable and biocompatible polymers.[1–3] Depending on their structure, polymers of this family are, for instance, currently employed for preparing bioresorbable suture threads, bioresorbable bone reparatory systems, and drug delivery systems from microspheres to subcutaneous disks or tablets.[4–10]

In principle, the simplest and cheapest process for preparing PLGAs would be the thermal polycondensation of the parent acids (see Scheme 1a). This method can indeed be used, but leads only to oligomeric products having molecular weights of the order of a few thousand.[7] High molecular weight PLGA samples are currently prepared by ring-opening polymerization processes, in the presence of catalysts, of 1,4-dioxane-2,5-dione and 3,6-dimethyl-1,4-dioxane-2,5-dione, i.e. of the dimeric cyclic self-condensation products of the same acids (see Scheme 1b). Most of the samples presently used in practice, and marketed, are prepared by this method.[11,12]

The oligomeric PLGA samples obtained by thermal polycondensation are still endowed with reactive terminal functions, the same as the parent acids, and therefore can be regarded as macromonomers. By taking advantage of this, we have described[13–20] a novel chain-extension process which, starting from PLGA thermal oligomers, leads to poly(ester-carbonates) whose molecular weights are at least as high as those of the PLGA samples currently obtained by ring-opening

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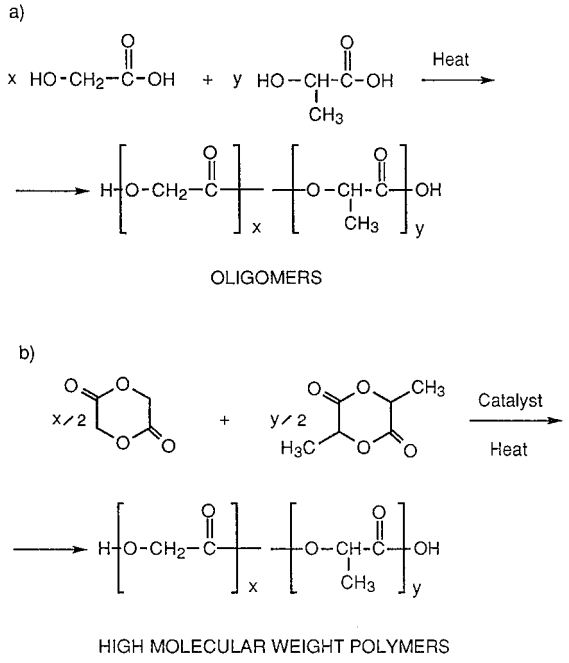
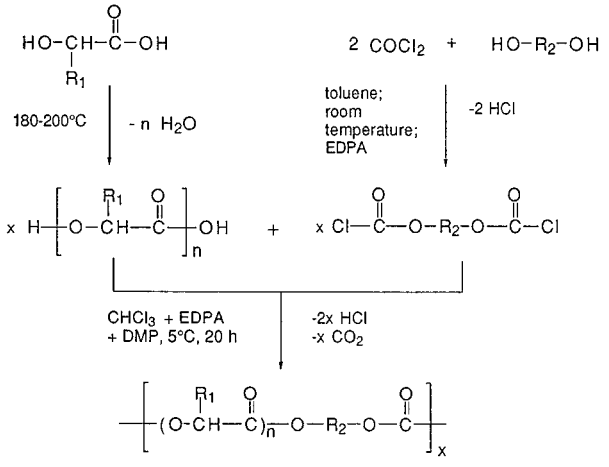


Scheme 1

polymerization. This process involves the reaction of PLGA oligomers with α,ω-bis(chloroformates), in turn obtained by reaction of phosgene with diols. The general synthetic pathway is summarized in Scheme 2.

In the first instances, the bischloroformates of simple diols were used. However, the scope of the above process can be extended. In fact, we have subsequently reported the preparation and properties of several novel high-molecular-weight multiblock copolymers containing PLGA segments coupled with segments derived from α,ω-oligomeric diols such as poly(ethylene glycols) (PEG),[13,14,16] and bishydroxy-terminated poly(ε-caprolactone) (PCL).[19,20] Because it would seem that no major limitations exist as regards the oligomeric diol to be coupled with PLGA oligomers, it is apparent that an



R_1 = H or CH_3 molar ratio 1:1;
R_2= aliphatic, alicyclic, or oligomeric groups;
EDPA = N-ethyl-N,N-diisopropylamine;
DMP=4-dimethylaminopyridine.

Scheme 2

endless variety of different structures can be obtained in principle, leading to novel polymeric materials which can be purpose-tailored according to specific requirements.

There are literature reports on di- or tri-block copolymers in which one PEG segment is coupled with one or two PLGA segments. These copolymers were obtained by simply adding PEG to a polymerizing system leading to PLGA.[21–23] Random and diblock copolymers[24,25] containing lactic acid, glycolic acid and 6-hydroxyhexanoic acid, synthesized by ring opening polymerization of the cyclic monomers, have also been reported.[26,27] To our present knowledge, the only multiblock copolymers resembling those reported in this paper have been recently prepared by copolymerization techniques from L-lactide and ethylene oxide.[28]

Several studies on the degradation behaviour of PLGA in aqueous media under conditions mimicking those found in biological fluids have been published.[23–27,29–31] The dissolution rate, for instance, has been found to depend on a number of factors, among which the composition in terms of molar ratio between lactic and glycolic acid moieties and the steric structure of the former apparently play a major role, probably owing to their influence on crystallinity and water absorption.[32,35] Moreover, other conditions being equal, the degradation rate depends on the size and shape of the samples, microspheres, for example, degrading at a slower rate than relatively large and thick sheets.[28,35–38] This rather unexpected result was attributed to the catalytic activity on the degradation reaction by acidic degradation products, which are less easily lost by diffusion from the latter, as a consequence of their lower surface area.

In this paper is described the degradation behaviour of the above mentioned new block copolymers containing PLGA-PEG segments in the form of both sheets and microspheres. The results are discussed in terms of composition and type of sample.

2. SYNTHESES

2.1 Preparation of PLGA prepolymers

The oligomeric PLGA was prepared by thermal polycondensation of mixtures of lactic and glycolic acids. The molecular weight of the oligomer was always determined by titration in benzyl alcohol according to the literature.[39] It usually ranged between 1500 and 3000.

We found that the best strategy consisted of titrating the oligomer and using it in the subsequent step immediately after the end of the polycondensation reaction, because on storage some breakdown of the polymer chain invariably occurred unless very strict precautions to exclude traces of moisture were taken. Even if the

degraded products could be used in the subsequent chain-extension step, the only difference from their precursors being a lower molecular weight, their use was not recommended, because the molecular weights of the resulting poly(ester-carbonates) were lower than that of the samples obtained from freshly prepared PLGA.

A typical preparation is the following. Glycolic acid (76·82 g, 1 mol) and D,L-lactic acid (123·40 g, 1 mol) were placed in a two-necked flask. A stream of nitrogen was bubbled through the mixture and the temperature gradually raised to 200°C by means of a thermostatic bath. After maintaining these conditions for 24 h, the nitrogen flushing was discontinued, and high vacuum applied for a further 24 h. The product was then cooled down to room temperature under vacuum. The oligomer settled into a hard mass and was recovered with some difficulty unless solvents were employed. The yield was almost quantitative.

The number average molecular weight of the oligomers was determined by titration in benzyl alcohol solution with aqueous sodium hydroxide in the presence of phenolphthalein as indicator, or better, by potentiometic titration in the same solvent with an isopropyl alcohol solution of tetrabutylammonium hydroxide. Values ranging between 1500 and 2500 were usually obtained. The corresponding intrinsic viscosities (in chloroform at 30°C) ranged between 0·08 and 0·12 dl g^{-1}.

2.2 Chain-extension reaction

The chain-extension reaction leading to high molecular weight poly(ester–carbonate)s was performed with α,ω-bischloroformates in chloroform solution and in the presence of *N*-ethyl-*N*,*N*-diisopropylamine (EDPA) and 4-dimethylaminopyridine (DMP). The overall process is figured in Scheme 2.

It should be noticed that two different reactions occur in the chain extension process. The first, i.e. the reaction of a chloroformate group with an hydroxyl group to give a carbonate group, is commonplace in organic chemistry. The second, i.e. the reaction of a chloroformate group with a carboxyl group to give an ester with the elimination of carbon dioxide, was first described on nonmacromolecular compounds by Adam and Trieschmann[40] and found to proceed in some cases up to about 80% yield.[41]

The bischloroformates used in the chain extension reactions were prepared from diol and phosgene (as 20% solution in toluene). A typical preparation is the following. A 20% toluene solution of phosgene (0·25 mol) was placed in a flask equipped with a stirrer, a gas inlet which could be lifted to various levels, a gas outlet connected with a sodium hydroxide trap and a dropping funnel with a pressure-equalizing side-arm, into which a solution of dry α,ω-dihydroxy(3,6-dioxa) octane, i.e. triethyleneglycol (15·02 g, 0·1 mol) and *N*-ethyl-*N*,*N*-diisopropylamine (25·86 g, 0·2 mol) in chloroform (50 ml) had been charged. This solution was added dropwise to the phosgene solution under stirring, while a stream of nitrogen was flushed above the level of the contents of the flask, which was cooled by means of an external bath at 5°C. After addition, stirring at 5°C was continued for a further 15 min, then the gas inlet was lowered and nitrogen bubbled through the reaction mixture for 30 min, in order to eliminate the excess phosgene. The whole process was complete in 2 h.

TABLE 1. Influence of reaction time on the intrinsic viscosity of PLGA–triethylene glycol copolymer[a]

Reaction time (h)	$[\eta]^b$ (dl g^{-1})
2·5	0·60
4·5	0·63
6·5	0·63
7·5	0·70
24	0·83

[a] Number average molecular weight of the starting oligomer: 2640.
[b] Intrinsic viscosity in chloroform at 32°C.

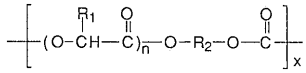
The same procedure was also followed in the case of oligomeric diols, such as PEG or PCL. The α,ω-bischloroformates obtained were not isolated, but directly treated with a solution of PLGA oligomer, EDPA and DMP in dry, alcohol-free chloroform. A typical procedure is the following. To a bischloroformate solution, a solution of PLGA oligomer (0·1 mol), *N*-ethyl-*N*,*N*-diisopropylamine (28·86 g, 0·2 mol) and 4-dimethyl aminopyridine (12·22 g, 0·1 mol) in chloroform (500 ml) was added under nitrogen atmosphere. The reaction mixture was maintained at 5°C for 8 h under stirring, and then for a further 10 h while rising to room temperature. After this time, the solvent was eliminated by evaporating under vacuum. The resulting product was then dissolved in chloroform, and precipitated, with excess ether. The precipitate was redissolved in chloroform, reprecipitated with excess isopropanol, and finally extracted with ether in a Soxhlet apparatus.

However, a study performed by measuring at intervals the intrinsic viscosity of the product prepared by using the bis-chloroformate of triethyleneglycol showed that the latter reaction reached a reasonably high value after a few hours. The results are reported in Table 1.

3. PREPARATION AND PROPERTIES OF DIFFERENT FAMILIES OF PLGA-BASED POLY(ESTER–CARBONATES)

The chain extension reaction between PLGA and low molecular weight diols, leads to high molecular weight

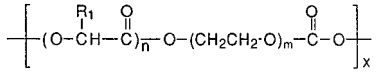
poly(ester-carbonates) (PLGAC) of the following general structure:



R_1 = H or CH_3 molar ratio 1 : 1
R_2 = $(CH_2CH_2O)_2CH_2CH_2$ (PLGAC-1);
$CH_2CH_2CH_2CH_2CH_2CH_2$ (PLGAC-2);
$CH_2{-}(CH_3)_2{-}CH_2$ (PLGAC-3)

The main products obtained are reported in Table 2.

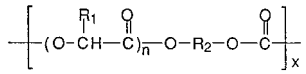
The chain extension reaction between PLGA and PEGs leads to high molecular weight PLGA-PEG segmented copolymers of the following general structure:



R_1 = H or CH_3 at different molar ratios
PLGA50 : 50-PEG1, m = 22·3;
PLGA50 : 50-PEG2, m = 45·0;
PLGA50 : 50-PEG3, m = 90·5;
PLGA50 : 50-PEG4, m = 181·4;
PLGA75 : 25-PEG1, m = 22·3;
PLA–PEG1, m = 22·3;
PLA–PEG2, m = 45·0.

The composition of typical segmented copolymers obtained from PLGA oligomers having different compositions of lactic and glycolic acids is reported in Table 3.

The chain extension reaction between PLGA and PCL diol-terminated oligomers leads to high molecular weight PLGA-PCL segmented copolymers of the following general structure:



R_1 = H or CH_3 at different molar ratios

R_2 = $-[-(CH_2)_5{-}COO-]_a-CH_2CH_2-[-OOC-(CH_2)_5-]_b-$

According to the synthetic pathway reported above, two series of samples were prepared by using as starting materials two PLGA oligomers having LA : GA molar ratios 75 : 25 and 50 : 50 respectively. In both series, the average molecular weights of PCL starting oligomers ranged between 530 and 2000 (see Table 4).

3.1 Molecular weight characterization

The molecular weights of the PLGA segmented copolymers have been estimated by gel permeation chromatography (GPC) using chloroform as mobile phase, against polystyrene standards. Intrinsic viscosities were also determined in the same solvent.

Some molecular weight characterizations of PLGACs, in comparison with those of the starting oligomers and of a typical commercial PLGA sample employed in drug delivery devices, are reported in Table 2. A 40-fold increase in the number average molecular weight of the PLGAC copolymers with respect to those of PLGA oligomers obtained by thermal polycondensation was observed. The resulting products, in fact, had molecular weights of the same order as, or higher than, most good quality PLGA samples presently marketed, which, as far as we know, are produced by ring-opening polymerization processes. The polydispersity index $\bar{M}_w/\bar{M}_n$ of PLGAC-1 was of the same order as that of the commercial sample, while in the case of PLGAC-2 and PLGAC-3 it was appreciably higher.

The results for PLGA-PEG copolymers are reported in Table 3. Their intrinsic viscosities were also determined in chloroform. They clearly show that the chain-extension reaction between PLGA oligomer and PEG leads to higher molecular weights than commercial PLGA. Moreover, the segmented PLGA-PEG copolymers have higher molecular weights than the PLGA chain-extension products obtained from 'simple' diols (see also Table 2 for comparison).

If we calculate the number average polymerization degrees $\bar{X}_n$ by considering as co-monomers the PLGA oligomer and the PEG employed (see Table 5, to which for comparison purposes the data relative to the PLGAC series have been added), we observe that in

TABLE 2. Main PLGAC copolymers synthesized via chain-extension reaction

Code	Oligomer			Diol	Product		
	LA : GA[a] molar ratio	$[\eta]^b$ (dl g^{-1})	$\bar{M}_n{}^c$		$[\eta]^b$ (dl g^{-1})	$\bar{M}_n{}^d$	$\bar{M}_w{}^d$
PLGAC-1	1 : 1	0·12	2 640	Triethylene glycol	0·83	91 000	180 000
PLGAC-2	1 : 1	0·08	1 590	1,6-Hexanediol	0·56	41 000	107 000
PLGAC-3	1 : 1	0·08	1 590	2,2-Dimethyl-1,3-propanediol	0·30	18 000	62 000
PLGA75 : 25	3 : 1	–	–	–	0·54	53 000	105 000

[a] LA and GA represent lactic and glycolic acid residues, respectively.
[b] Intrinsic viscosity in chloroform at 32°C.
[c] Number average molecular weight determined by end-group titration.
[d] Calculated by GPC using a polystyrene calibration curve.

TABLE 3. Main PLGA-PEG copolymers synthesized via chain-extension reaction

Code	R^2 [a]	Starting PLGA Oligomer[b]			Copolymer		
		LA : GA[b] molar ratio	$[\eta]$[c] ($dl\,g^{-1}$)	$\bar{M}_n$[d] $\times10^{-3}$	$[\eta]$[c] ($dl\,g^{-1}$)	$\bar{M}_n$[e] $\times10^{-3}$	$\bar{M}_w$[e] $\times10^{-3}$
PLGA50 : 50-PEG1	PEG1000[f]	1 : 1	0·09	1·92	1·74	239	434
PLGA50 : 50-PEG2	PEG2000[f]	1 : 1	0·09	1·92	1·55	197	370
PLGA50 : 50-PEG3	PEG4000[f]	1 : 1	0·09	1·92	n.d.	194	370
PLGA50 : 50-PEG4	PEG8000[f]	1 : 1	0·09	1·92	1·16	111	276
PLGA75 : 25-PEG1	PEG1000[f]	3 : 1	0·13	2·15	1·43	102·1	253·4
PLA-PEG1	PEG1000[f]	–	0·17	3·05	1·20	86·0	208·3
PLA-PEG2	PEG2000[f]	–	0·17	3·05	1·63	119·5	301·2

[a] General formula $[-O-CO-(-CHR^1-O-CO-)_x-R^2-]_y$ where R^1 is H or CH_3.
[b] LA and GA represent lactic and glycolic acid residues, respectively.
[c] Intrinsic viscosity in chloroform at 32°C.
[d] Number average molecular weight determined by end-group titration.
[e] Calculated by GPC using a polystyrene calibration curve.
[f] The number refers to the PEG's $\bar{M}_n$; the values are those reported by the suppliers.

PLGA50 : 50-PEG series, up to PEG4000, PEG derivatives seem to give better reaction yields than simple aliphatic diols, among which 1,6-hexanediol performs better than 2,2-dimethyl-1,3-propanediol, probably because of the steric hindrance exerted by the methyl substituents of the latter. PEG8000 gives in this respect rather poor results, but it may be considered that in this case it is less easy to achieve an exact stoichiometric ratio between the reagents with the exclusion of molarly significant traces of water. However, the PEG oligomer being the same, the number average polymerization degrees of the copolymers decrease with increasing lactic/glycolic acid ratio in the starting PLGA oligomer. This can possibly be ascribed to the less pronounced reactivity of secondary hydroxylic groups derived from lactic acid moieties, when present at PLGA chain-ends.

It should be noted that the above discussion is based on molecular weight determinations performed using polystyrene standards. These might not be equally appropriate, or for that matter equally inappropriate, for all the samples considered, which vary widely in composition. However, we feel it unlikely that such a difference, if any, would be sufficiently large to completely upset, at least from a qualitative standpoint, our considerations.

The results of molecular weight determinations of PLGA-PCL copolymers, estimated by GPC using chloroform as mobile phase, are reported in Table 4. It is

TABLE 4. Main PLGA-PCL copolymers synthesized via chain-extension reaction

Code	R^2 [a]	Starting PLGA oligomer			Copolymer		
		LA : GA[b] molar ratio	$[\eta]$[c] ($dl\,g^{-1}$)	$\bar{M}_n$[d] $\times10^{-3}$	$[\eta]$[c] ($dl\,g^{-1}$)	$\bar{M}_n$[e] $\times10^{-3}$	$\bar{M}_w$[e] $\times10^{-3}$
PLGA50 : 50-PCL1	PCL530[f]	1 : 1	0·11	1·93	0·40	8·4	31·5
PLGA50 : 50-PCL2	PCL1250[f]	1 : 1	0·11	1·93	1·20	22·0	114·7
PLGA50 : 50-PCL3	PCL2000[f]	1 : 1	0·11	1·93	0·82	27·0	113·7
PLGA50 : 50-PCL1	PCL530[f]	3 : 1	0·13	2·83	1·49	18·0	97·1
PLGA75 : 25-PCL2	PCL1250[f]	3 : 1	0·13	2·83	1·45	26·6	133·7
PLGA75 : 25-PCL3	PCL2000[f]	3 : 1	0·13	2·83	0·81	22·8	123·9

[a] General formula $[-O-CO-(-CHR^1-O-CO-)_x-R^2-]_y$ where R^1 = H or CH_3.
[b] LA and GA represent lactic and glycolic acid residues, respectively.
[c] Intrinsic viscosity in chloroform at 32°C.
[d] Number average molecular weight determined by end-group titration.
[e] Calculated by GPC using polystyrene calibration curve.
[f] The number refers to the PCL's $\bar{M}_n$; the values are those reported by the suppliers.

TABLE 5. Number average degrees of polymerization $\bar{X}_n$ of PLGA copolymers[a]

Code	$\bar{X}_n$
PLGA 50 : 50-PEG1	163·7
PLGA 50 : 50-PEG2	100·5
PLGA 50 : 50-PEG3	65·5
PLGA 50 : 50-PEG4	22·4
PLGA 75 : 25-PEG1	64·8
PLA-PEG1	42·5
PLA-PEG2	47·3
PLGAC-1	66·2
PLGAC-2	48·0
PLGAC-3	21·3

[a] Calculated by considering as co-monomers the starting PLGA oligomer, and diol.

apparent that both number-average and weight-average molecular weights of all PLGA-PCL samples are lower than those of PLGAC and PLGA-PEG, while their polidispersity is substantially larger. Moreover, a strict correlation between the molecular weight of the materials and their composition, both in terms of lactic/glycolic acid molar ratio and length of PCL segments, was not evident.

3.2 Solubility properties

The knowledge of the solubility behaviour in organic solvents of PLGA copolymers is essential, especially in view of possible transformation processes from solutions (see for instance microsphere preparations).

The solubility behaviour of the materials was determined by a standard procedure, which consisted of weighing our known amounts (40 mg) of the products, and treating them with the solvent (2 ml) for 4 h at room temperature. After this time, if complete dissolution was not observed, the mixtures were warmed to the boiling point and the result observed.

The results of the solubility tests for PLGAC copolymers are reported in Table 6. Broadly speaking, the solubility behaviour of PLGACs in organic solvents differs to some extent from that of commercial PLGA. Nevertheless, there are some differences inside the series which cannot be accounted for only on the basis of differences in molecular weight. For instance, triethyleneglycol moieties, with respect to hydrocarbon moieties, increase the affinity towards ethyl acetate, but decrease the affinity towards other solvents.

The results of solubility tests for PLGA-PEG copolymers are reported in Table 7. It may be observed that all the samples exhibit on the whole, a similar behaviour despite their widely different composition regarding the relative weights of PLGA and PEG segments and the lactic/glycolic acid molar ratio in PLGA segments. Major differences, as expected, are found in their behaviour towards hydroxylated solvents. As a further observation, PLGA50 : 50-PEG4 exhibits some unusual features if compared with the other members of the series. It dissolves completely in warm alcohols, and nearly completely in water. From several solvents, it reprecipitates in a 'dry' state on cooling, a feature which is probably related to its tendency to crystallize.

The results of the solubility experiments on PLGA-PCL are reported in Table 8. It may be observed that the products are soluble in many organic solvents such as chloroform, acetone, acetonitrile and ethyl acetate, and insoluble in aqueous media. Moreover, the solubility in ethyl acetate decreases by increasing the lactic

TABLE 6. Solubility properties of PLGAC copolymers

Solvent	PLGAC-1		PLGAC-2		PLGAC-3		PLGA 75 : 25	
	RT[a]	BP[a]	RT	BP	RT	BP	RT	BP
Water	i[b]	i	i	i	i	i	i	i
Methanol	i	i	i	i	i	i	i	i
Ether	i	i	i	i	i	i	i	i
Dioxane	i	i	sw[b]	sw	sw	sw	s[b]	s
Chloroform	s	s	s	s	s	s	s	s
Ethyl acetate	i	s	sw	sw	sw	sw	s	s
Acetone	i	s	sw	s	s	s	s	s
Toluene	i	i	i	sw	i	s	sw	s
DMF[c]	i	s	s	s	s	s	s	s
DMSO[d]	i	i	s	s	i	i	s	s
n-Heptane	i	i	i	i	i	i	i	i
Cyclohexane	i	i	i	i	i	i	i	i

[a] RT, at room temperature; BP, at solvent boiling point.
[b] i, Insoluble; s, soluble; sw, swellable.
[c] *N*,*N*-dimethylformamide.
[d] Dimethyl sulphoxide.

TABLE 7. Solubility properties of PLGA-PEG copolymers

Solvent	PLGA50 : 50-PEG1		PLGA50 : 50-PEG2		PLGA50 : 50-PEG3		PLGA50 : 50-PEG4		PLGA75 : 25-PEG1		PLA-PEG1		PLA-PEG2	
	RT[a]	BP[a]	RT	BP	RT	BP	RT	BP	RT	BP	RT	BP	RT	BP
Water	i[b]	i	i	i	i	i	cd[b]	cd	i	i	i	i	i	i
Methanol	sw[b]	sw	sw	sw	sw	sw	cd	s	i	i	sw	sw	sw	s[b]
Ether	i	i	i	i	i	i	i	i	i	i	i	i	i	i
Dioxane	s	s	s	s	s	s	s	s	i	i	i	i	i	i
Chloroform	s	s	s	s	s	s	s	s	s	s	s	s	s	s
Ethyl acetate	s	s	s	s	i	s	cd	s	sw	sw	i	i	i	i
Acetone	s	s	s	s	sw	s	cd	s	s	s	s	s	s	s
Toluene	sw	sw	sw	s	s	s	cd	cd	i	sw	i	i	i	sw
DMF[c]	s	s	s	s	s	s	cd	s	s	s	s	s	s	s
DMSO[d]	s	s	s	s	s	s	cd	s	s	s	s	s	s	s
n-Heptane	i	i	i	i	i	i	i	i	i	i	i	i	i	i

[a] RT, at room temperature; BP, at boiling point.
[b] i, Insoluble; s, soluble; sw, swellable; cd, cloud dispersion.
[c] *N*,*N*-dimethylformamide.
[d] Dimethyl sulphoxide.

acid moieties content of PLGA segments. All the samples exhibit similar behaviour, irrespective of the length of PCL segments.

3.3 Water absorption experiments

Owing to the strict correlation existing between the degradation behaviour of biodegradable materials and their hydrophilicity, water absorption experiments were performed on PLGAC, PLGA-PEG and PLGA-PCL copolymers. The procedure consisted of weighing a known quantity of the product, in the form of 0.5 mm thick sheet, and allowing it to stay in contact with a liberal excess of distilled water for 8 h at 20°C.

The results of these short-term water absorption experiments are reported in Table 9. It may be observed that the water absorption capability of PLGAC copolymers is similar within the series, and larger than commercial PLGA by an order of magnitude. Within the PLGA-PEG series, as expected, the amount of water absorbed is directly related to the length of the PEG segments and always larger than in the case of PLGAC samples. Because, as previously mentioned, in PLGA-based polymers the rate of degradation in aqueous media mainly depends on the degree of swelling,[20] which in the PLGA-PEG series depends in turn on the PEG sample used as starting material, we have a simple and reliable tool for tailoring the permanence of the new PLGA-PEG copolymers within living organisms. As regards the PLGA-PCL series, all the samples show low water absorption values. The hydrophilicity of the copolymers decreases, all the other conditions being equal, by increasing the lactic/glycolic acid molar ratio in the PLGA segments. However, the influence of the

TABLE 8. Solubility properties of PLGA-PCL copolymers

Solvent	PLGA 50 : 50-PCL530		PLGA 50 : 50-PCL1250		PLGA 50 : 50-PCL2000		PLGA 75 : 25-PCL530		PLGA 75 : 25-PCL1250		PLGA 75 : 25-PCL2000	
Water	i[a]	i	i	i	i	i	i	i	i	i	i	i
Phosphate buffer	i	i	i	i	i	i	i	i	i	i	i	i
Isopropyl alcohol	i	i	i	i	i	i	i	i	i	i	i	i
Acetone	s[a]	s	s	s	s	s	s	s	s	s	s	s
Ethyl acetate	cd[a]	cd	cd	cd	cd	cd	s	s	s	s	s	s
Acetonitrile	s	s	s	s	s	s	s	s	s	s	s	s
Chloroform	s	s	s	s	s	s	s	s	s	s	s	s
Ether	i	i	i	i	i	i	i	i	i	i	i	i
Heptane	i	i	i	i	i	i	i	i	i	i	i	i

[a] i, Insoluble; s, soluble; cd, cloudy dispersion.

TABLE 9. Water absorption capacity of PLGA-based copolymers[a]

Sample	Water absorption[b]
PLGAC-1	0·30
PLGAC-2	0·30
PLGAC-3	0·45
PLGA 75 : 25	0·02
PLGA50 : 50-PEG1	1·06
PLGA50 : 50-PEG2	3·61
PLGA50 : 50-PEG3	11·21
PLGA50 : 50-PEG4	Suspendible
PLGA75 : 25-PEG1	0·86
PLA-PEG1	0·73
PLA-PEG2	2·15
PLGA50 : 50-PCL1	0·20
PLGA50 : 50-PCL2	0·11
PLGA50 : 50-PCL3	0·12
PLGA75 : 25-PCL1	0·03
PLGA75 : 25-PCL2	0·03
PLGA75 : 25-PCL3	0·02

[a] After 8 h at 20°C.
[b] Evaluated by using the following equation: WA = $(W_s - W)/W$, where WA is water absorption, W_s is the weight of the swollen polymer after 8 h, and W_0 is the weight of the polymer in the dry state.

length of PCL segments on hydrophilicity is not clearly delineated. The products derived from PCL1250 show lower water absorption values than those derived from PCL2000, in spite of the fact that the latter have longer segments, i.e. a higher proportion of the more hydrophobic component, as well as a higher degree of crystallinity.

3.4 Thermal characterization

The glass transition temperatures of PLGAC-1, PLGAC-2 and PLGAC-3 are fairly different being 45, 33·7 and 26·2°C, respectively. They apparently depend more on the molecular weight of the products than on the nature of the diol moieties. None of the samples showed evidence of crystallinity.

Some DTA data on PLGA-PEG samples and the starting PEGs are reported in Table 10. The T_g of PLGA50 : 50-PEG3 and PLGA50 : 50-PEG4 and those of the starting PEGs were not revealed using our DTA instrument. In all the other cases it could be unambiguously detected. It should be observed that, the complementary oligomer being the same, higher molecular weight PEGs give copolymers having lower T_g values. However, the influence on the T_g of the composition of PLGA segments is not straightforward.

The thermograms of PLGA50 : 50-PEG2–4 exhibits sharp evidence of crystallinity, with melting points 44·6, 57·1, and 57·4°C, respectively. The corresponding heats of fusion ΔH_m increased from PLGA50 : 50-PEG2 to PLGA50 : 50-PEG4, and the calculated degrees of crystallinity increased correspondingly. Moreover, the melting points of these samples are not far apart from those of the parent PEGs. All these results lead to the conclusion that the crystallization process involves the separation of ordered PEG domains, whose amount is obviously quantitatively larger in the case of PLGA-PEGs having longer PEG segments, i.e. larger PEG contents.

As regards the PLGA-PCL series, the thermal properties of all samples, as well as those of the starting PLGA and PCL oligomers, were also studied by means of DTA. Calorimetric data are summarized in Table 11.

TABLE 10. DTA data on PEGs and PLGA-PEG copolymers[a]

Sample	PEG (w%)	T_g (°C)	M.p.[b] (°C)	ΔH_m[c] (J g^{-1})	Degree of crystallinity[d] (%)
PEG1000	–	–	36·9	154·7	–
PEG2000	–	–	52·8	179·2	–
PEG4000	–	–	59·9	188·1	–
PEG8000	–	–	62·4	180·5	–
PLGA50 : 50-PEG1	34	−5·7	–	–	–
PLGA50 : 50-PEG2	51	−27·1	44·6	42·2	46
PLGA50 : 50-PEG3	67·6	–	57·1	79·7	63
PLGA50 : 50-PEG4	80·6	–	57·4	123·6	85
PLGA75 : 25-PEG1	6·6	19·7	–	–	–
PLA-PEG1	31·8	−3·5	–	–	–
PLA-PEG2	24·7	6·5	–	–	–

[a] Scanning rate 10·0°C min^{-1}.
[b] Melting point.
[c] Heat of fusion.
[d] Degree of crystallinity: $(\Delta H(\text{PLGA-PEG})/(\Delta H(\text{PEG}) \times \text{w\% of PEG}) \times 10^4$.

TABLE 11. DTA data on starting PCL and PLGA-PCL segmented copolymers[a]

Sample	PCL[b] (wt%)	T_g (°C)	M.p.[c] (°C)	ΔH_m[c] (J g^{-1})	C[e] (%)
PCL530		−77·1	31·3	41·9	
PCL1250		−70·3	45·7	70·7	
PCL2000		−59·1	49·7	75·4	
PLGA50 : 50-PCL530	21·54	25·7	–	–	–
PLGA50 : 50-PCL1250	39·31	−57·9	40·3	16·6	59·7
PLGA50 : 50-PCL2000	50·89	−45·3	49·4	24·4	63·6
PLGA75 : 25-PCL530	15·77	22·6	–	–	–
PLGA75 : 25-PCL1250	30·64	20·2	–	–	–
PLGA75 : 25-PCL2000	41·41	−66·5	35·6	6·1	19·5

[a] Scanning rate 10·0°C min^{-1}.
[b] Weight percentage PCL within the copolymer.
[c] Melting point.
[d] Heat of fusion.
[e] Percentage crystallinity evaluated using the equation $C = \Delta H_m\ 100/\Delta H_m^0 w$, where ΔH_m^0 is the heat of fusion of the starting PCL, ΔH_m is the heat of fusion of the segmented copolymer and w is the weight ratio of PCL in the copolymer.

It should be observed that products PLGA50 : 50-PCL1, PLGA75 : 25-PCL1 and PLGA75 : 25-PCL2 are amorphous, with T_g values in the range 20–30°C. All the other samples showed some degree of crystallinity, related to the presence of long PCL segments which presumably constitute separate domains. Melting points within the range 30–50°C were observed.

It may also be noticed that the percentage crystallinity increases with the molecular weight of the PCL segment, i.e. with the weight fraction of PCL. Moreover, the starting PCL being the same, the degree of crystallinity decreases by increasing the lactic/glycolic acid molar ratio in PLGA segments.

3.5 Degradation behaviour

The degradation behaviour in aqueous media of PLGAC copolymers and PLGA50 : 50-PEG segmented copolymers, as well as, for comparison purposes, a typical commercial sample of PLGA was investigated according to the methods summarized in Table 12. The samples used for the experiments were in the form of sheets of dimensions 1 cm × 0·5 cm × 0·4 cm and of microspheres.

The sheets were obtained by compression moulding at 80°C and 25 kg cm^{-2} for 10 min. This procedure did not induce appreciable degradation, because no significant changes of the intrinsic viscosity of the samples occurred, and there was no discoloration of the products. The sheets were translucent, pliable, and fairly tough.

The microspheres were prepared by solvent evaporation from stirred suspensions in water of solutions of the polymers in chloroform, or methylene chloride, according to the literature.[36] In order to ensure size uniformity among samples, the microspheres were sieved using 120–230 mesh sieves. The microspheres were examined by optical microscopy and scanning

TABLE 12. Degradation conditions and tests

Degradation conditions	Sample form	Degradation tests
Temperature 37°C, 0.025 M phosphate buffer pH 7·4, ionic strength 0.13 with NaCl	Sheets	Water absorption with time Weight loss with time Variation of the molecular weight of the residue with time Variation of the molecular weight of the solubilized part with time Variation of pH of the solution with time Dissolution time
	Microspheres	Morphological variation with time Dissolution time

electron microscopy (SEM). Differences were observed between the microspheres obtained from methylene chloride, and those obtained from chloroform, the former having a shrunken and porous aspect, probably because of the higher volatility of the solvent.

The degradation experiments were in all cases performed under static conditions by dipping a known amount of material (usually 80 mg) in 0.025 M phosphate buffer pH 7.4 (5 ml) inside sealed glass tubes, and placing the mixture in a thermostatic bath at 37°C.

Water absorption with time. Figure 1 reports the results of water absorption tests for both PLGAC-1 and commercial PLGA. They show a steady increase in water absorption capacity for both materials, even at very prolonged times. This can be related to their tendency to degrade in aqueous solutions, becoming more and more hydrophilic. The tendency of PLGAC copolymers to degrade is higher than that of PLGA, as could be predicted from their higher hydrophilicity.

As regards the PLGA-PEG series, only PLGA50 : 50-PEG1 was considered for this test, because copolymers PLGA50 : 50-PEG3 and 4 were very hydrophilic per se, and were not studied owing to their poor mechanical properties in the swollen state. The results are shown in Fig. 2.

Weight loss with time. Curves of weight loss versus time for PLGAC-1, and for commercial PLGA are shown in Fig. 3. It is apparent that the rate of degradation is higher for PLGAC-1 than for commercial PLGA, in agreement with other tests.

Data of weight loss versus time for samples PLGA50 : 50-PEG1, PLGA50 : 50-PEG2 and PLGA50 : 50-PEG3, in the form of sheets are shown in Fig. 4. It is apparent that rate of weight loss increases in the order: commercial PLGA$\langle$PLGA50 : 50-PEG1 < PLGA50 : 50-PEG2 < PLGA50 : 50-PEG3.

The weight loss measured after 14 days for PLGA-PCL copolymers in water and in aqueous media are reported in Table 13, together with dissolution times measured in phosphate buffer. It can be observed that samples PLGA50 : 50-PCL1 and PLGA75 : 25-PCL1 show degradation rates similar to that of a commercial

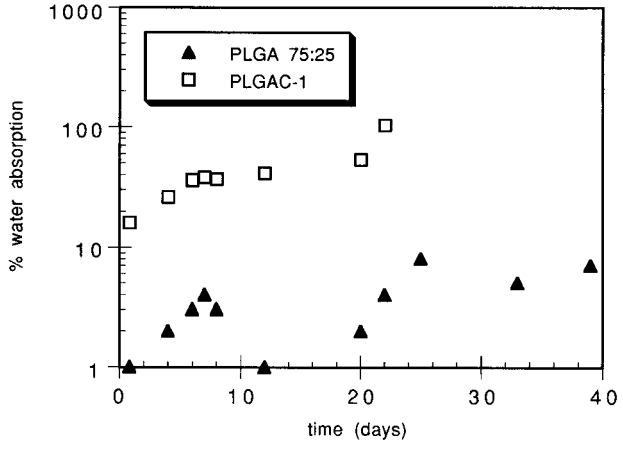


Fig. 1. Water absorption with time of PLGAC-1 and commercial PLGA.

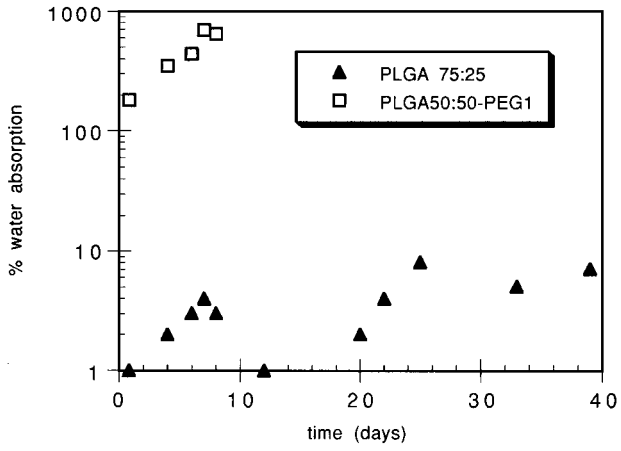


Fig. 2. Water absorption with time of PLGA50 : 50-PEG1 and commercial PLGA.

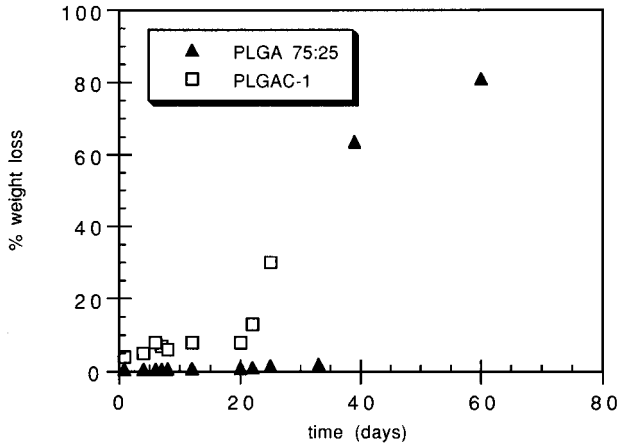


Fig. 3. Weight loss with time of PLGAC-1 and commercial PLGA.

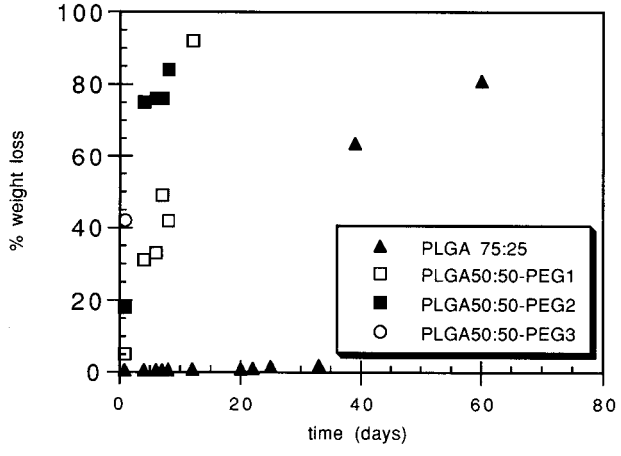


Fig. 4. Weight loss with time of PLGA50 : 50-PEG copolymers and commercial PLGA.

TABLE 13. Weight loss and dissolution times of PLGA-PCL copolymers

Sample	Weight loss[a]		Dissolution time (months)
	In water	In buffer	
PLGA50 : 50-PCL1	9·1	8·0	1·5
PLGA50 : 50-PCL2	11·7	16·7	n.c.d.[b]
PLGA50 : 50-PCL3	9·2	14·1	n.c.d.[b]
PLGA75 : 25-PCL1	4·6	5·1	2·5
PLGA75 : 25-PCL2	4·1	3·9	n.c.d.[b]
PLGA75 : 25-PCL3	4·9	4·7	n.c.d.[b]

[a] After 14 days at 37°C.
[b] Not completely dissolved after 8 months.

PLGA75 : 25 sample (see Table 14), while all the other samples exhibit much lower degradation rates. Moreover, the other conditions being equal, the degradation rate decreases by increasing the lactic/glycolic acid molar ratio in PLGA segments, and decreases even more strongly by increasing the length of PCL segments. This behaviour can be tentatively explained by the lower hydrolytic susceptibility of the ester groups of PCL compared with those of PLGA, and also by the presence of crystalline domains in PLGA-PCL samples with longer PCL segments.

Variation with time of the molecular weight of the residue. The variations of the number average molecular weight of the undissolved residues versus time for PLGAC-1 and PLGA are reported in Fig. 5. The degradation rate is higher for PLGAC-1, possibly because of the higher hydrophilicity of its macromolecular structure, as already stated. Moreover, it may be noted that, while in the case of commercial PLGA the molecular weight decreases linearly with time during the degradation experiments, in the case of PLGAC-1 an irregular pattern is observed.

The variations of number average molecular weight of the undissolved residues versus time of PLGA-PEG samples are reported in Fig. 6. It may be observed that all samples exhibit similar behaviour.

TABLE 14. Dissolution times for PLGA-PEG copolymers

Sample	Dissolution time (days)	
	Microspheres	Sheets
PLGA 75 : 25	>60	82
PLGA50 : 50-PEG1	41	12
PLGA50 : 50-PEG2	7	4
PLGA50 : 50-PEG3	4	1

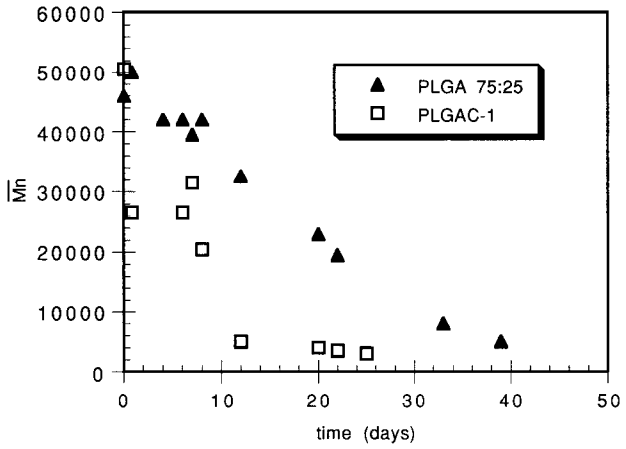


Fig. 5. Time-dependence of the number-average molecular weight of the residue for PLGAC-1 and commercial PLGA.

Variation with time of the molecular weight of the solubilized part. A possible explanation of the irregular pattern obtained in the experiments reported in the previous paragraph lies in the solubilization of relatively high molecular weight PLGA segments by attached diol or PEG segments. This led us to analyse by GPC in aqueous media the molecular weight of the solubilized part of the samples used in the degradation experiments.

Typical results relative to PLGAC-1 and corresponding to two different degradation times are reported in Fig. 7. The GPC traces reported clearly indicate, at intermediate degradation times (see curve b), the presence of species having molecular weight higher than those of monomeric lactic and glycolic acids and of triethylene glycol, probably consisting of segments of PLGA covalently bound to diol residues.

Similar results were obtained for PLGA-PEG samples. For instance, the results relative to PLGA50 : 50-PEG1 are reported in Fig. 8. For comparison purposes, we report here that under our conditions the starting PEGs had retention times of

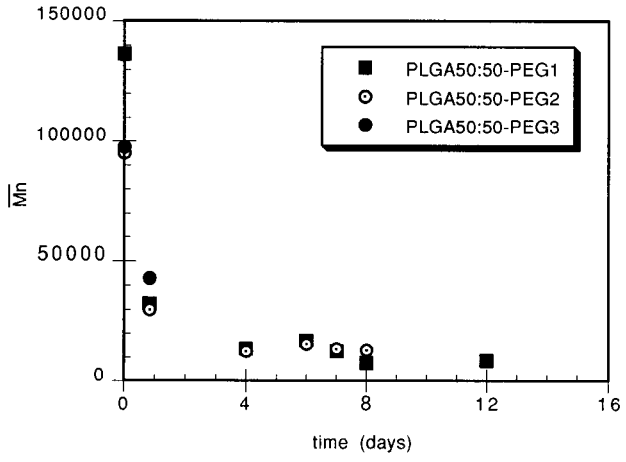


Fig. 6. Time-dependence of the number-average molecular weight of the residue for PLGA50 : 50-PEG1–3.

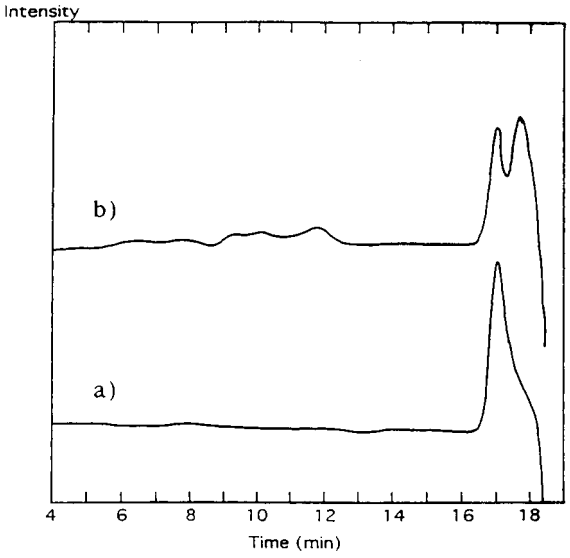


Fig. 7. GPC curves of the buffer solution in contact with PLGAC-1 at different times: (a) after 20 h; (b) after 33 days.

16·81 min (PEG1000), 16·01 min (PEG2000) and 15·27 min (PEG4000). The corresponding peak widths at half-height were 0·96 min, 0·94 min and 1·10 min, respectively. In this case also, species having molecular weight higher than monomeric lactic acid and glycolic acid are present at intermediate degradation times (see curve b of Fig. 8). In particular, the peak at 10·4 min, which is very close to the column exclusion limit, corresponds to high molecular weight, macromolecular soluble species. The peak at 15·72 min, which is intermediate between those of PEG2000 and PEG4000, corresponds to molecular species larger than PEG1000. It can be related to short PLGA fragments covalently linked to PEG chains. It can also be observed that the area of this peak steadily increases with time. For long reaction times the GPC trace shows evidence only of monomeric acids and parent PEGs, as expected, as a consequence of complete hydrolysis of the soluble products. The results obtained with PLGA50 : 50-PEG2 and 3 were qualitatively similar to those of PLGA50 : 50-PEG1.

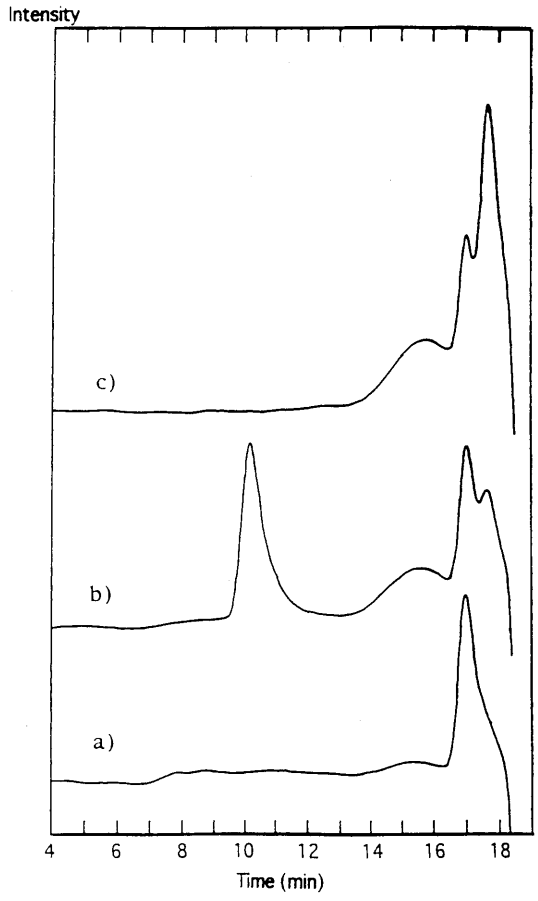


Fig. 8. GPC curves of the buffer solution in contact with PLGA-PEG1 at different times: (a) after 20 h; (b) after 33 days; (c) after 44 days.

Contrary to the results obtained with PLGAC and PLGA-PEG samples, the commercial PLGA sample under the same conditions did not show any evidence of the presence of high molecular weight species in solution. Only a peak at 17.02 min retention time was detectable, corresponding exactly to the retention time of both lactic and glycolic acids, which are not separated under our conditions.

Time-dependence of the pH of the supernatant during degradation experiments. A clear indicator of the proceeding of the degradation reaction is the variation of pH of the buffer solution in contact with samples throughout the degradation experiments.

In Fig. 9 the pH variation as a function of time for PLGAC-1 and commercial PLGA is reported. It should be noted that, while no significant variation of pH over a wide range of degradation times is observed for either material, in the case of PLGAC-1 after 20 days, the pH steadily decreases with time, because of the presence in solution of acidic degradation products. The results of the same experiment performed with PLGA50 : 50-

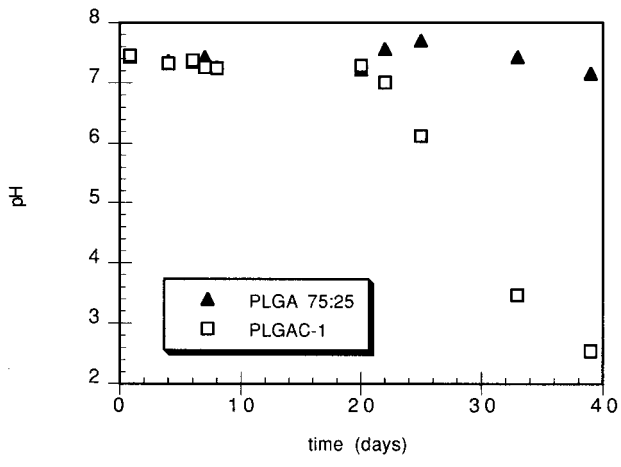


Fig. 9. Time dependence of pH of the buffer solution in contact with PLGAC-1 and PLGA.

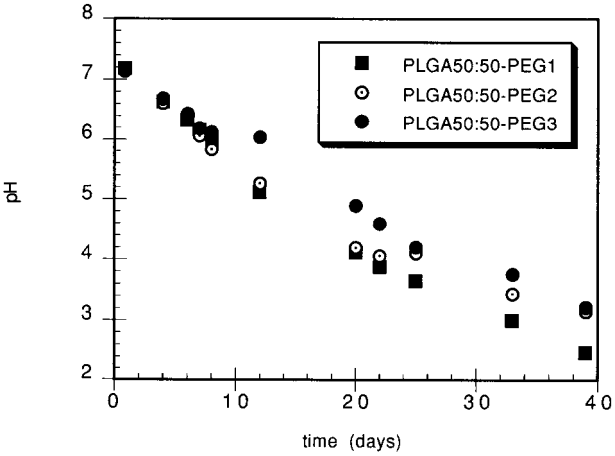


Fig. 10. Time dependence of pH of the buffer solution in contact with PLGA50 : -PEG1–3.

PEG1-3 are shown in Fig. 10. The pH of the solution decreases monotonically with time for all copolymers. This can probably be ascribed to the presence of degradation products into solution at early reaction times of PEG segments.

Dissolution time. The dissolution times for microspheres and sheets are 60 and 33 days, respectively, in the case of PLGAC-1, and more than 60 and 82 days, respectively, in the case of commercial PLGA.

The dissolution times increase as the total surface area of the specimens decreases, sheets dissolving considerably faster than microspheres. This is in agreement with previous literature reports on plain PLGA samples[27,29,37,38] and has been explained by the fact that the first degradation products do not diffuse away easily from specimens with a relatively low surface area and, being acidic, may exert their catalytic activity on the degradation reaction for significant lengths of time.

In the case of PLGA-PEG samples, dissolution times for both sheets and microspheres are reported in Table 14. In both cases, the dissolution times are decreased by increasing the length of the PEG segments present in the samples, e.g. the PEG content on a w/w basis. As pointed out above, at the same composition, the dissolution times of microspheres are invariably longer than those of sheets, the difference being higher at lower PEG content. Apparently, also in the PLGA-PEG series the first degradation products do not diffuse away easily. Their slow diffusion rate, in spite of the swollen state of the specimens, adds further evidence to the macromolecular nature of at least a portion of the degradation products.

4. CONCLUSIONS

The polycondensation reaction of PLGA thermal oligomers with the bischlorofomates of simple diols, and also of α,ω-bishydroxylated oligomers, proved to be a novel and effective procedure for obtaining new PLGA-based biodegradable polymeric materials. This chain-extension process leads to poly(ester-carbonates) whose molecular weight is as high as, or higher than, those of PLGA samples currently obtained by ring-opening polymerization. When telechelic oligomers are utilized as starting reagents, multiblock copolymers are obtained. By varying the nature and the length of the starting simple or oligomeric diols, together with the lactic/glycolic acid molar ratio in the starting PLGA oligomers, products with widely different physicochemical properties, such as thermal properties, hydrolytic susceptibility, swelling in water and solubility in organic solvents can be synthesized. In particular, dissolution times in aqueous media can be tuned within a large range, from a few days to several months. This provides exceptional opportunities for designing products whose properties are tailored for specific applications in the biomedical field.

REFERENCES

1 Vert, M., Schwarch, G. & Coudane, J., *Plast. Eng.*, (1995) 29.
2 Vert, M., Schwarch, G. & Coudane, J., *J. Macromol. Sci., Pure Appl. Chem.*, **A32** (1995) 787.
3 Mauduit, J. & Vert, M., *S.T.P. Pharma Sci.*, **3** (1993) 197.
4 Vert, M., Chabot, F., Leray, J. & Christel, P., *Makromol. Chem. Suppl. 5* (1981) 30.
5 Barrows, T. H., Synthetic bioabsorbable polymers, in *High Performance Biomaterials, a Comprehensive Guide to Medical and Pharmaceutical Applications*, ed. M. Szycher, Technomics Publishing, Lancaster, Pennsylvania USA, 1991, pp. 243–258.
6 Vert, M., Li, S. & Garreau, H., *J. Controlled Rel.*, **16** (1991) 15.
7 Asano, M., Fukuzaky, H. & Yoshida, M., *J. Contr. Rel.*, **9** (1989) 111.
8 Christel, P., Li, S. M., Vert, M. & Patat, J. L., Eur. Pat. Appl. 1991 EP564, 369.
9 Vert, M., Li, S. M., Spenlehauer, G. & Guerin, P., *J. Mater. Sci., Mater Med.*, **3** (1992) 432.
10 Anselme, K., Flautre, B., Hardouin, P., Chanavaz, M., Ustariz, C. & Vert, M., *Biomaterials*, **14** (1993) 44.
11 Fukuzahy, H., Yoshida, M. & Asano, M., *Eur. Polym. J.*, **24**, (1988) 1029.
12 Boyle, W. J., Allied-Signal, WO 89/05664 (1989).
13 Ferruti, P., Ranucci, E., Bignotti, F., Int. Pat. Appl. PCT/EP92/01262 (1992).
14 Ferruti, P., Penco, M., Ranucci, E. & Bignotti, F., Mediolanum Farmaceutici S.p.A.; Ital. Pat. Appl. 93/2364 (1993); Appl. WO 94/EP3560 (1994); Chem. Abstr., **123**, 287271.
15 Penco, M., Ranucci, E., Bignotti, F. & Ferruti, P., *Macromol. Rapid Commun.*, **15** (1994) 683.
16 Ferruti, P., Penco, M., D'Addato, P., Ranucci, E. & Deghenghi, R., *Biomaterials*, **16** (1995) 1423.
17 Penco, M., Marcioni, S., Ferruti, P., D'Antone, S. & Deghenghi, R., *Biomaterials*, **17** (1996) 1583.
18 Penco, M., Becattini, M., Ferruti, P., D'Antone, S. & Deghenghi, R., *J. Polym. Adv. Tech.*, **7** (1996) 536.
19 Ferruti, P., Penco, M. & Ranucci, E., Europeptides, Ital. Mi 96/A 00238 (1996).
20 Penco, M., Donetti, R., Ferruti, P. & Mendichi, R., *Biomaterials*, submitted.
21 Casey, D. J., Jarret, P. K. & Rosarf, L., American Cyanamid Company, U.S. Pat. 4,716,203 (1987).
22 Rashkov, I., Manolova, N., Li, S. M., Espartero, J. L. & Vert, M., *Macromolecules*, **29** (1996) 50.

23 Rashkov, I., Manolova, N., Li, S. M., Espartero, J. L. & Vert, M., *Macromolecules*, **29** (1996) 57.
24 Jacobs, C., Dubois, Ph., Jerome, R. & Teyssie, Ph., *Macromolecules*, **24** (1991) 3027.
25 Vanhoorne, P., Dubois, Ph., Jerome, R. & Teyssie, Ph., *Macromolecules*, **25** (1991) 37.
26 Li, S. M., Garreau, H. & Vert, M., *J. Mater. Sci., Mater. Med.*, **1** (1990) 123.
27 Holland, S. J., Tighe, B. J. & Gould, P. L., *J. Controlled Rel.*, **4** (1986) 155.
28 Chen, X., McCarthy, S. P. & Gross, R. A., *Macromolecules*, **30** (1997) 4295.
29 Shah, S. S., Cha, Y. & Pitt, C. G., *J. Controlled Rel.*, **18** (1992) 261.
30 Shih, C., *J. Controlled Rel.*, **34** (1995) 9.
31 Pitt, C. G., Gu, Z. *J. Controlled Rel.*, **4** (1987) 283.
32 Kulkarni, R. K., Moore, E. G., Hegyeli, A. F. & Leonard, F., *J. Biomed. Mater. Res.*, **5** (1971) 169.
33 Leeslang, J. W., Pennings, A. J., Bos, R. R. M., Rozema, F. R. & Boering, F., *Biomaterials*, **8** (1987) 311.
34 Pitt, C. G., Gratzi, M. M., Kimmel, G. L., Surles, J. & Schindler, A., *Biomaterials*, **2** (1981) 215.
35 Li, S. M., Garreau, H. & Vert, M., *J. Mater. Sci., Mater. Med.*, **1** (1990) 198.
36 Zhu, J. H., Shen, Z. R., Wu, I. T. & Yang, S. L., *J. Appl. Polym. Sci.*, **42** (1991) 2099.
37 Vert, M., Mauduit, J. & Li, S. M., *Biomaterials*, **15** (1994) 1209.
38 Grizzi, I., Garreau, H., Li, S. M. & Vert, M., *Biomaterials*, **16** (1995) 305.
39 Zhang, X., Wyss, U. P., Pichora, D. & Goosen, M. F. A. *Polym. Bull.*, **27** (1992) 623.
40 German Patent 890-792 (1955); Adam, K. & Trieschmann, H. G., *Chem. Abstr.*, **50** (1956) 12,099h.
41 Green, M., *Chem. Ind.*, (1961) 435.