Release of 5-fluorouracil by biodegradable poly(ester-ether-ester)s. Part I: release by fused thin sheets

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The 5-fluorouracil release by biodegradable ε-caprolactone and L-lactide copoly(ester-ether-ester)s was tested. The drug-copolymer mixture was formed by fusion in thin sheets, which were dipped in Dulbecco's PBS for time intervals ranging from one hour to two months. Each experiment shows a fast initial release, which subsequently slows down and stops at a limiting value, depending on the copolymer composition. This behavior was attributed to an extraction of the drug present on the sheet surface, due only to its shape, and to hydrogen bonds between the drug and the copolymers. The results obtained lead to a possibility of using such copolymers as ''time-delayed'' drug-releasing systems, when formed in specimens with smaller surface-to-volume ratio, which could minimize the fast initial extraction.

1. Introduction

In pharmacological therapy, it is necessary to maximize the therapeutic effect of drugs, minimizing any adverse effect, which can be achieved by delivering the drug at the site of action. A drug administered systemically is distributed in the body to various organs and tissues perfused with blood, so that only a relatively small amount reaches its target tissue.

Implanting a biodegradable device loaded with antineoplastic agent in the cavity created by a tumor provides high local concentrations of the drug, killing malignant cells which survived surgery; it also prevents the systemic side effects of chemotherapy, normally associated with intravenous administration [1].

In recent years many groups have investigated the use of two bioresorbable polyesters, poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA), as implantable or injectable biodegradable carriers for the controlled release of drugs. Transport of drugs from depot systems based on these polyesters is governed by the degradation properties of the polymers and by the relative drug loading of the implant [1, 2].

A drug delivery system with prolonged release of an anticancer drug needs the use of high molecular weight biodegradable polymers. However, such polymers lack suitable hydrophilicity. The use of poly(ether-ester) block copolymers can solve this problem: indeed the ether blocks assure a suitable hydrophilicity to the material, whereas the length of the polyester blocks can be varied corresponding to different degradation times of the copolymers. Some years ago, Zhu *et al*. tested *in vitro* poly(D,L-lactide)-*block*-poly(ethylene glycol) copolymers (PLA-PEG), for the release of norethisterone in water [3]. Unfortunately, until quite recently, such materials were synthesized using organometallic catalysts, like stannous octoate, which may leave potentially toxic tin residues in the copolymer.

More recently our group developed a thermally initiated synthesis of three-block poly(ester-ether-ester)s, through a ring-opening mechanism needing no catalyst [4, 5]. Such copolymers have been found to be biocompatible [6], bioresorbable [7] and bioactive [8] materials. We tested the possibility of using these poly(ester-ether-ester)s as drug-releasing materials by examining the *in vitro* release, into Dulbecco's phosphate buffered saline, of two drugs, namely vitamin B_{12} and tetracycline, by three different poly(L-lactide)-*block*-poly(oxyethylene)-*block*-poly(L-lactide) copolymers as biodegradable matrixes [9]. The release seemed very dependent on the technique of forming the specimens to be tested, which were quite thick tablets, sintered at room temperature by compression under

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vacuum. In particular, we observed two concurrent releasing processes, extraction of the drug by the buffer solution and its release into the solution due to the copolymer hydrolytic degradation. The release kinetics were also affected by the formation of buffer solution gradients within the tablets. As a consequence, we thought that a reduction of both the porosity and the thickness of the specimens, obtained by forming them by fusion into thin sheets, could minimize such difficulties.

The present paper deals with the release of the anticancer drug 5-fluorouracil (FU) by thin sheets, made by poly(ε-caprolactone)-*block*-poly(oxyethylene)-*block*-poly(ε-caprolactone) (PCL-POE-PCL) and poly(L-lactide)-*block*-poly(oxyethylene)-*block*-poly(L-lactide) (PLA-POE-PLA) copolymers, into Dulbecco's PBS (pH 7.3 ± 0.3).

2. Materials and methods

2.1. Copolymer synthesis

The PCL-POE-PCL and PLA-POE-PLA three-block copolymers were synthesized by reacting poly(ethylene glycol), of 35 000 Dalton average molecular mass (PEG-35 000, Merck), with ε-caprolactone (Fluka) at 185 °C and with L-lactide (Aldrich) at 140 °C in bulk, without catalyst, under vacuum, using reagents purified as already reported [4–9]. The copolymer formation occurs through the already described ring-opening mechanism [4, 5], where an active hydrogen atom of the preformed PEG causes a selective acyl-oxygen cleavage of an ester group of the monomer ring (see Fig. 1). After the initial formation of an intermediate bis-hydroxy-diester, a step-by-step addition of monomer units occurs with formation of two external polyester blocks, the length of which depends on the amount of cyclic ester monomer used in the feed. The compositions and number average molecular masses of the copolymers, calculated from ^{1}H nuclear magnetic resonance spectra, are reported in Table I.

2.2. Drug-releasing tests

Samples of the copolymers in Table I were mechanically mixed in a mortar with FU (Sigma), in a drug-to-copolymer weight ratio of 10 : 90; the resulting mixtures were formed in thin sheets of 500 μm thickness, by means of a SPECAC heating press connected to a HELLMA thermoregulator. The powder was compressed at about two tons for 1.5 min, at 60 °C. The same procedure was used to form ''blanks'', containing only the same quantities of copolymer as the sheets made by the drug-copolymer mixture.

The sheets were laid onto polycarbonate microporous (pore size 12.0 μm) culture plate inserts (Millicell), and dipped in Dulbecco's PBS (pH 7.3 ± 0.3) at 37 °C; at fixed time intervals, ranging from 1 h to 2 months, the eluates were collected and replaced with fresh buffer solution. The release of FU was measured spectrophotometrically at 300 nm, using as reference solutions the eluates of the corresponding ''blanks'', by a Shimadzu 2100 UV-visible spectrophotometer. The percentage of the drug released at each time was calculated by adding the FU concentrations found in the eluates, dividing by the theoretical concentration corresponding to a complete release of the same drug, and then multiplying by 100.

3. Results and discussion

The molar compositions and the hydrophilicity levels of the copolymers used as drug-releasing materials are shown in Table I. In each series of copolymers the hydrophilicity increases with increasing molar percentage of oxyethylene units in the chain; the PLA-POE-PLA copolymers are generally more hydrophilic than the

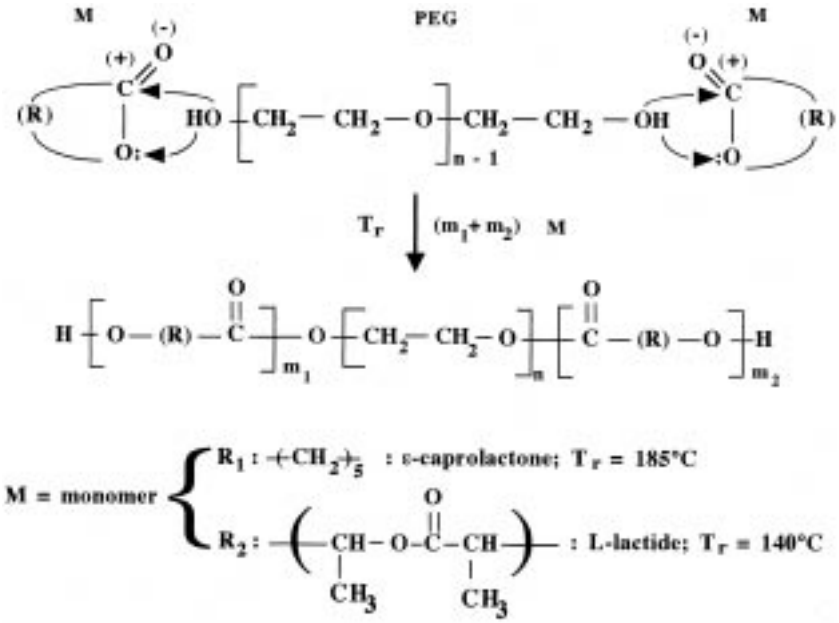


Figure 1 General scheme of reaction for the chemical synthesis of PCL-POE-PCL and PLA-POE-PLA three-block copolymers.

TABLE I PCL-POE-PCL and PLA-POE-PLA three-block copolymers. OE: molar percentage of repetitive oxyethylene units; CL: molar percentage of repetitive oxycaproyl units; LA: molar percentage of repetitive lactyl units; LV: limiting value of the weight percentage of 5-fluorouracil release

Copolymer	OE	CL or LA	Hydrophilicity	10^{-4} M_n	LV $\pm$ SD
CL28	52	48	medium	11.76	79.2 $\pm$ 5.7
CL26	65	35	medium-high	8.32	70.1 $\pm$ 2.7
CL24	86	14	high	4.99	52.4 $\pm$ 6.8
LA3	27	73	low	18.93	42.3 $\pm$ 0.3
LA6	53	47	medium	8.26	33.6 $\pm$ 7.6

PCL-POE-PCL ones having the same molar composition [7]. Fig. 2 shows the hydrolytic degradation mechanism of the copolymers.

The release of FU by PCL-POE-PCL after up to 96 h of dipping in Dulbecco's PBS is reported in Fig. 3. All the curves show quite a fast release of FU in the first 10 h; afterwards the release slows down, and finally reaches a limiting value, always less than 100%, after dipping times different for the different materials. In particular, the release by CL26 is near the limiting value after 9 h, and that by CL28 after 24 h, while CL24 keeps on releasing small quantities of FU after more than a week of dipping (data not shown).

The behavior of both LA6 and LA3, shown in Fig. 4, is quite similar to that of CL26; within about 9 h both copolymers reach their limiting values of FU release, lower than all the values reached by PCL-POE-PCL copolymers. The limiting values of the release by all the copolymers tested are reported in Table I; they decrease in the order CL28 $>$ CL26 $\gg$ CL24 $>$ LA3 $>$ LA6.

In a precedent paper [9] dealing with drug release by PLA-POE-PLA sintered tablets, we generally found the presence of two releasing processes, a drug extraction by the solvent followed by the copolymer hydrolytic degradation; we also found that very low percentages of LA3 and LA6 are hydrolyzed after 9 h of dipping. Since the PCL-POE-PCL copolymers degrade more slowly than the PLA-POE-PLA ones [7], their hydrolysis can also be assumed to be very scarce when the release by these materials reach their limiting values. As a consequence, the release observed in all the tests seems due to the extraction rather than to the hydrolytic degradation.

In this work we tried to minimize FU extraction by the buffer solution, as well as formation of solution gradients within the sintered tablets, by reducing both the thickness and porosity of the specimens to be tested. For this reason, we carried out the release tests on fused thin sheets.

The results shown in Table I and in Figs 3 and 4 seem, on the contrary, to indicate that no sensible drug release due to the hydrolytic degradation of the copolymers occurs up to two months, while the extraction of a portion of FU, likely that present on the sheet surface, occurs in the initial hours of dipping. The statistical nature of such ''superficial'' quantities of FU can explain the great SD values present in most release data (see Figs 3 and 4).

The decreasing of the limiting release values in the order CL28 $>$ CL26 $\gg$ CL24 $>$ LA3 $>$ LA6 (see Table I) indicates that in each series of copolymers, the higher the oxyethylene percentage in the chain, the lower the limiting value. In addition, such limiting values are

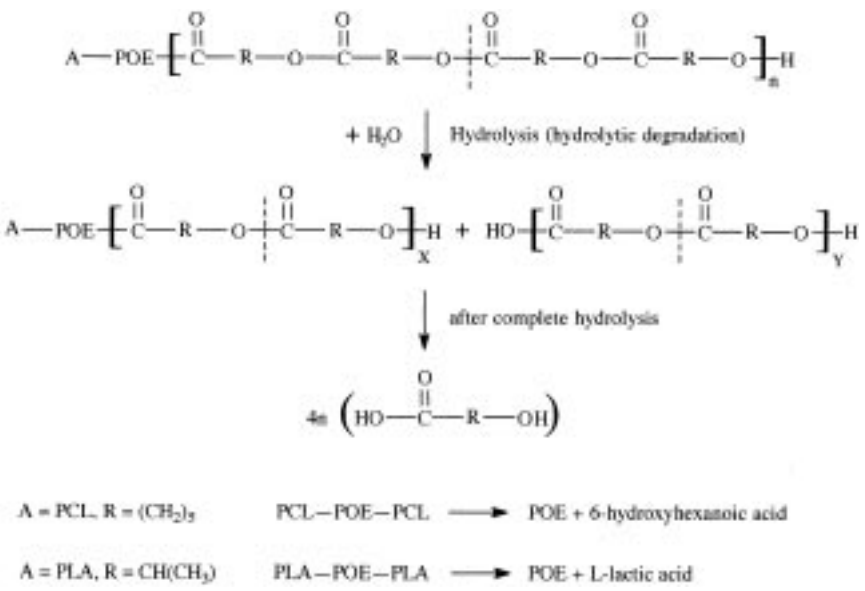


Figure 2 General scheme for the hydrolytic degradation of PCL-POE-PCL and PLA-POE-PLA three-block copolymers.

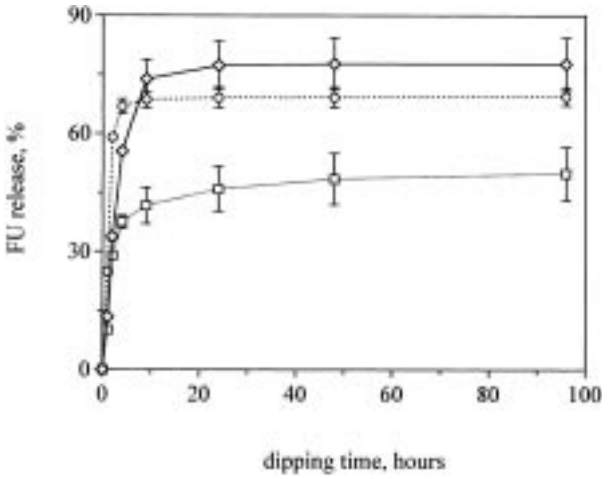
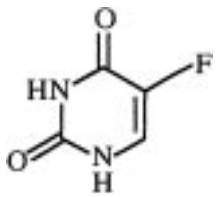


Figure 3 Release of FU by PCL-POE-PCL sheets into Dulbecco's PBS at 37 °C up to 96 hours. Releasing materials: CL28 (◇) CL26 (○) CL24 (□). The data are the mean ± SD of four determinations.

markedly lower for the PLA-POE-PLA macromolecules than for the PCL-POE-PCL ones; for example, the LA6 copolymer releases less than the half the FU released by the CL28, which has about the same monomeric unit ratio. Such a behavior may be explained by the interactions between FU and the poly(ester-ether-ester) macromolecules.

The FU molecule has the following structure:



The hydrogen atom, linked to the imide nitrogen 3 of the pyrimidine ring, is a weakly acidic one, having an apparent pK_a of 7.98 [10]; so, it is partially dissociated $(\alpha = 0.2)$ in a pH 7.3 buffer solution. However, the oxygen atoms present in the poly(ester-ether-ester) chains (see Figs 1 and 2) can undergo hydrogen bonds with both the –NH groups of the uracil ring also in the solid state. Since the polyether moieties are more flexible than the polyester ones, they can more easily arrange themselves around the FU molecules in the melt, to permit the formation of hydrogen bonds. Conversely, the oxygen atoms of ester groups may have some difficulty in undergoing all the theoretically possible hydrogen bonds with FU, especially those of PCL-POE-PCL, where the distance between two ester groups is greater than in PLA-POE-PLA. The FU extraction by the buffer solution is clearly easier for the ''unbound'' FU molecules; so the formation of the hydrogen bonds can explain the inverse linear dependence of the release limiting value on the molar percentage of repetitive oxyethylene units in PCL-POE-PCL, as well as the very lower limiting values of the release by PLA-POE-PLA (see Fig. 5).

The results obtained have pointed out the consequences on the release of dispersing FU in a molten poly(ester-ether-ester) matrix and of forming the melt in thin sheets. The melting-compression technique used reduces the specimen porosity to a minimum and enhances the interactions between the drug and the copolymer. As a consequence, no drug extraction by the buffer is possible inside the solid mass, and the release by copolymer hydrolysis is not detectable for two months. Conversely, formation in thin sheets causes quite a fast extraction of the FU present on the surface. Since this forming technique was chosen to avoid the formation of buffer gradients, which does not seem to occur with melt-formed specimens, the fast initial extraction may be strongly reduced by modifying the specimen shape. We could then make the surface-to-volume ratio of the specimens as little as possible, for example by forming them in small cylinders having height equal to radius. If such a shape modification will minimize the fast initial extraction, it will be possible to investigate the ability of these copolymers to release drugs at a very slow rate. In addition, a test carried out by dissolving both PEG and FU in Dulbecco's PBS, in

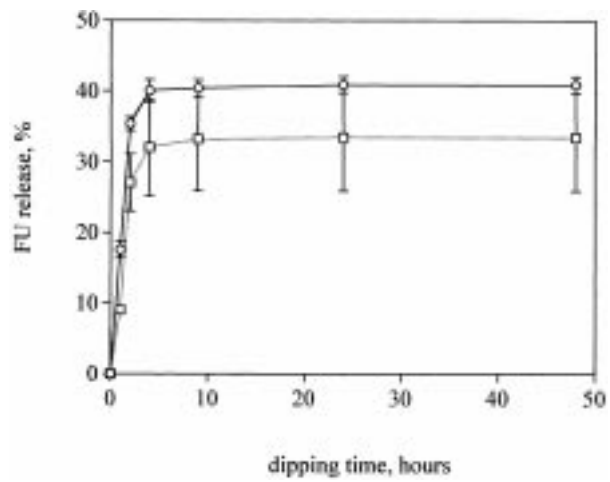


Figure 4 Release of FU by PLA-POE-PLA sheets into Dulbecco's PBS at 37 °C up to 48 hours. Releasing materials: LA3 (○) and LA6 (□) The data are the mean ± SD of four determinations.

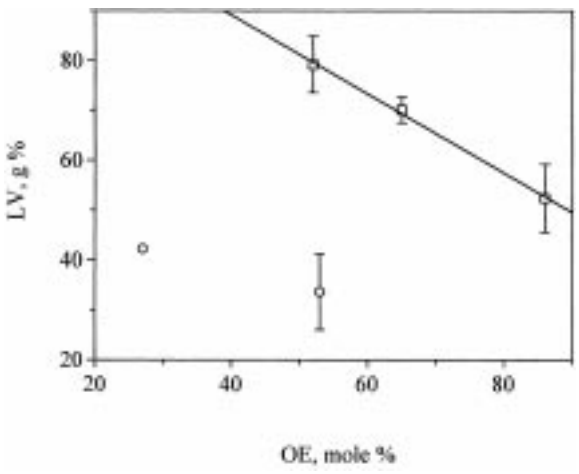


Figure 5 Dependence of the limiting value of the FU release (LV) on the repetitive oxyethylene units (OE) in PCL-POE-PCL (□) and in PLA-POE-PLA (○). The data are the mean ± SD of four determinations.

concentrations similar to those obtainable after complete copolymer hydrolysis, and by measuring spectrophotometrically the concentration of the ''free'' FU present, shows that it is ''analytical''. This fact indicates that the hydrogen bonds between the drug and the oxyethylene groups are not relevant in the buffer solution, so that the FU activity is likely unaffected and the drug remains spectrophotometrically detectable. As a consequence, such materials could be tested as ''time-delayed'' drug-releasing systems, by investigating the release by copolymer hydrolytic degradation also at times long after two months.

In conclusion, the most relevant aspect of these preliminary results is a future possibility of using poly(ester-ether-ester)s, like LA6 or one with an even lower ''limiting value'' of release, as a time-delayed drug releasing system. Attempts to form fused drug-copolymer mixtures in small cylinders are now in progress. The study of drug release by similarly shaped specimens will be the object of future work.

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