Bioresorbable microspheres as devices for the controlled release of paclitaxel

Giulio D. Guerra, Caterina Cristallini, Niccoletta Barbani, and Mariacristina Gagliardi

***Abstract*—** The release of the anti-cancer drug paclitaxel (PTX) from microspheres of both a bioresorbable poly(ε-caprolactone-oxyethylene-ε-caprolactone) tri-block copolymer and of polyurethanes containing either copolymers with the same composition and different molecular weights or poly(ε-caprolactone) diol as soft segments was studied. The microspheres, both loaded and not with PTX, were prepared by emulsion-evaporation technique, then characterized by SEM and DSC. The quantities of PTX released were measured by HPLC. The results showed slow and very regular releases, which fit very well the Peppas equation, $M_t/M_\infty = k \cdot t^n$, where M_t is the amount of solute released at the time t, M_∞ is the amount of drug released at the plateau condition, k represents the Peppas kinetic constant and n the diffusion order. Most n values are consistent with non-Fickian release mechanisms, with the exceptions of two less hydrophilic polyurethanes.

***Keywords*—**Controlled release, microspheres, paclitaxel, poly(ester-ether-ester)s, polyurethanes.

I. Introduction

THE poly(ester-ether-ester) tri-block copolymers have been proposed as bioresorbable materials since many years [1], [2]; biodegradable polyurethanes containing either them or polyester diols as soft segments were synthesized and tested more recently [3]-[6]. The development of the non-catalyzed synthesis of the tri-block copolymers [7]-[9] allowed to avoid the use, as a catalyst, of 2-ethylhexanoic acid, tin(II) salt, commonly known as stannous octoate, which was found to be cytotoxic [10]. The copolymers having poly(ε-caprolactone) chains as the polyester blocks (PCL-POE-PCL) were tested for cell adhesion and proliferation and cytotoxicity [11], as well as for cytocompatibility and hemocompatibility [12]. All the copolymers were found to be biocompatible with respect to the tests carried out. The degradation *in vitro* of the copolymers both in the absence and in the presence of cells was also tested [13], and the degradation product, 6-hydroxyhexanoic acid, were found to not alter the endothelial metabolism [14], and to modulate the endothelin release by human umbilical vein endothelial cells, with no significant alteration of the vasoconstrictor-vasodilator balance [15]. Polyurethanes were found to be enzymatically degradable by chymotrypsin [6]. PCL-POE-PCL was melt-spun to make fibers to be used as bioresorbable suture threads [16]. Composites of hydroxyapatite and copolymers with the same monomer composition were prepared both by direct copolymer synthesis [17] and by blending; the latter material was employed to make periodontal membranes, which were successfully tested for the bioresorption *in vitro* and *in vivo* [18]. A parallel ε-caprolactone homopolymerization, initiated by hydroxyapatite [17], [19], glasses [20] and alumina [21], was also found. In the last system, however, the presence in the product of bioresorbable polyester chains bonded to the Al^{3+} ions, which are toxic especially for neuronal cells [22], makes uncertain the complete biocompatibility of the composite. The release of the anti-cancer drug 5-fluorouracil by thin sheets of PCL-POE-PCL was also investigated [23]; the dependence of the release kinetics on the interactions between the copolymers and the drug was evaluated with *ab initio* calculations at the Hartree-Fock and second order Møller-Plesser levels [24]. In recent years, new therapies for tumors are being proposed, like anti-angiogenic treatments [25] and recombinant manganese superoxide dismutase [26].

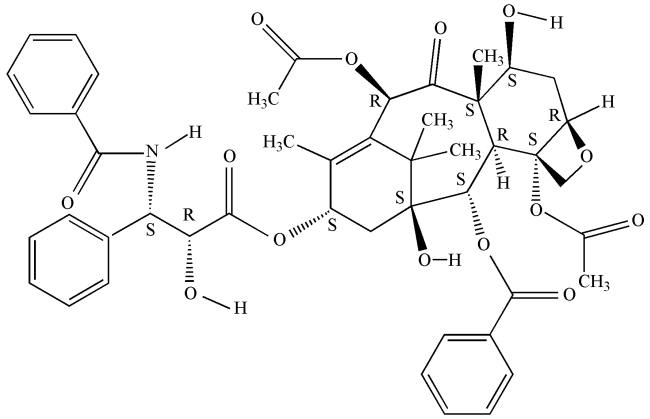

Fig. 1 structural formula of paclitaxel

This paper regards the release of a "classical" anti-cancer drug, the 5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one-4,10-diacetate-2-benzoate 13-ester with (2*R*,3*S*)-N-benzoyl-3-phenylisoserine (paclitaxel, PTX). PTX (see Fig.1) is a diterpenoid first isolated from the bark of the

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western yew *Taxus brevifolia*. It aids the polymerization of tubulin dimers to form microtubules, which are not only very stable, but also dysfunctional, leading to cell death; this property makes PTX a very effective anti-tumor agent [27]. The use of PTX in cancer therapy gives rise to the problem of its administration to the patients. Direct intravenous administration was found to have undesirable side effects on healthy cells [27]-[30], so that different PTX administration techniques were needed. Films of chitosan-poly(vinyl alcohol) blends made by casting, both as such and cross-linked with pentane-1,5-dial (glutaraldehyde), released a very scarce percentage of the PTX initially put into them [31]. A more efficacious drug delivery technique is the use of nanoparticles [32] or microspheres [33]. Since the release of PTX from microspheres of biodegradable macromolecular materials was tested successfully [33]-[40], an attempt has been made to study the controlled release of PTX from microspheres of both a PCL-POE-PCL and of polyurethanes containing either PCL-POE-PCLs with different molecular weights or a poly(ε-caprolactone) diol (PCLdiol) as soft segments.

II. Experimental

A. Materials

The PCL-POE-PCL copolymer used as such was the previously synthesized C27 sample [12], having an ester (CL) to ether (OE) units 66:34 molar ratio, a central polyether chain (POE) with a mean number molecular weight *(M_n)* value of 3.5×10^4, and a total mean M_n value of 20.37×10^4. The polyurethanes were synthesized using the technique described in a previous paper [6], with: PCL-POE-PCL (CL:OE = 65:35, POE M_n = 600, total M_n = 3300), 2,6-diisocyanatohexanoic acid ethyl ester (LDI), and 1,4-cyclohexane dimethanol (CDM, Aldrich) as the chain extender (PUC35, total $M_n = 1.94 \times 10^4$); PCL-POE-PCL (CL:OE = 50:50, POE M_n = 600, total M_n = 2200), 2,6-diisocyanatohexanoic acid ethyl ester (LDI), and 1,4-cyclohexane dimethanol (CDM, Aldrich) as the chain extender (PUC50, total $M_n = 1.67 \times 10^4$); PCLdiol (Aldrich, mean M_n = 2000), LDI, and CDM (PUH, total $M_n = 4.23 \times 10^4$); PCLdiol (mean M_n = 2000), LDI, and L-lysine ethyl ester as the chain extender (PUL, total $M_n = 9.22 \times 10^4$). PTX (Sorin, Italy) was used as supplied.

B. Microsphere fabrication

The microspheres of both the PCL-POE-PCL and the polyurethanes, loaded with PTX, were prepared adding to 5 mL of each macromolecule solution 60 g/L in CH_2Cl_2 either 15 or 60 mg of PTX, in order to obtain drug/polymer percentages of 5% and 20% w/w, respectively. Each solution was rapidly poured into 100 mL of 25 g/L aqueous solution of poly(vinyl alcohol) (PVA, Aldrich) as an emulsifier in a three-neck flask, and the two-phases liquid was stirred at 800 rpm for 2 h; 1,5 mL (1,62 g) of Tween 80 (TW, Acros) were added, as an additional emulsifier, to one of the solutions containing PUC50, then the so obtained micro-emulsions were stirred at 250 rpm overnight, keeping the flask opened to allow CH_2Cl_2 evaporation. The microspheres were separated by centrifugation, washed tenfold with H_2O and lyophilized. The percentage encapsulation efficiency *(%EE*) was calculated according to Freiberg and Zhu [41]. The mean values of *%EE* of the materials used to fabricate the microspheres are listed in Table I. "Placebo microspheres" without PTX were also prepared by the same procedure.

Table I Percentage PTX encapsulation efficiencies (*%EE*) of the different microspheres used for the release

Material	Emulsifier	% PTX w/w	Mean *%EE*
C27	PVA	5	86.9 ± 1.7
		20	82.6 ± 1.3
PUC35	PVA	5	83.7 ± 0.9
		20	79.3 ± 1.0
PUC50	PVA	5	97.3 ± 0.8
		20	95.6 ± 0.9
	PVA + TW	5	26.4 ± 1.3
		20	26.8 ± 0.7
PUH	PVA	5	56.5 ± 0.7
		20	52.7 ± 1.3
PUL	PVA	5	67.9 ± 1.0
		20	64.2 ± 1.2

C. Characterization of the microspheres

Scanning Electron Microscopy (SEM) was carried out, using a JEOL T-300 instrument, on samples coated with 24 carat gold in a vacuum chamber. Differential scanning calorimetry (DSC) was carried out in triplicate with a Perkin Elmer DSC7 apparatus, on 5 to 7 mg of both PTX loaded and placebo microspheres in Al pans, from 40.00 to 240.00°C at 20.00°C per min under N_2 flux.

D. Paclitaxel release

Microspheres in the delivery solution

10 mg of PTX-loaded microspheres, loaded with both 5% and 20% in weight of drug, were weighted and immersed in 5 ml of the delivery medium composed of a phosphate buffered solution (PBS) at pH 7.4 containing 0.05% (w/v) in sodium dodecyl sulphate (SDS, Sigma). In order to avoid the tendency of the microspheres to aggregate, samples were sonicated after the immersion in the delivery medium. The use of the SDS surfactant was finalized to increase the solubility of the PTX in water and also to reproduce a condition more similar to the *in vivo* one. Samples were maintained in a lightly stirred bath at a fixed temperature (37°C ± 1°C) through all the testing period (34 days). Withdraws from the delivery medium were carried out at established times and the delivery solution was replaced by fresh solution after each withdraw, in order to maintain a sink condition. The sink condition occurs when the amount of solute delivered is lower than 10-20% or even 30% of the maximum solubility of the solute in the dissolution medium [42]. For this reason, the solubility limit of PTX in

the delivery solution at 37°C has to be known. This value was gathered from literature [43] and used to verify if the sink condition was always verified.

Released paclitaxel determination by HPLC

Withdraws from the delivery medium were analyzed using high performance liquid chromatography (HPLC) at room temperature. The quantities of released PTX were calculated by means of a previously made calibration curve. HPLC was carried out using the following apparatus: a Perkin Elmer 410 LC Pump, an Alltech C8 5U Alltima column having 4.6 mm diameter and 10 cm length, and a Perkin Elmer LC 90 UV Spectrophotometric Detector. The moving phase was a 58:42 v/v CH_3CN-H_2O solution; the flux speed was 1 mL/min; the injected volume was 100 μL; the detector wavelength was kept at 230 nm; the PTX retention time was 3.04 min.

Release kinetics evaluation

The solute delivery kinetics was studied using two different mathematical models, expressed by the equations of Peppas (1) and Hopfenberg (2), respectively [44]:

$$\frac{M_t}{M_\infty} = k \cdot t^n \tag{1}$$

$$\frac{M_t}{M_\infty} = 1 - \left(1 - \frac{k_0 \cdot t}{c_0 \cdot a}\right)^m \tag{2}$$

M_t represents the amount of solute released at the generic time t, M_∞ is the amount of drug released when the system reaches the plateau condition, k and n represent the Peppas kinetic constant and the diffusion order respectively, k_0 is the Hopfenberg kinetic constant, c_0 is the uniform starting drug concentration, a is the mean radius of the microspheres, m is a "shape factor" and was assumed to be 3 because of the spherical geometry of the delivery platform [44]. In the present work, the value of M_∞ was considered equal to M_0, considering that polymer used to obtain microspheres is biodegradable and it ensures that the entire amount of drug contained in the delivery platform is released at infinite times. Values of M_0 were calculated considering the *%EE* values in Table I, using which the actual amounts of drug loaded within samples were estimated. Equations (1) and (2) are valid only for the first 60% of the total drug released [45]. Release orders allowed establishing the regime governing the release process. In particular, for spherical swellable samples, three different controlled release mechanisms can be identified [46]: $n = 0.43$ (Fickian diffusion, concentration gradient-controlled regime), $0.43 < n < 0.85$ (anomalous non-Fickian transport) and $n = 0.85$ (case-II transport, swelling-controlled regime). A not very different behavior can be supposed for the rather hydrophilic C27 copolymer. The hydrophilicity order of the other macromolecules, all less hydrophilic than C27, is the following: PUC50 > PUC35 > PUL > PUH.

III. Results and Discussion

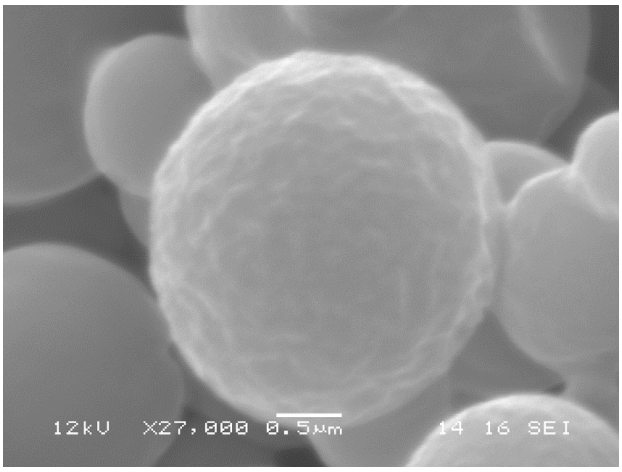

Fig. 2 SEM image of a single PUC50 microsphere; dimension bar = 0.5 μm

The SEM images of the microspheres, loaded with 5% PTX, are shown in Fig. 2 and Fig. 3. The regular spherical shape of the particles and their non-porous surface were confirmed by the SEM image of a single PUC50 microsphere, shown in Fig. 2. The microspheres made with C27 (Fig. 3a) appear very regular, with diameters ranging between 1.2 μm and 3.5 μm, whereas the PUC35 ones (Fig. 3b) show a less uniform diameter distribution. The distribution is more uniform for the microspheres of PUC50 made with only PVA as an emulsifier (Fig. 3c), and even more for those made with both PVA and TW (Fig. 3d). On the contrary, the microspheres made with the polyurethanes having PCL as a soft segment show the least uniform diameter distributions, although that of PUL (Fig. 3e) is more similar to that of PUC35 than that of PUH (Fig. 3f); however, some PUL particles have diameters more than double than the greatest ones of PUC35. Such characteristics can be related to the different structures of the macromolecular compounds. The presence of PEG in the macromolecules influences also the microsphere dimensions; indeed, those made with C27, PUC35, and PUC50 (Fig. 3 a-c) have mean dimensions smaller than those containing PCL (Fig. 3 e-f). As it is well known, the stability of a droplet of an organic solution dispersed in water phase depends on its total surface and on the interfacial tension, due to the different values of the electric field within the droplet and in the surrounding aqueous phase. The stability is inversely proportional to the surface energy, E_s, equal to the product between the specific interfacial tension, γ, and the total surface, increasing when the diameter of each droplet decreases. Since the system tends to the minimum energy state, smaller microspheres are obtained with the macromolecules having better interactions with the aqueous phase, and then lower γ values. That also a more effective emulsifier decreases the γ value can be seen comparing the microspheres in Fig. 3 (d) with those in Fig. 3 (c).

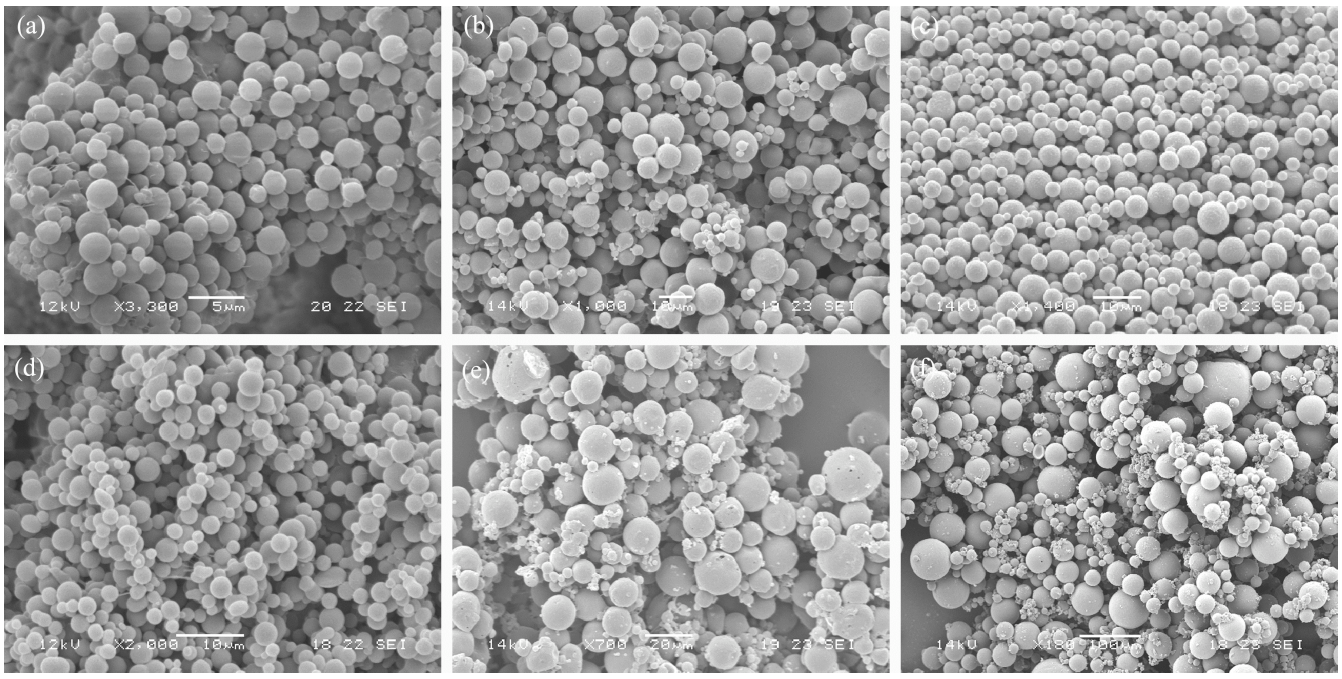

Fig. 3 SEM images of microspheres of C27 (a), dimension bar = 5 μm; PUC35 (b), dimension bar = 10 μm; PUC50 only PVA (c), dimension bar = 10 μm; PUC50 PVA + TW (d), dimension bar = 10 μm; PUL (e), dimension bar = 20 μm; PUH (f), dimension bar = 100 μm

Table II Thermal properties of the microspheres, calculated from the DSC curves

Material	Microspheres			
	PTX loaded		Placebo	
	ΔH (J/g)	T_m (°C)	ΔH (J/g)	T_m (°C)
C27	66.3	56.3	84.9	60.3
PUC35	40.7	51.3	43.3	52.7
PUC50 PVA	24.9	45.3	44.0	51.2
PUC50 PVA+TW	41.8	50.3	44.2	51.3
PUH	49.8	47.3	52.0	50.2
PUL	64.3	48.0	73.1	55.7

The thermal properties, calculated from the DSC curves, of the materials in the form of microspheres, both loaded and not loaded with 20% PTX, are reported in Table II. The presence of the drug diminishes both the enthalpy foffrence (ΔH) and the melting temperature (T_m), indicating a lower degree of cristallinity of the macromolecules. The greatest lowering occurs for the microspheres of C27 and PUC50 made with only PVA as an emulsifier; conversely, polyurethanes containing less or no OE unit, as well as PUC50 made with TW as an additional emulsifier, show little lowering of ΔH and T_m values. These values are in agreement with those of *%EE* reported in Table I; indeed, the strongest interactions occur between the polyether segments present in the polymer and the PTX, whose –NH and –OH groups can form hydrogen bonds with the "naked" electron pairs of the ether oxygen atoms, enhancing both the drug encapsulation and the perturbation of the macromolecular structure. Since the PUC50 microspheres made with both emulsifiers show a very low *%EE* value, the few PTX molecules present can cause only weak perturbations. As regarding the drug, Fig. 4 shows that the pure PTX melting endotherm at 223°C in curve (a) is totally absent in curve (b) of the loaded microsphere, as for the amorphous PTX [47]; the splitting of the C27 melting signal, with respect to that of the placebo particle in curve (c), indicates the presence of two phases, less and more ordered, of the copolymer. The behaviors of the other macromolecules are those summarized in Table II.

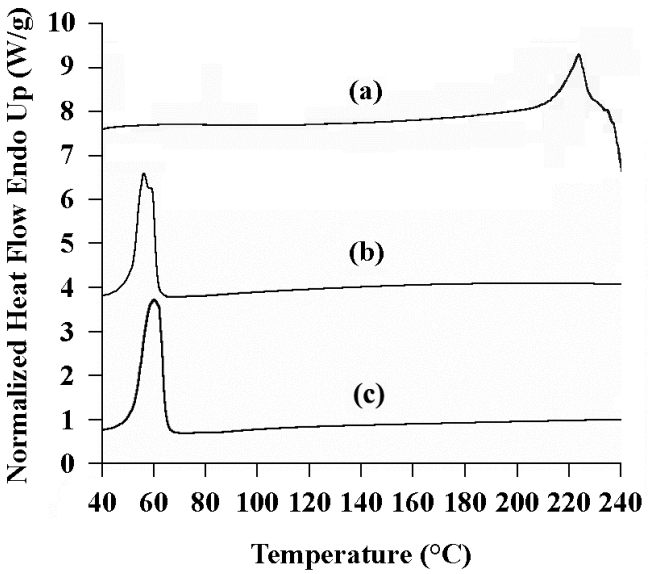

Fig. 4 DSC traces of pure PTX (a) and of the microspheres, loaded (b) and not loaded (c) with 20% PTX

Table III Kinetic parameters evaluated using the Peppas (P) and the Hopfenberg (H) models

Material	%PTX (w/v)	P			H
		k (d^n)	n	correlation	k_0 (μm/d)
C27	5	0.012 ± 0.003	0.523 ± 0.004	0.9888	$7.30 \cdot 10^{-5} \pm 1.59 \cdot 10^{-5}$
	20	0.005 ± 0.002	0.567 ± 0.011	0.9924	$1.47 \cdot 10^{-4} \pm 4.05 \cdot 10^{-5}$
PUC35	5	0.029 ± 0.004	0.610 ± 0.021	0.9948	$5.89 \cdot 10^{-4} \pm 5.28 \cdot 10^{-5}$
	20	0.009 ± 0.004	0.643 ± 0.096	0.9918	$7.03 \cdot 10^{-4} \pm 1.73 \cdot 10^{-4}$
PUC50 PVA	5	0.035 ± 0.005	0.493 ± 0.002	0.9996	$3.37 \cdot 10^{-4} \pm 4.95 \cdot 10^{-5}$
	20	0.020 ± 0.005	0.510 ± 0.047	0.9993	$7.74 \cdot 10^{-4} \pm 1.55 \cdot 10^{-4}$
PUC50 PVA + TW	5	0.045 ± 0.007	0.695 ± 0.035	0.9981	$1.42 \cdot 10^{-4} \pm 1.26 \cdot 10^{-5}$
	20	0.020 ± 0.006	0.691 ± 0.051	0.9994	$2.37 \cdot 10^{-4} \pm 4.58 \cdot 10^{-5}$
PUH	5	0.005 ± 0.002	0.452 ± 0.046	0.9966	$1.82 \cdot 10^{-4} \pm 7.35 \cdot 10^{-5}$
	20	0.002 ± 0.001	0.566 ± 0.062	0.9967	$3.11 \cdot 10^{-4} \pm 8.98 \cdot 10^{-5}$
PUL	5	0.031 ± 0.007	0.419 ± 0.013	0.9973	$6.46 \cdot 10^{-4} \pm 1.07 \cdot 10^{-4}$
	20	0.013 ± 0.006	0.481 ± 0.133	0.9977	$1.02 \cdot 10^{-3} \pm 2{,}51 \cdot 10^{-4}$

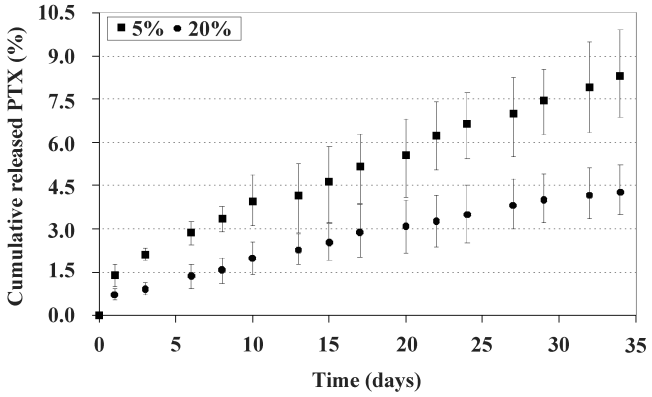

Fig. 5 Profiles of the PTX released from C27 microspheres loaded with both 5% (■) and 20% (●) PTX

In Fig. 5 the values of M_t/M_∞ versus time are reported for C27. Other materials also show a good fitting with the Peppas calculated curve, as can be seen from the correlation values reported in Table III. From literature, solubility limit of PTX in the PBS solution containing SDS was found to be 0.08±0.01 mg/ml at 37°C [43]. Then, the perfect sink condition is satisfied if the concentration of PTX detected in the delivery medium is smaller than $2.4 \cdot 10^{-3}$ mg/ml, that represent the upper limit of 30% in respect of the maximum solubility. This condition was verified for all tested samples in each withdraw. Releasing profiles obtained showed that the fraction of drug released from samples loaded with a smaller amount of PTX was greater than the fraction eluted from microparticles loaded with 20% in PTX. It could be reasonably attributed to greater interactions established between drug molecules in the samples loaded with a greater amount of active principle in respect to the samples loaded with a smaller amount of drug, where drug molecules are more dispersed in the polymeric matrix. In addition, a contained burst effect was detected in the first days (< 1% for C27 microspheres loaded with 20% in PTX and < 1.5% for 5%) and a linear trend after the first week of test. This behaviour is desirable in the case of the release of drugs that could show toxic effects, such as Paclitaxel [48].

Kinetic parameters of releasing profiles were evaluated using equations (1) and (2) and obtained results are summarised in Table III. For the Peppas model, kinetic

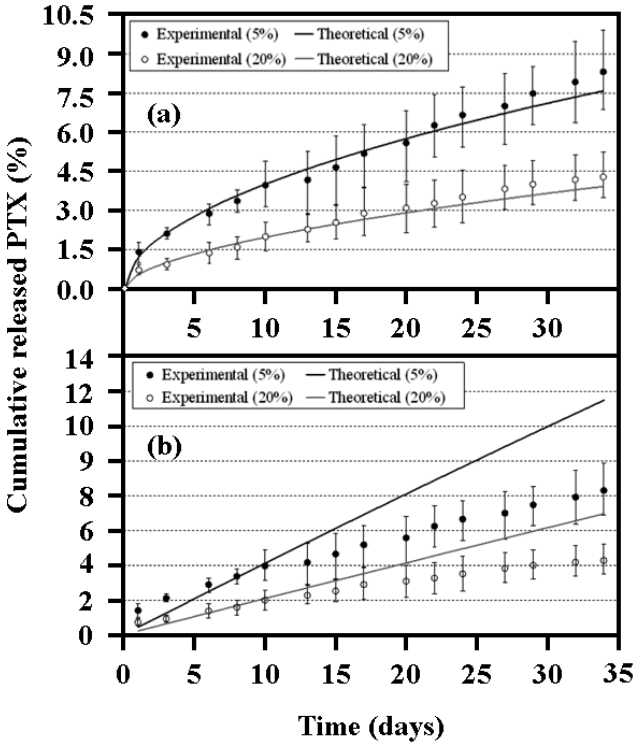

Fig. 6 Comparison between experimental and theoretical data obtained through the Peppas (a) and the Hopfenberg (b) models for the PTX released from C27 microspheres loaded with both 5% (●) and 20% (○) PTX

constant decreased with the increase in PTX and it could be attributed to the slower kinetic release of the drug in the presence of a greater amount of active principle, according as previously stated. Concerning drug release orders, most values are comprised in the range from 0.43 to 0.85, indicating an anomalous non-Fickian transport mechanism for swellable spheres. This transport mechanism is typical of polymeric systems not showing a solute transport controlled by the concentration gradient nor the macromolecular relaxation rate but the superposition of both diffusion and relaxation phenomena influence the delivery kinetics. Conversely, the microspheres made with PUH and PUL, have a kinetic order $n \leq 0.43$, indicating a Fickian transport mechanism typical of spheres made with scarcely hydrophilic or rather hydrophobic materials. Concerning the Hopfenberg model, kinetic constants k_0 increase with the increase of the starting drug loading for all the microspheres.

The comparison between the experimental releasing profiles of C27 and theoretical trends, evaluated using the kinetic parameters obtained through the theoretical models in the equations (1) and (2), are shown in Fig 6. From the correlation values reported in Table III, the theoretical models evaluated can be considered accurate and the Peppas equation can be used to describe the release behaviour of these systems. Also the release from the less hydrophilic materials fit the Peppas model, as can be seen from Fig. 7. On the contrary, Hopfenberg model is less accurate for the description of releasing profiles. It could be justified considering that Hopfenberg model was developed for surface-eroding polymer matrices while copolymer CL27 could be supposed to undergo a bulk hydration, considering that its macromolecules contain a fraction (~0.34) of POE that is highly hydrophilic, and then tend to absorb water within the whole structure of the microparticle. However, a further investigation of the degradation mechanism is necessary to validate this hypothesis. The particulate structure of the tested samples seems to overcome one of the major limits related to the release of drugs from biodegradable matrices, represented by a delivery kinetics showing a discontinuous trend. This characteristic was highlighted, for poly(L-lactide)-*block*-poly(oxyethylene)-*block*-poly(L-lactide) copolymers, in a paper reporting the analysis of tetracycline release from sintered tablets [49]. In the case of the PTX release from PCL-POE-PCL microparticles, two distinct release mechanisms were not shown, likely since the degradation of the C27 is still very scarce after 35 days of dipping [13], and then only the PTX extraction by absorbed water occurs; this behaviour offers the advantage of more controlled delivery kinetics.

IV. Conclusion

In the present work a preliminary morphological, chemicophysical and functional characterization of a micro-particulate delivery platform, obtained using PCL-POE-PCL copolymer and polyurethanes containing either copolymers with the same composition and different molecular weights or PCLdiol as soft segments, was reported.

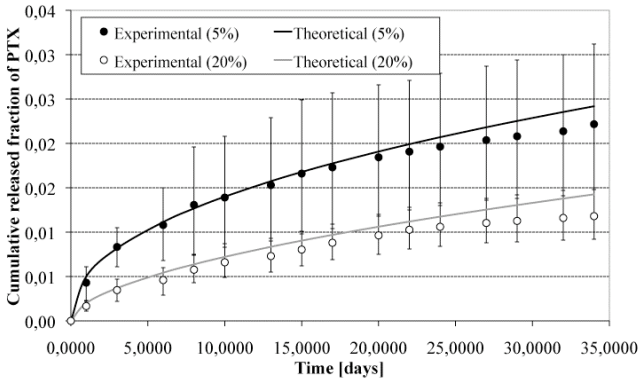

Fig. 7 Profiles of the PTX released from PUH microspheres loaded with both 5% (●) and 20% (○) PTX, and fitting with the Peppas model

Morphological analysis, carried out by SEM, showed that the obtained particles are spherical and not aggregated, with a radius distributions depending on the structure of the macromolecular material. DSC showed that the presence of PTX affects the thermal properties of the macromolecules.

Drug delivery tests showed that the starting drug payload, contained within the sample, affects the release kinetics; in particular, a faster release was detected in the samples loaded with the smaller amount of PTX. However, both systems showed the tendency to release the drug slowly, and it may portend that the delivery of the overall amount of PTX occurs with a vey sustained kinetics. It is a desirable characteristic of anti-mitotic delivery systems, because it provides a very prolonged treatment, so that it is not necessary to administer the drug subjecting the patient to several administrations.

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