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Research paper

Development and characterization of CyA-loaded poly(lactic acid)–poly(ethylene glycol)PEG micro- and nanoparticles. Comparison with conventional PLA particulate carriers

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Abstract

Cyclosporin A (CyA) loaded poly(lactic acid)–poly(ethylene glycol) (PLA–PEG) micro- and nanoparticles have been developed using an emulsion-solvent evaporation method. Physico-chemical properties, peptide loading content and in vitro release profiles of these novel CyA carriers were compared with those corresponding to conventional PLA micro- and nanoparticles. Results obtained confirm the previously described disposition of PEG chains on the surface of the PLA–PEG formulations. In addition, they revealed the presence of CyA molecules on the surface of both PLA and PLA–PEG systems. Further determination of the surface chemical composition by electron spectroscopy for chemical analysis (ESCA) allowed us to quantify the amount of CyA in the nanospheres' top layers, this amount being higher for nanoparticles than for microparticles, and higher for the PLA systems than for those based on PLA–PEG. In vitro release experiments revealed that PLA–PEG particles provided a more adequate control of CyA release than conventional PLA micro- and nanoparticles. Physico-chemical characterization of the systems during the release studies showed that the developed PLA and PLA–PEG micro- and nanoparticles were not degraded, which suggest a diffusion-mediated release mechanism. Furthermore, we have hypothesized that the hydrophilic outer shell of PEG provides a stationary layer for the diffusion of CyA.

Keywords: Poly(lactic acid); Poly(lactic acid)–poly(ethylene glycol); Microparticle; Nanoparticle; Controlled release; Cyclosporin A; Peptide delivery

1. Introduction

Previous reports have described the ability of nanoparticles made of block copolymers of poly(ethylene glycol) (PEG) and poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) to extend the blood circulation times of bioactive materials by diverting them away from the mononuclear phagocytic system (MPS) [1,2]. Therefore, the development of these Stealth nanoparticles has been initially focused to provide targeted delivery after intravenous administration. Nevertheless, recent reports have shown the attractive features of PLA–PEG micro- and nanoparticles as new protein delivery systems for oral and nasal administration [3,4]. Compared with conventional particulate carriers (i.e. PLA micro- and nanoparticles), PLA–PEG-based systems have shown improved stability in the gastro-intestinal (GI) tract, thus favouring the protein transport through the intestinal barrier. An additional feature of these systems includes their specific disposition to reach the lymphatic system after oral and nasal administration and, consequently, their great potential for the delivery of proteins to the lymphatic system.

These interesting properties provide new therapeutic opportunities and open up viable possibilities to improve conventional therapies. Cyclosporin A (CyA) is an example of a peptide which can benefit from the above-mentioned features. This highly hydrophobic peptide is used in the prevention of xenograft rejection following organ transplantation and it is also applied in the treatment of patients with selected autoimmune diseases [5]. However, it is well known that CyA presents a major problem related to its highly variable and incomplete absorption from its conventional oral

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formulation, which leads to a tremendous variation in drug pharmacokinetics and, consequently, to an uncertain relation between drug dosage and in vivo exposure [6].

Recently a new oral CyA formulation was developed (Sandimmun Neoral), which incorporates the peptide in a microemulsion. This microemulsion formulation displays significantly less interindividual variation [7]. However, it has been also reported that this formulation does not improve the predictability of trough levels, and therefore one cannot be assured of a uniform CyA exposure after a given dose of Sandimmun Neoral [6]. In fact, although the microemulsion formulation is supposed to improve the absorption characteristics, thus leading to a reduction in the doses administered, toxic and sub-therapeutic levels have been reported [5,8]. From this evidence, some authors have questioned the improved efficacy of the microemulsion formulation [5] and, presently, whether patients stabilized on conventional formulation should be converted to the microemulsion depends on patient's and physician's preference [9]. In this respect, a recent study has demonstrated that low bioavailability is due to extensive CyA metabolism in the gut wall [10]. Therefore, the improved bioavailability observed with the microemulsion formulation would be presumably related to protection from metabolic degradation [5]. If this was the case, the protection against degradation in the GI tract would be a first key parameter to improve marketed CyA oral formulations.

On the other hand, the immunosuppressive activity of CyA is related to a selective action against T lymphocytes, which mainly circulate in the lymphatic system. Consequently, targeting to lymphatics has been suggested as a second key parameter to improve commercial CyA formulations [11]. In view of both the lipophilic nature of CyA and the composition of the oily commercial dosage forms, one might expect an extensive lymphatic absorption of CyA. Nevertheless, studies with radiolabelled CyA in humans and rats [12] revealed a preferential distribution to several other tissues (i.e. liver, spleen and kidneys). The design of emulsions of different compositions [11] and lipid microparticles [13] are some of the formulation approaches towards the targeting to the lymphatics. However, the high variability in lymph concentrations and the lack of a comparison with the commercial formulation limit the interest of these formulations.

Based on these considerations, and in view of both the stabilizing effect over proteins encapsulated into PLA–PEG micro- and nanoparticles [14,15], and the lymphatic transport provided by these systems [4], they emerge as promising new CyA vehicles for oral administration. In addition, and bearing in mind the ability of these particles to reach lymphatic systems after nasal administration [3], a new route of CyA delivery could be proposed using these nano–microparticulate systems.

Here we report on the development of these systems, their physico-chemical characterization and the evaluation of their release properties.

2. Materials and methods

2.1. Materials

The homopolymer PLA with a molecular weight (M_w) of 38 000 g/mol was supplied by Phusis (Versoud, France). The diblock copolymer PLA–PEG (PLA M_w 45 000 g/mol and PEG M_w 5000 g/mol) was synthesized as previously described [14], by the ring-opening polymerization at 110°C of lactide in toluene, in the presence of monomethoxy PEG, using stannous octanoate as a catalyst. Average molecular weight and polydispersity were determined by size-exclusion chromatography coupled to a refractive index and a multi-angle light-scattering detectors (MALLS, Wyatt Dawn model F, Milton Roy, Wyatt Technology). The polydispersity indices were 1.4 and 1.2 for PLA and PEG-PLA, respectively. CyA was kindly donated by Novartis (Basel, Switzerland). [^{3}H]CyA was obtained from Amersham (Buckinghamshire, UK). PVA_{18k} (13 000–23 000 g/mol) and PVA_{50k} (30 000–70 000 g/mol) were purchased from Aldrich (France).

2.2. Preparation of CyA-loaded PLA and PLA–PEG micro- and nanoparticles

PLA and PLA–PEG micro- and nanoparticles with theoretical loadings in CyA of 17% (mg of CyA/100 mg of micro- and nanoparticles) were prepared using an oil-in-water emulsion-solvent evaporation method, conveniently adapted to obtain nanoparticles, 'small-sized' microparticles (MS_s) (mean diameter around 1 μm) and 'large-sized' microparticles (MS_l) (mean diameter around 40 μm). Briefly, CyA was dissolved in 2 ml of dichloromethane containing 25 mg (nanoparticles) or 50 mg (microparticles) of PLA or PLA–PEG. A volume of 25 ml of a 0.3% (w/v) Polyvinyl alcohol (PVA) aqueous solution (PVA_{18k} for nanoparticles and PVA_{50k} for microparticles) was added over the organic solution and the two phases were emulsified by using a vortex or by ultrasonication (40 W) (30 s vortex + 60 s sonication for nanoparticles; 10 s sonication for MS_s; 60 s vortex for MS_l). The organic solvent was then rapidly eliminated by evaporation under vacuum. Finally, the particles were isolated by centrifugation and washed three times with water.

For the determination of the encapsulation efficiency and in vitro release studies, the above-described formulations were prepared incorporating trace amounts of [^{3}H]CyA, in order to quantify the encapsulated and released CyA by liquid scintigraphy.

2.3. Physico-chemical characterization of the developed formulations

The morphological examination of the developed formulations was performed by scanning electron microscopy (SEM) (JEOL JSM T330, Japan), following a coating with gold and palladium to a thickness of about 70 Å, and by

transmission electron microscopy (TEM) (Philips), following freeze-fracture (Balzers, Lichtenstein) and subsequent coating with platinum and carbon as described elsewhere [14].

The mean particle size, particle size distribution and zeta potential of nanoparticles were determined, respectively, by photon correlation spectroscopy (PCS) (Malvern 4600, Malvern, UK) and laser Doppler anemometry (LDA) (Zetasizer 4, Malvern, UK). Zeta potential was measured in NaCl 10^{-3} M solutions. The mean particle size and size distribution of microparticles were determined using a Coulter Counter (Coultronics, US) and a Master Sizer granulometer (Malvern Instruments, UK).

The presence of residual PVA in the formulations was determined by a 'direct' and an 'indirect' method. In the indirect method, the residual PVA was calculated by the difference between the total amount used to prepare the systems and the amount of PVA present in the supernatant aqueous phase of the washing steps. For this purpose, a colorimetric method was used [16], based on the formation of PVA–iodine complexes in presence of boric acid (0.6 M), and subsequent absorbance determination at 620 nm, taking into account standards ranging from 0.1 to 0.6 mg/ml. In the 'direct' method, the particles were digested in 0.1 M NaOH for 20 h. The solutions thus obtained were neutralized using 0.1 M HCl, diluted with phosphate buffer (PBS) (pH 7.4), and analyzed for the PVA content as described above. Corrections were made in the case of PEG-PLA particles, because PEG resulting from polymer degradation interferes with the PVA dosage.

The presence of CyA at or near the surface of the systems was investigated by electron spectroscopy for chemical analysis (ESCA) (1) (Hewlett Packard HP 5950, USA) using the K_a ray of aluminium and by time-of-flight secondary ion mass spectrometry (TOF-SIMS) (PHI 7200). Using the first technique it is possible to quantify the CyA at or near the surface, taking into account that an elemental composition in N (N(%)) of 12.9% corresponds to 100% of CyA at or near the surface. Thus, the percentage of CyA at or near the surface in the developed formulations (surface %CyA) can be calculated according to

$$\text{Surface } \%\text{CyA} = \text{N}(\%) \times 100/12.9$$

Water uptake of the developed formulations was determined using an electronic suspension microbalance (Sartorius 4201) and under controlled conditions of water vapour activity and temperature (37°C). Details of the technique are given elsewhere [14].

2.4. Determination of the encapsulation efficiency (E.E.)

[^{3}H]CyA-loaded micro- and nanoparticles (specific activity 0.04 μCi/mg) were prepared to determine the E.E., as described above. The E.E. was calculated from the difference between the total amount used to prepare the formulations and the amount of non-encapsulated [^{3}H]CyA (present in the supernatant after isolation of the particles and washing steps) by liquid scintillations counting (Beckman LS 6000 LL, Beckman, USA), using Aquasol-2 (DuPont, Belgium) as the scintillation cocktail.

2.5. In vitro release studies

Samples of CyA/[^{3}H]CyA loaded micro- and nanoparticles were suspended in 10 ml of water and incubated at 37°C. At predetermined time intervals, the samples were centrifuged, the supernatant was removed and replaced with fresh water. Released CyA during each time interval was determined by liquid scintillation counting, as described above. Results were shown as the percentage of CyA released with respect to the amount of encapsulated CyA.

Size, zeta potential, and morphology of the releasing formulations were assessed as described above. The quantity of CyA in the particle top layers during the release studies was also determined as described above.

PLA and PLA–PEG molecular weight evolution was followed by gel permeation chromatography (GPC), using two GPC columns (Lichrogel PS20 and PS400, 300×7.8 mm, Merck, Germany) mounted in series and polystyrene standards ranging from 2×10^3 to 10^6 g/mol and THF as eluent.

PEG release from the PLA–PEG incubating micro- and nanoparticles was followed by a colorimetric method [17] based on the formation of PEG complexes in presence of an iodine and KI solution, and subsequent absorbance determination at 500 nm.

Table 1
Physico-chemical characteristics of the developed CyA-loaded micro- and nanoparticles[a]

Formulation	Particle size	Loading % (w/w)	E.E. (%)	Surface appearance	PVA content % (w/w)
PLA–PEG nanoparticles	206 ± 3 nm	14.4 ± 0.9	86 ± 4	Smooth	0.6 ± 0.8
PLA–PEG 'small' microparticles	2.3 ± 0.2 μm	15.9 ± 1.1	90 ± 3	Spongy	ND
PLA–PEG 'large' microparticles	37 ± 5 μm	16.2 ± 0.9	96 ± 4	Spongy	3.0 ± 1.0
PLA nanoparticles	181 ± 2 nm	14.7 ± 0.8	83 ± 3	Smooth	8.8 ± 0.9
PLA 'small' microparticles	1.0 ± 0.3 μm	15.1 ± 1.2	85 ± 2	Smooth	ND
PLA 'large' microparticles	33 ± 3 μm	16.7 ± 1.1	96 ± 3	Smooth	3.6 ± 1.6

[a] Means of 3–4 independent formulations. ND, Not determined.

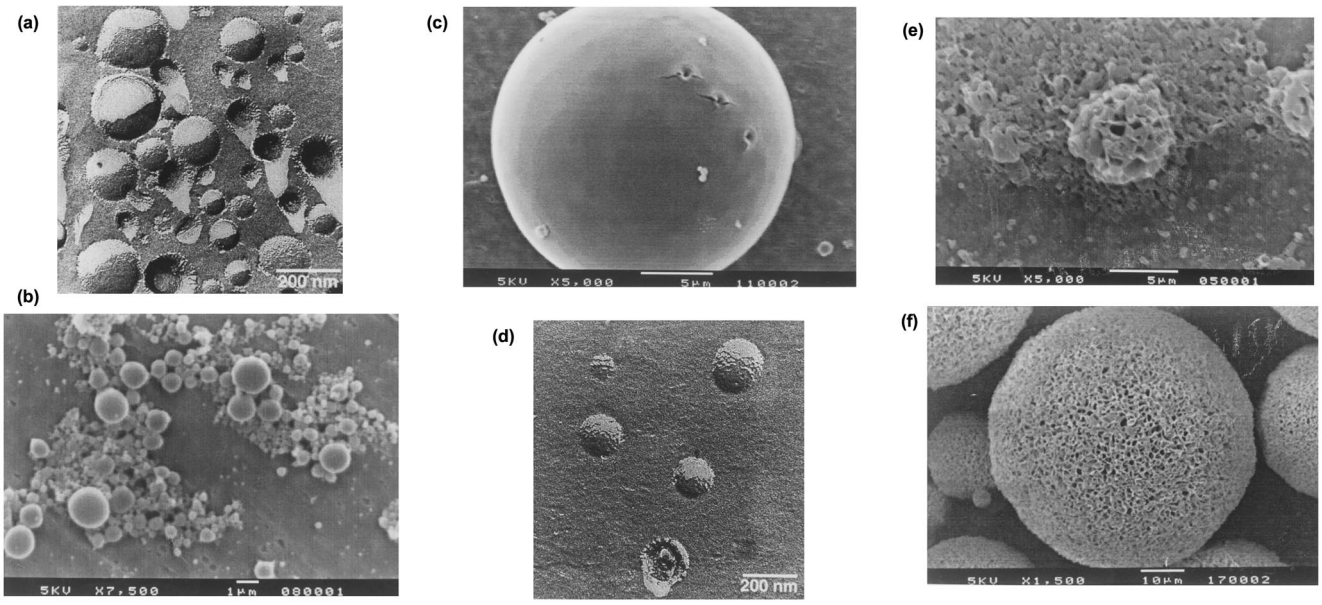

Fig. 1. Scanning electron micrographs (SEM) and transmission electron micrographs (TEM) of the developed CyA-loaded PLA and PLA–PEG micro- and nanoparticles (A, PLA nanoparticles; B, PLA 'small' microparticles; C, PLA 'large' microparticles; D, PLA–PEG nanoparticles; E, PLA–PEG 'small' microparticles; F, PLA–PEG 'large' microparticles).

3. Results and discussion

Two major problems of the marketed cyclosporin A (CyA) oral formulations have been identified so far: (i) the significant and variable degradation of CyA from these formulations in the GI tract, thus accounting for a low and variable CyA bioavailability; (ii) the low specificity of such formulations for the lymphatic system, which is the major target site for CyA. From our previous work there is evidence that, after oral or nasal administration, PLA–PEG micro- and nanoparticles offer unique features to provide effective protein protection and transport to the lymphatic system. Therefore, PLA–PEG micro- and nanoparticles appear to be attractive new CyA carriers for oral or nasal administration. Thus, the purpose of the present work has been to design CyA-loaded PLA–PEG particulate carriers with different particle sizes, to characterize the developed systems and to investigate their release properties.

CyA-loaded PLA–PEG or PLA micro- and nanoparticles were prepared using an emulsion-solvent evaporation technique with PVA as emulsion stabilizer. As shown in Table 1, the preparation technique could be conveniently adapted to obtain nanoparticles, 'small-sized' microparticles (MS_s) (mean diameter around 1 μm) and 'large-sized' microparticles (MS_l) (mean diameter around 40 μm). Size distributions were narrow and monodisperse (results not shown). In addition, Table 1 shows that high encapsulation efficiencies of CyA (E.E.) were achieved in all the formulations (ranging from 83 to 96%), regardless of their size.

Fig. 1A–C shows the scanning electron micrographs (SEM) and transmission electron micrographs (TEM) of the developed PLA micro- and nanoparticles. These images reveal that PLA particles have non-porous spherical shape with a smooth surface. On the contrary, PLA–PEG 'small' or 'large' microparticles (MS_s or MS_l) (Fig. 1D–F) present a spongy surface structure, which could be due to a different organization of the PEG and PLA blocks at the organic solvent/water interface, during solvent evaporation.

Table 2
Atomic composition of the developed CyA-loaded micro- and nanoparticles and quantification of CyA at or close to their surface, as determined by ESCA

Sample	ESCA elemental ratio (%)			Surface %CyA[a]
	C	N	O	
CyA	73.0	12.9	14.1	
PLA–PEG nanoparticles	63.4	3.4	33.2	8.10
PLA–PEG 'large' microparticles	61.6	5.4	33.0	0.06
PLA nanoparticles	67.8	6.4	25.8	16.60
PLA 'large' microparticles	64.1	2.4	33.5	0.03

[a] Percentage of encapsulated CyA at or close to the surface of the nano- and microparticles (calculation method according to Section 2).

The PVA content of the particles, determined both by the 'direct' and 'indirect' methods (see Section 2), gave similar results. The values in Table 1 are averages between the values obtained by these methods. The results show that residual PVA content of the PLA–PEG nanoparticles is negligible (0.6%) compared with the one in PLA nanoparticles (8.8%) of similar diameter (around 200 nm). Possibly, the presence of the PEG 'brush' at the surface of the PLA–PEG particles hinders the adsorption of PVA at their surface. The amount of PVA on PLA nanoparticles is high, as reported by other authors in the case of nanoparticles [18,19]. As an example, the residual PVA amount in PLA nanoparticles varied from 4.7 to 7.8% depending on the PLA molecular weight [19]. This is an interesting finding since PVA is potentially carcinogenic [20] and therefore, its amount in the particles should be reduced as much as possible. On the other hand, the PLA microparticles, because of their smaller surface area, adsorbed a lower PVA amount (3.6%) than the nanoparticles.

The presence of the PEG chains on the surface of PLA–PEG nanoparticles could explain the lower zeta potential values determined for PLA–PEG nanoparticles (-8.7 ± 0.1 mV) as compared with PLA ones (-14.9 ± 0.2 mV). The PEG coating layer shifts the shear plane of the diffuse layer to a larger distance from the nanoparticles, thus resulting in a decrease of the measured absolute value of zeta potential [21,22].

In order to determine the presence of trace CyA molecules at the surface of the micro- and nanoparticles, we used a sensitive analytical tool, TOF-SSIMS, which provides the mass spectrum of the top 10 Å of a surface [23]. The spectra obtained for the developed PLA–PEG formulations with this technique showed characteristic peaks corresponding to PEG and CyA (results not shown). These results confirm not only the previously described core-shell structure of PLA–PEG micro- and nanoparticles [1], but also the presence of CyA molecules at the surface of the systems. However, taking into account that the size of the CyA molecule (10-21 Å) [24], we were not able to quantify CyA at the surface using the SSIMS technique. The surface chemical composition was then determined by ESCA. This technique provides quantitative elemental and chemical state information (functional group analysis) on the composition of the material under investigation in the top layers (around 10 nm depth) [25]. The atomic composition and the amount of CyA at the surface of the nano- and microparticles are presented in Table 2. The differences between the percentage of CyA located at the surface of nano- and microparticles (values of 8–16% for nanoparticles in respect to those of 0.03–0.06% for microparticles) can be explained by the higher specific surface of the former, thus accounting for a higher percentage of superficial CyA.

Fig. 2 compares the release profiles of CyA from the PEG-coated systems with those provided by conventional PLA-based systems. As can be seen, PLA–PEG micro- and nanoparticles lead to higher total amounts of CyA released.

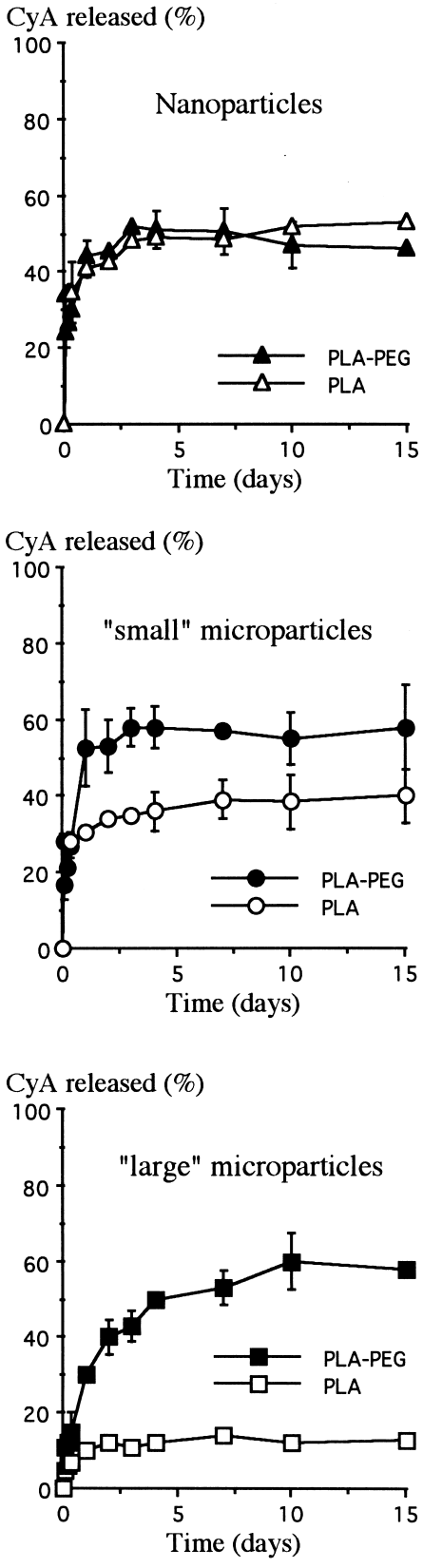

Fig. 2. Release profiles of CyA from PLA and PLA–PEG micro- and nanoparticles.

Table 3
Mean size on the PLA and PLA–PEG 'large' microparticles (MS_l), and nanoparticles (Ns) and CyA content (%CyA) at or near their surface during the release studies

Time (days)	PLA–PEG Ns		PLA–PEG MS_l		PLA Ns		PLA MS_l	
	Size (nm)	%CyA[a]	Size (nm)	%CyA	Size (nm)	%CyA	Size (μm)	%CyA
0	206 ± 3	8.100	37 ± 5	0.060	181 ± 2	16.600	33 ± 3	0.030
6	207 ± 2	ND	37 ± 3	ND	180 ± 3	ND	31 ± 5	ND
15	209 ± 4	8.500	36 ± 7	0.030	182 ± 6	< 0.004	29 ± 5	0.004

[a] Percentage of encapsulated CyA at or close to the surface of the nano- and microparticles, as determined by ESCA.

It should be noted, however, that the percentage of CyA released in the initial burst phase (Fig. 2) is higher than the corresponding percentage of superficial CyA (Table 2). This fact indicates that inner encapsulated CyA is released by diffusion in this initial phase. This diffusion process can be also responsible of the release in the subsequent time-periods. In fact, microscopic examination (SEM and TEM) of the micro- and nanoparticles during the release study did not reveal any sign of degradation after 15 days (results not shown), which suggest a release based on a diffusion process, rather than a degradation following by erosion phenomena.

The absence of degradation was corroborated by analysis of the releasing particles in terms of molecular weight evolution of the forming polymer, by gel permeation chromatography (GPC). The GPC chromatograms did not show any significant modification of the molecular weight of the PLA or PLA–PEG during the release studies, which indicates the absence of degradation on the polymer matrices. Additionally, Table 3 shows that the mean sizes of the particles remain unchanged during the release studies, thus confirming the absence of degradation in such period. Lemoine et al. [26] have previously reported the above-mentioned stability of the PLA nanoparticles. In addition, Landry et al. [27] reported that the degradation of PLA particles could be controlled by protecting the nanoparticles with coating agents. To corroborate such hypothesis, and to further confirm the absence of degradation of the PLA–PEG polymer during the release studies, we determined the modification of the PEG coating during such studies. Taking into account that the ester linkage between PLA and PEG is very accessible and, consequently, susceptible to hydrolysis in the course of the release process, we determined the percentage of PEG released from the particles and also, the zeta potential values of the nanoparticles, which are conditioned by the PEG presence, as previously discussed. The results presented in Fig. 3 show that only very small percentages of PEG were released from the PLA–PEG formulations. The very limited release of the PEG chains from PLA–PEG nanoparticles (less than 4% (w/w) in 50 days) is also evidenced by the absence of significant modifications in their zeta potential values, which remained unchanged after the release studies (Table 4).

Considering that CyA is released by a diffusion mechanism, the different total amounts of CyA released from each formulation could be explained by factors such as water uptake and porosity. Fig. 4 shows the water uptake profiles of the developed formulations. These profiles indicate that water can easily diffuse through the PLA and PLA–PEG matrix. The presence of the hydrophilic PEG chains in the matrix increases the total amount of water absorbed, as has been previously shown [14]. The high water uptake in the 'large' PLA–PEG microparticles (Fig. 4) could be attributed to their inner porosity (Fig. 1).

The release (Fig. 2), water uptake (Fig. 4) and surface composition (Table 2) results corroborate the mechanism of CyA release from the developed micro- and nanoparticles by a diffusion process. In this respect, the higher burst effect observed for the nanoparticles as compared with the microparticles (with a higher initial water capture than nanoparticles), has been attributed to their higher superficial CyA content and surface area.

The ability of PLA–PEG microparticles to display a more adequate control of in vitro release than conventional PLA micro- and nanoparticles can be attributed to the outer

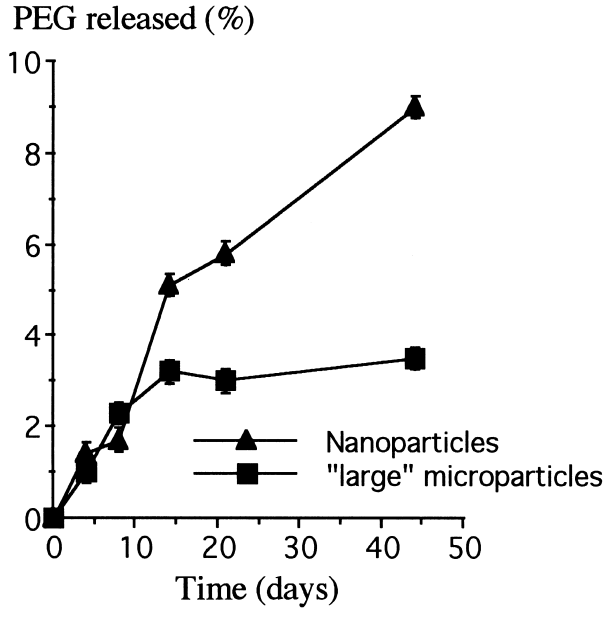

Fig. 3. Percentage of soluble PEG released in the incubating suspensions of the PLA–PEG formulations during the release studies.

Table 4
Zeta potential values of PLA–PEG and PLA nanoparticles during the course of the release studies

Time (days)	PLA–PEG nanoparticles zeta potential (mV)	PLA nanoparticles zeta potential (mV)
0	−8.7 ± 0.1	−14.9 ± 0.2
3	−5.1 ± 0.2	−13.3 ± 0.2
15	−8.1 ± 0.1	−13.6 ± 0.3

hydrophilic PEG coating of the PLA–PEG systems and the limited solubility of CyA in aqueous media. We hypothesize that the hydrophilic outer shell of PEG provides a reservoir phase or stationary layer for the diffusing CyA from the hydrophobic inner core to the external release medium. This hypothesis is supported by the CyA content at the surface of the systems during the course of the release studies (Table 3). Thus, after 15 days of release studies, negligible amounts of CyA are present at the surface of the PLA particles, indicating that the superficial CyA has been removed, and the CyA released at this stage comes from the core of the particles. On the contrary, the small percentage of CyA at the surface of PLA–PEG particles remains unchanged after the same time period, even when around 50% of the total encapsulated CyA has been released (Fig. 2). According with the above stated hypothesis, it was therefore understood that as water enters the particles, CyA diffuses to the limited aqueous volume of the hydrophilic PEG outer shell, thus generating CyA depots at the surface of the systems, from which CyA is released in a controlled manner.

On the other hand, the stability of the PEG coating around the particles is essential to explain the reported stability in digestive fluids and the in vivo fate of PLA–PEG nanoparticles following oral administration [4]. Thus, the described stability and the inherent characteristics of PLA–PEG micro- and nanoparticles are crucial issues for favouring the interaction and further transport of the intact nanoparticles through the intestinal barrier. In this respect, we should remember that marketed oral formulations for CyA present a major problem related to their low and variable bioavailability, which makes difficult the prediction of trough levels (and subsequent toxic/non-therapeutic effects) [6]. This important limitation has been recently related to extensive CyA metabolism in the gut wall [10]. In this context, the stable PEG coating around the developed particles could provide an additional protective effect against degradation in the gastric fluids to the encapsulated peptides and proteins. Therefore, the developed 'peggylated' micro- and nanoparticles emerge as very attractive new carriers for CyA oral administration. In addition, the great potential of these carriers for the delivery of peptides and proteins to the lymphatic system after oral or nasal administration [3,4] revealed their objective in targeting the encapsulated CyA to its suggested main action site [11].

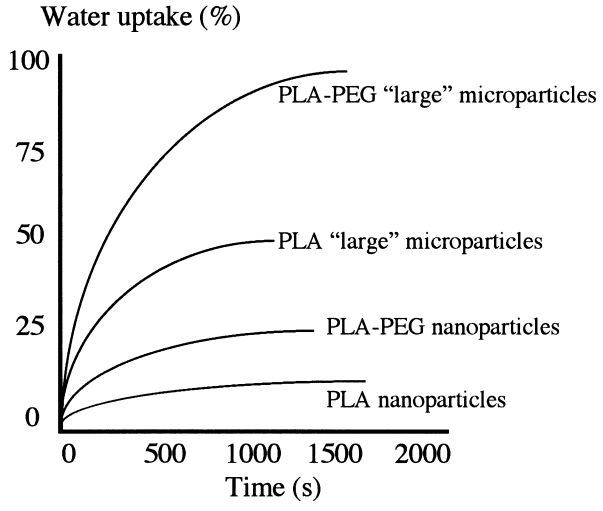

Fig. 4. Water uptake profiles of the developed CyA-loaded micro- and nanoparticles.

4. Conclusions

The results presented in this paper indicate that PLA–PEG particulate carriers with different particle sizes can be designed as new CyA carriers, showing promising characteristics as compared with conventional PLA micro- and nanoparticles. Taking into account the previously reported potential of PLA–PEG systems, CyA-loaded PLA–PEG micro- and nanoparticles provide new opportunities to improve present marketed CyA formulations, which opens up viable possibilities to improve present CyA-based therapies and to widening the possible areas of CyA biomedical application.

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