

RESEARCH ARTICLE

Hydrocortisone-loaded poly(ϵ -caprolactone) nanoparticles for atopic dermatitis treatment

Catarina Rosado¹, Catarina Silva^{1,2}, and Catarina P. Reis²

¹Universidade Lusófona (CBIOS – Experimental Dermatology Unit), Campo Grande 376, 1749-024 Lisboa, Portugal and ²Universidade Lusófona (CBIOS – Laboratory of Nanoscience and Biomedical Nanotechnology), Campo Grande, 376, 1749-024, Lisboa, Portugal

Abstract

Atopic dermatitis (AD) is a chronic inflammatory skin condition that affects mostly young infants. The purpose of this research was to achieve a prolonged drug release and the reduction of side effects with hydrocortisone-loaded nanoparticles (NPs), for AD treatment. Poly(ϵ -caprolactone) (PCL) NPs were prepared by modified solvent displacement method and were characterized in terms of size, potential zeta, morphology, entrapment efficiency (EE), Fourier transform infrared (FT-IR) spectrometry and *in vitro* permeation studies using Franz cells. Toxicology of this nanosystem was also assessed. The obtained NPs EE showed an increased size and a more homogenous size distribution after loading and were negatively charged. EF was around 62%. *In vitro* release studies demonstrated a controlled release of drug from the NPs over time. FT-IR analysis showed the system stability for one week. Permeation studies revealed significant differences in the permeation of encapsulated and free hydrocortisone. *In vitro* toxicity studies showed no effect of drug toxicity after encapsulation. The study seems to indicate that encapsulation of hydrocortisone in PCL NPs could enable a faster control of the disease and a decrease in the side effects associated to the long-term application of corticosteroids.

Keywords: Atopic dermatitis, poly(ϵ -caprolactone), prolonged drug release, hydrocortisone acetate, nanoparticles

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin condition that affects mostly lower age groups.^[1,2] AD has acute flare-ups and periods of remission, which have a significant impact on mobility and quality of life. Patients may suffer from itching, associated with sleep disturbances, daytime tiredness, social stigma caused by the visible skin condition, and all of these symptoms can only be mitigated with frequent application of topical ointments.^[1,3–5] Besides nonpharmacological measures, essential to the control of AD,^[4] topical corticosteroids (TC) are the oldest and most commonly used treatment. However, these strategies generally lead to several systemic side effects, such as tachyphylaxis, hypothalamic-pituitary-adrenal suppression, hypertension, glaucoma and also local adverse effects like skin atrophy, rosacea, erythema, striae and skin thinning.^[1,6,7]

For the past several years, nanotechnology has gained an important role in developing innovative drug delivery systems.^[8] Polymeric nanoparticles (NPs) are generally made of biocompatible and biodegradable polymers, which allow drug bioavailability increase, better drug release over time, optimized therapy for specific targets, reduced toxicity and undesirable effects.^[9–14] Poly(ϵ -caprolactone) (PCL) is a hydrophobic polymer and a good candidate for controlled and long-term drug delivery due to its high stability and slow degradation.^[14,15] In addition, PCL NPs appear to improve drug residence in the skin and reduce its permeation through deeper skin layers.^[16,17] In order to reduce the majority of the corticosteroids side effects, we propose hydrocortisone acetate (HCA)-loaded PCL NPs using solvent displacement method. This preparation method is generally very simple, relatively safe and

Address for Correspondence: Catarina Pinto Reis, Universidade Lusófona (CBIOS – Laboratory of Nanoscience and Biomedical Nanotechnology), Campo Grande 376, 1749-024 Lisboa, Portugal. E-mail: catarina.reis@ulusofona.pt

(Received 02 April 2012; revised 28 June 2012; accepted 09 July 2012)

allows high drug encapsulation efficiency.^[18] Other studies have showed promising results in encapsulating hydrocortisone.^[19–25] The main purpose of this study was to obtain and characterize PCL NPs loaded with HCA, assess the application of this system to the treatment of a local pathology aiming a prolonged drug release and a reduction in side effects. Ideally, patients would no longer need to apply corticosteroids several times per day, since the nanosystem would improve drug residence at epidermal and dermal layers, lead to prolonged targeting and highly local drug concentrations, allied with reduced systemic distribution.^[16,26] In addition, encapsulation in NPs can potentially lead to an improvement in the sensorial characteristics of TC conventional formulations, such as in greasiness or stickiness, which may enhance the patient compliance with added efficient action and safety.^[27]

Since chronic skin diseases like AD involve long-term glucocorticoid treatment, fear of adverse effects may lead to underutilization of drugs, especially in children.^[1,28,29] Patient adherence is, therefore, crucial for successful clinical outcomes.^[28,29] Advances in AD treatment include the use of systemic immunosuppressants, such as cyclosporine, methotrexate and IFN- γ ; however, these drugs are only adequate for a severe and refractory state of the disease and arise some concerns when administrated in young infants.^[30] In addition, PCL NPs and nanosystems can be applied to delivery other drugs such as potent immunosuppressants,^[27] lowering the dose of drug administered and entrapped into the NPs. This property may allow the minimization of the side effects and the improvement of clinical effectiveness and safety.

Materials and methods

HCA (MW: 404.50 g.mol⁻¹), Pluronic® F-68 (Poloxamer 188), sodium chloride, disodium hydrogen phosphate dihydrate and stearic acid and PCL, (MW: 14,000 g.mol⁻¹) were supplied by Sigma-Aldrich (Steinheim, Germany). Polysorbate 80 was obtained from Fluka (Steinheim, Germany). Acetone and methanol were purchased from José Manuel Gomes dos Santos Lda. (Lisbon, Portugal) and Valente e Ribeiro Lda. (Lisbon, Portugal), respectively. Sodium lauryl sulfate (SLS), methylparaben, propylparaben and cetyl alcohol were purchased from José M. Vaz Pereira SA (Lisbon, Portugal). Sodium dihydrogen phosphate dehydrate was purchased from Riedel-de-Haen (Seelze, Germany). White wax was purchased from Farma-Química Sur SL (Magala, India) and petrolatum was supplied by Fagron Iberica SA (Terrassa, Spain). Polydimethylsiloxane (PDMS) membranes with a thickness of 75 μ m were kindly donated by Dow Corning Europe S.A. (Seneffe, Belgium).

Preparation of the PCL NPs

Blank and HCA-loaded PCL-NPs were prepared by a modified technique of the solvent displacement method previously described.^[25] Briefly, 125 mg of PCL dissolved

in 25 mL of acetone and 5 mg of HCA suspended in methanol were added to an aqueous phase consisting of 50 mL of distilled water containing 2% (v/v of the aqueous phase) of Polysorbate 80. The emulsion was stirred at 100 rpm using a mixer equipped with a marine impeller. The obtained suspension was then concentrated by solvent removal under reduced pressure in a rotary evaporator. The NPs were then centrifuged at 41,200 x g for 40 min (High Speed Centrifuge Z36HK, Hermle Labortechnik, Germany). The supernatant was withdrawn and the NPs were dried and stored at refrigeration temperature around 4°C. Firstly, different parameters were tested with batches of empty PCL NPs, such as the stirring rate, the type and the concentration of the stabilizer used (Table 1). For these studies, water soluble surfactants, with FDA approval, such as Poloxamer 188 and Polysorbate 80 (nonionic surfactants), and also SLS (anionic surfactant) were used for improving NPs properties^[13] such as drug release enhancement and control of the particle size.

Particle size analysis

The mean particle size, polydispersity index (PI) and zeta potential of the NPs were measured with a Coulter Nano-sizer Delsa Nano™C (Fullerton, CA, USA). Experiments were made in triplicate. A low value of PI factor indicates a more stable and less dispersed NP distribution in size.

Morphology

The morphological assay of the HCA-loaded NPs was conducted by using transmission electron microscopy (TEM, CM12 Philips, the Netherlands). The samples were stained with 2% phosphotungstic acid, and placed on copper grids with Formvar® films for viewing by TEM.

Determination of HCA content and entrapment efficiency

A sample of the nanoparticulate suspension centrifuged at 41,200 x g for 40 min were weighted and redispersed in 20 mL of methanol. The dispersed NP were then lysed after heating (>60°C) and sonicated every 10–15 min during 60 min. Samples were collected at appropriated time intervals and centrifuged at 68,600 x g for 30 min. The amount of HCA entrapped within polymeric NPs was determined spectrophotometrically at 242 nm

Table 1. Formulation and processing conditions of HCA-loaded PCL NPs (means $\pm$ SD, n = 3).

Formulation	Stirring rate (rpm)	Surfactant (%)	Type of surfactant
I	100	0	None
II	100	2	Polysorbate 80
III	100	2	Poloxamer 188
IV	100	2	Sodium lauryl sulfate
V	100	3	Polysorbate 80
VI	100	4	Polysorbate 80
VII	200	3	Polysorbate 80
VIII	400	3	Polysorbate 80
IX	600	3	Polysorbate 80
X	800	3	Polysorbate 80

(UV-visible spectrophotometer, Evolution 600, UK), by measuring the drug content in the supernatant solution (nonencapsulated drug). Measurements were made in triplicate. A standard calibration curve was performed with the HCA solution in methanol and the entrapment efficiency (EE) was then calculated. The established linearity range was 1–20 µg/mL ($R^2 = 0.9992$).

***In vitro* drug release**

HCA-loaded NPs (10mg) were placed in a recipient containing 20mL of PBS (pH 7.4) (USP XXX) without enzymes and incubated at room temperature under constant stirring (130rpm). At appropriated time intervals, aliquots of the release medium were collected and replaced immediately with fresh solution. A clear supernatant was obtained after an ultrafiltration centrifugation technique ($68,600 \times g$ for 30 min). HCA concentration in the supernatant was determined in triplicate using a UV-visible spectrophotometer. A standard calibration curve was performed with the HCA solution in PBS 7.4. The established range was 1–25 µg/mL ($R^2 = 0.9992$).

Stability studies and interaction analysis by fourier transform infrared spectroscopy

Drug stability was assessed for 7 days, at $37 \pm 1^\circ\text{C}$, under constant stirring (130 rpm), in the same medium as used for the *in vitro* release studies, by measuring the size and zeta potential over time and by analyzing interactions of both polymer and drug into the nanoparticulate formulation by fourier transform infrared (FT-IR) spectroscopy. At appropriate time intervals, a sample of the NPs was collected and freeze-dried in order to obtain a powder for FT-IR analysis. The sample analysis was performed by comparing the raw polymer, the raw drug, the blank NPs, the HCA-loaded NP and a physical mixture of blank NPs and HCA (1:1). The KBr pellet method was chosen for this assay and the FT-IR was recorded by using a Bruker Spectrometer IFS-66V (Karlsruhe, Germany).

Preparation of semi solid formulations

In order to study the permeation profile of HCA from several vehicles, two traditional formulations were used for comparative purposes. A conventional oil-in-water (O/W) type cream containing 1% (w/w) HCA was prepared as follows. Firstly, methylparaben and propylparaben were dissolved with SLS in water (75°C). Secondly, a mixture of cetyl alcohol and stearic acid was added to the solution, gently and with constant stirring, at the same temperature. An ointment containing 1% (w/w) of HCA was prepared as described. First, white wax was merged (70 – 75°C). Then, petrolatum was added to the mixture, as the temperature of the two excipients was identical and they were completely merged. The formulation was removed from the bath and mixed until solidification.

***In vitro* drug permeation studies**

Samples of 1% of HCA-loaded NPs were suspended in 200 µL of oleic acid. A hydrophobic ointment, an O/W

emulsion and a solution of HCA in oleic acid were used as controls for this experiment. *In vitro* diffusion studies with Franz cells were first conducted using a PDMS membrane as a simplified model of human epidermis. PDMS membranes have been used extensively in preliminary permeation studies because of their simple structure and reproducible nature.^[31–33] Equal amounts of drug in the different formulations were placed in the donor compartment of the diffusion cells. The effective diffusion area was 0.95 cm² and the mean volume of the receptor chamber was approximately 3.9 mL. The receptor chamber was filled with PBS at pH 7.4 and stirred constantly at 130 rpm. The receptor compartments of the diffusion cells were maintained at 37°C using a recirculating water bath. Donor compartments were covered by glass slips. Samples were taken at regular intervals for UV spectrophotometry assay at 242 nm and replaced immediately with an equal volume of fresh PBS. A standard calibration curve was performed with the HCA solution in PBS 7.4. All experiments were performed fivefold. The multiple steady-state fluxes of HCA and the percentage of drug permeation were determined.

***In vitro* toxicity studies**

Cell viability of HCA-loaded PCLNPs was evaluated based on the *Saccharomyces cerevisiae* model.^[34–36] This model consists of the inoculation of yeast cell cultures in complete medium YPD (yeast extract 1%, peptone 0.5% and glucose 2%) containing 1.5% agar in a 3 mL cuvettes. After inoculation, the cultures were placed in the LEEC incubator (LEEC Automatic CO₂ Incubator Model GA3010) to grow at 30°C , under constant agitation (130 rpm). Cells were incubated with HCA-loaded NPs suspension from 0.0018 to 0.09 mg/mL. Control experiments were carried out for the equivalent HCA concentrations of free drug solution, as well as for empty PCL NPs from 0.009 mg/mL to 0.45 mg/mL. Different ranges were conducted in order to establish a relation between the system's toxicity and the cellular response. At designated time intervals, the cultures were vortexed and cell density was determined spectrophotometrically. The absorbance was measured at 525 nm and the number of cells/mL was calculated by the previously described standard curve $Y = 6.8219E-8X + 0.0327$.^[36] Each sample was measured in fourfold. Cell viability for each concentration was calculated by the following equation:

$$\text{Cell viability (\%)} = \frac{\text{Abss}}{\text{Abscontrol}} \times 100$$

where Abss is the absorbance of the cells incubated with the HCA-loaded NPs, the free drug solution and the empty PCL NPs, and Abscontrol is the absorbance of the cells incubated with the culture medium only.

The number of inoculated cells and the incubation time, up to 6 h, were adjusted in order to include a linear part of the log phase. The log phase appeared after 2 h of incubation at 30°C .

Statistical methodology

Results are expressed as mean $\pm$ SD. The significance of differences was assessed using One-way ANOVA (Tukey's Test) for multiple comparisons between the physicochemical parameters of the formulation control, the *in vitro* drug permeation assay and the *in vitro* toxicity evaluation. A 0.05 significance level was adopted.

Results and discussion

Effect of processing conditions on the physical properties of HCA-loaded PCL NP

Several batches of empty PCL NPs were prepared by varying parameters such as the stirring rate and the type and concentration of the stabilizer used. The influence of manufacturing conditions on the PCL NPs main physical parameters such as particle size and mean diameter, zeta potential and polydispersity was assessed (Table 2). Measurement analysis of the particle size indicated a significant decrease of the mean particle diameter in the presence of a surfactant agent in the external phase ($p < 0.05$). NPs prepared in the absence of surfactant were significantly larger. Previous studies reported that this effect may be caused by a decrease in the interfacial tension developed at the surface of the globules formed in the emulsifying medium.^[37] Polysorbate 80 produced the smallest NP. Also as expected, the addition of a surfactant increased the mean value of the zeta potential and decreased the polydispersity value, contributing for the NPs increased stability. No precipitate was observed after NPs preparation. Change in the surfactant type did not show an effect on the mean particle diameter as well as demonstrated with the increase of the surfactant concentration from range 2% to 4%. Furthermore, no influence on the zeta potential or on the polydispersity was demonstrated. Apart from this fact, NPs prepared with SLS increased the absolute value of zeta potential (compared with the NPs prepared without surfactant), ensuring the particles stability and avoiding their aggregation. Besides, formulations I (no surfactant) and IV (with SLS) demonstrated a similar charge value, which may suggest a low fixation of this surfactant at the NPs surface. Since

the polymer shows also an anionic character,^[15] weak electrostatic interactions may lead to small adhesion of the negative charged surfactant to the NPs, reducing its value. However, SLS is considered a disrupting substance for the skin barrier, exacerbating the skin contact eczema which may intensify the inflammatory response^[38,39] and because of this the referred excipient was not included for further studies. Finally, increasing the speed of stirring from 100 to 800 rpm showed no change on the mean particle size, zeta potential or PI (Table 2).

In the presence of the drug, mean particle size increased slightly after loading, with a range between 190 and 230 nm. In terms of the zeta potential, the particles were negatively charged (between -3 and -5 mV) and PI (0.010–0.030) indicated a higher stability for the loaded NPs, when comparing with the blank NPs. EE for HCA-loaded NPs was around 62%. In spite of the great capacity of PCL to enhance encapsulation of lipophilic drugs, through hydrophobic interactions, the entrapment of HCA is still limited, as demonstrated in other studies.^[23,24,40] In the present study, HCA initial loading was 80 μ g per mg of polymer. Although higher EE was expected, lower values were obtained probably due to the PCL highly hydrophobic domains that increase the instability of the primary emulsion, during the NP' formation^[40] or, most likely, because of the HCA diffusion from the polymer matrix, before acetone evaporation, since the drug has a high affinity for this organic solvent.^[41,42] Finally, electrostatic charge may modulate the interactions between the NPs and the skin, thereby providing specific attachment to the desired local action.^[43–45] Since the human skin has zeta potential around $+23$ mV as previously described^[46], opposite charge may increase contact time between drug and skin, and this electrostatic interaction could be a technological advantage. However, skin hair follicles are also reported to have a negative charge.^[44] Thus, this subject is still controversial. On the one hand, cationic nanocarriers such as liposomes^[47] are described as capable of penetrating deeply the skin, reaching high follicular depths but on the other hand, another study conducted by Kholi and coworker showed that negatively charged particles were capable of skin permeation independently from their size, while neutral and positive charged particles were not^[48]. The authors propose that this outcome is a consequence of repulsive forces between the skin lipids and the NPs, permitting drug permeation through the formation of transitory channels. Indeed, Raiman et al.^[43] suggest that skin surface may vary from a negative charge at higher pH values (~ 6 – 7), to a neutral one at pH ~ 4 . According to the literature,^[49] the skin surface is considered acidic with a normal pH range between 4.2 and 5.6, and this acidic character might also interfere with the penetration of NP through the skin by decreasing the electrostatic forces and creating aggregates of the NPs^[49]. Further, recent studies report some local skin toxicity associated with the induction of keratinocytes apoptosis by empty cationic lipid vesicles.^[49,50]

Table 2. Effect of formulation and processing conditions on the properties of PCL NPs (mean $\pm$ SD, $n = 3$).

Formulation	Particle diameter (nm)	Polydispersity index (PI)	Zeta potential (mv)
I	471.4 $\pm$ 66.1	0.121 $\pm$ 0.11	-42.09 $\pm$ 22.6
II	201.5 $\pm$ 23.2	0.068 $\pm$ 0.04	-10.10 $\pm$ 0.9
III	262.6 $\pm$ 9.8	0.059 $\pm$ 0.02	-8.91 $\pm$ 15.2
IV	210.0 $\pm$ 11.2	0.022 $\pm$ 0.02	-58.68 $\pm$ 13.5
V	204.9 $\pm$ 7.7	0.074 $\pm$ 0.01	-8.32 $\pm$ 0.6
VI	213.4 $\pm$ 11.8	0.077 $\pm$ 0.01	-7.06 $\pm$ 1.2
VII	216.6 $\pm$ 16.5	0.085 $\pm$ 0.02	-4.42 $\pm$ 12.8
VIII	203.2 $\pm$ 15.1	0.071 $\pm$ 0.02	-7.02 $\pm$ 4.2
IX	210.9 $\pm$ 11.8	0.037 $\pm$ 0.01	-10.42 $\pm$ 3.5
X	204.1 $\pm$ 19.0	0.090 $\pm$ 0.03	-3.70 $\pm$ 3.6

PI, polydispersity index.

Morphology

The morphological appearance of the loaded PCL NPs was assessed by TEM. The images shown in Figure 1 demonstrate that the nanosystems have a spherical shape and have a size near 200 nm (a). General background also evidence

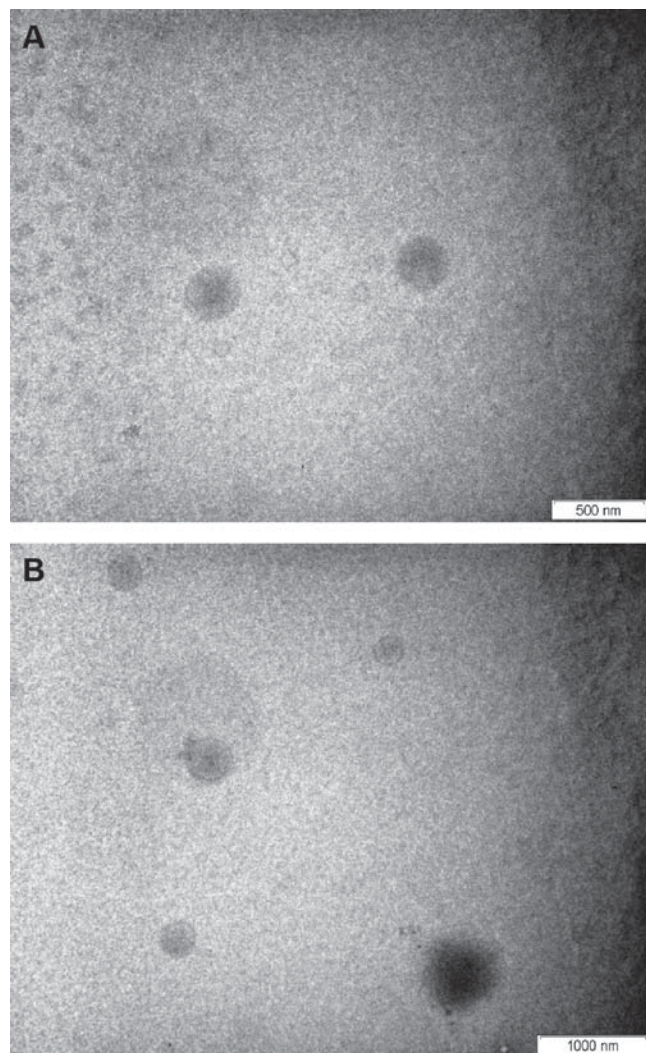


Figure 1. Electron transmission micrographs of (A) HCA-loaded PCL NPs; (B) general background of HCA-loaded PCL NPs.

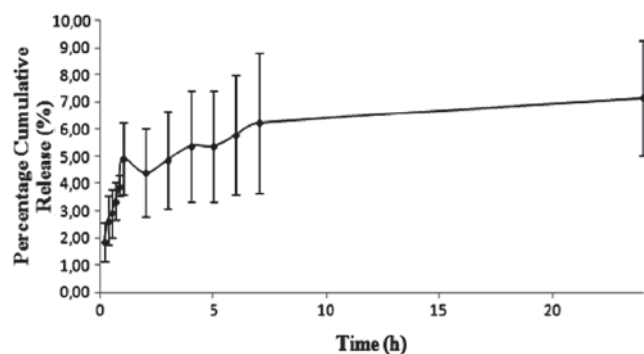


Figure 2. Kinetics of *in vitro* percent cumulative release profiles of HCA from PCL NPs suspended in PBS buffer (pH 7.4) (Mean $\pm$ SD, n = 3).

that there is a heterogeneous population of NPs but also show approximately the same type of structure (b).

In vitro drug release studies

Drug release profiles from PCL NPs in the release medium showed a prolonged release profile from the PCL NPs, as demonstrated in Figure 2. After 24 h, approximately 7% of the drug was released from the NPs. Such low value for the cumulative amount of released HCA may be explained by the ability of PCL, as a hydrophobic polymer, to retain mostly highly hydrophobic drugs like HCA, which may lead to the drug slow release or by some degradation of the drug over the time.

Stability studies and interaction analysis by FT-IR spectroscopy

The stability of the HCA-loaded NPs by measuring their size and zeta potential for up to one week. The results (Figure 3) showed a slight decrease in the NPs zeta potential after one week. In addition, the values of the size remained constant during the period of study. Typical FT-IR spectra of raw HCA, PCL and NPs (blank and physical mixture 1:1) are represented in Figure 4 (a, b, c, d). Also in this figure, HCA-loaded NP's stability over 7 days was compared (e, f, g, h). It can be seen that the FT-IR spectrum of raw HCA shows specific absorption at 3429, 3339, 1747, 1723, 1630 and 1374 cm^{-1} [51], in which the bands corresponding to the three carbonyl groups (C=O) of HCA are 1747, 1723 and 1630 cm^{-1} and the ones corresponding to the hydroxyl groups (OH) are 3429 and 3339 cm^{-1} [52]. As for raw PCL characteristic absorption spectra, bands positions are: 1189 (O-C-O stretching), 1726 (C=O stretching) cm^{-1} [53] and 2945 and 2866 cm^{-1} [54]. Blank NPs show some differences from the polymer as the band 2945 cm^{-1} and suffered a shift to 2926 cm^{-1} ; the other bands overlap, in exception of the band concentrated at 1189 cm^{-1} , which maintained constant but turn out more enlarged. Entrapment of HCA resulted in alterations in the spectrum transmittance with intensity attenuation after the NPs drug loading. HCA spectrum at 1747 cm^{-1} and PCL (and blank NPs) spectrum at 1726 cm^{-1} are shifted to 1730 cm^{-1} after the drug entrapment. This location is, actually, a probable site for interaction since it can form hydrogen bond with OH of HCA[53]. In contrast, the bands correspondents to the physical mixture of blank NPs and HCA (1:1) show characteristics of both features.

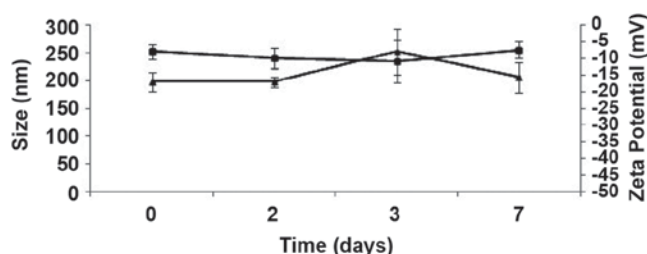


Figure 3. NP size (▲) and zeta potential (■) measured at 37°C at pH 7.4 over one week (mean $\pm$ SD, n = 3).

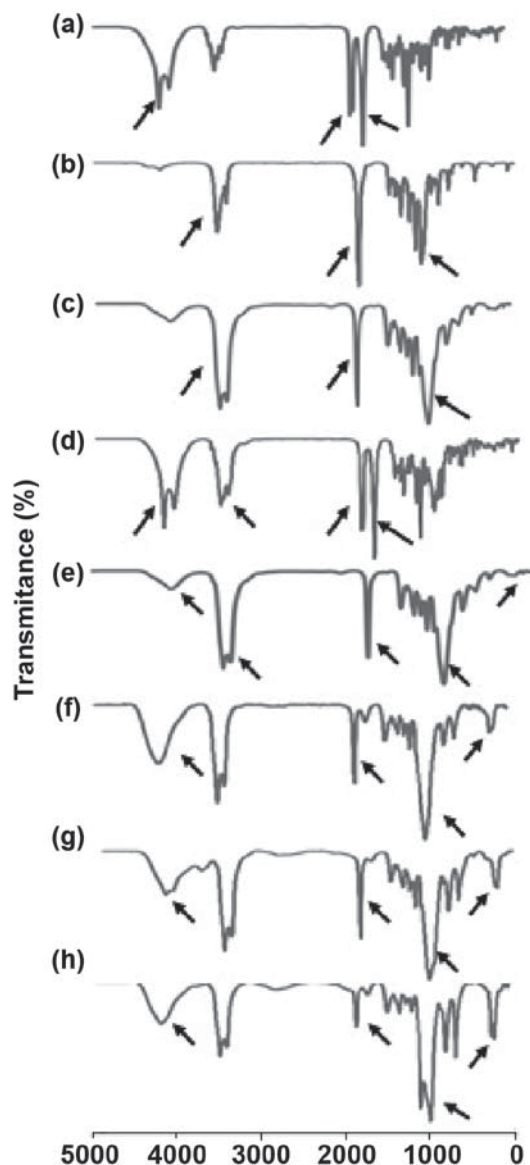


Figure 4. FT-IR spectra of (a) HCA; (b) PCL; (c) Blank NPs; (d) Blank nanoparticles: HCA physical mixture (1:1); (e) HCA-loaded nanoparticles (0h); (f) HCA-loaded nanoparticles (48h to 2 days); (g) HCA-loaded nanoparticles (72h to 3 days) and (h) HCA-loaded nanoparticles (168h to 7 days).

Finally, HCA-loaded NPs spectrum showed little variation over time (7 days) with general intensity decay in the specific bands and also new absorption bands appear at lower wavelengths. In addition, the bands for HCA hydroxyl groups suffer a shift to 3352 cm^{-1} (after loading) with an increase to 3438 cm^{-1} over time, also present in the specific polymer spectra. The FT-IR may indicate that the NPs are stable for one week and reorientated the original structures as the drug is released.

In vitro drug permeation studies

Diffusion patterns of the permeation of HCA through PDMS in each formulation are shown on Figure 5. Depending on the formulation system, different flux values were obtained (Table 3), but the steady-state was quickly reached in all cases and the cumulative amount

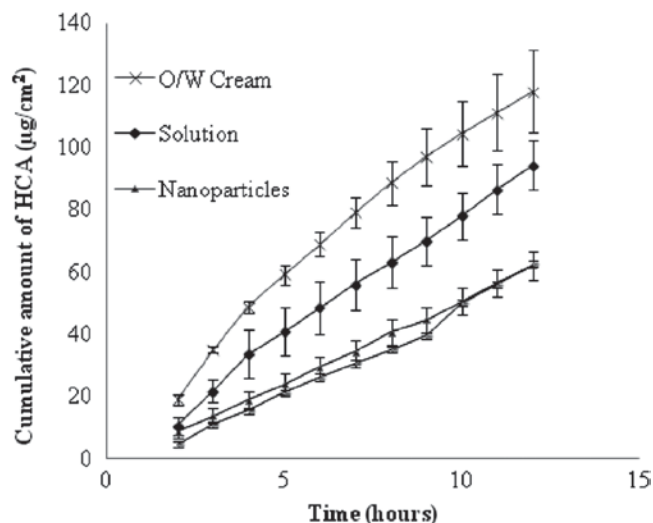


Figure 5. *In vitro* permeation profiles of HCA in PBS buffer at 37°C and pH 7.4 after 12h (mean $\pm$ SD, $n = 5$, except for the solution and O/W cream with $n = 4$).

Table 3. Fluxes ($\mu\text{g}/\text{cm}^2/\text{h}$) and cumulative drug amount (%) for the different delivery systems in the permeation assay after 12h (mean $\pm$ SD, $n = 5$, except for the solution and O/W cream with $n = 4$).

System	PDMS membrane	
	Flux	Amount
NPs	5.35 ± 0.37	6.19
O/W cream	9.66 ± 1.50	11.79
Solution	7.52 ± 0.61	8.64
Ointment	5.56 ± 0.28	6.24

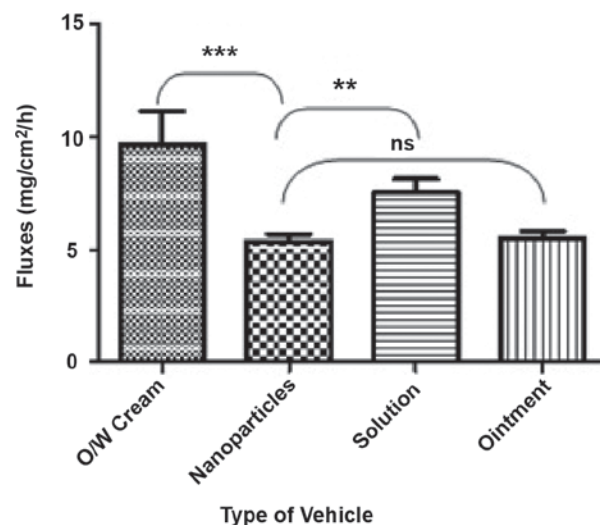


Figure 6. Relationship between the type of vehicle and the permeation fluxes (ns: $p > 0.05$, ** $p < 0.01$, *** $p < 0.001$). Data are shown as mean $\pm$ SD ($n = 5$), except for the O/cream and the solution with $n = 4$.

of diffused drug increased along with the permeation profile. The highest fluxes were obtained with the O/W cream and the HCA solution. On the other hand, the lowest value was found for the ointment and HCA-loaded NPs. In fact, the nanosystem showed a diffusion pattern similar to the ointment ($p > 0.05$), as demonstrated in

Figure 6. The same trend was observed in the % of drug that permeated after 12 h.

Such differences in permeation can be attributed to variations in the resistance opposed by the different formulations to the diffusion of the drug. This effect is very marked in the ointment, but almost negligible in the solution.

In vitro toxicity studies

Cytotoxicity experiments were carried out for HCA-loaded NPs, as well as for blank NPs and free HCA (Figure 7). Higher concentrations were not conducted since early high absorbances enabled the reading of the continuous cell growth, within the detectable range. Experimental results show a significant reduction in the *S. cerevisiae* proliferation rate ($p < 0.05$), in a concentration-dependent manner in the three systems. Moreover, there was a statistical significant difference between the loaded NPs initial concentration ($C = 0.0018 \text{ mg/mL}$) and the other concentrations for the same system ($C = 0.009 \text{ mg/mL}$ and $C = 0.9 \text{ mg/mL}$) ($p < 0.05$). No reduction of the toxicity level was obtained with the encapsulation of HCA into NPs. It is known that there is a correlation between NPs increased surface area,

as a consequence of its small size, and the establishment of induced inflammation (via oxidative stress) in human keratinocytes.^[55] Further studies should assess *in vitro* toxicity in epidermal keratinocytes and LC, since these are the main targets of AD treatment and may be involved in this pathology.^[56,57] Another important aspect in toxicity studies is the degradation time of the PCL NPs. PCL remains active during at least 1 year.^[15,58,59] For this reason, slowly degradable particles used in drug delivery may show persistence and accumulation on the site of drug delivery, perhaps resulting in chronic inflammatory reactions.^[60] Finally, further experiments should be made in animals for determination of NP's systemic toxicity, both acute and subchronic.

Conclusions

Therapeutics with TC remains a challenge in many autoimmune disorders, such as AD. This study describes the preparation of PCL NPs for the encapsulation of HCA. Experimental results allow the conclusion that the optimization of the NPs procedure, by solvent displacement method, lead to the achievement of small size particles, with a homogeneous distribution and an average negatively charged surface after loading, which may be crucial for *in vivo* interaction with the cell membrane. Based on particle size, zeta potential and polydispersity measurements, the optimal conditions for the preparation of HCA-loaded PCL NPs occurred when the stirring velocity was 100 rpm and the surfactant was Polysorbate 80 at 2% (v/v). The encapsulation of HCA into PCL NPs may offer a potential system for local delivery to the skin surface and a controlled release of the drug, minimizing absorption of topically applied HCA. Work in progress confirmed that it is possible to reduce the HCA release from the NPs.

The overall conclusion confirms that PCL is a useful and safe polymer for drug delivery to the skin surface and for the potential application of nanotechnology in transdermal delivery systems. Further studies should assess the accomplished results and further develop this work.

Acknowledgements

The authors would like to thank to Professor Maria José Alonso from University of Santiago de Compostela in Spain for the support and for providing facilities for the microscopy and FTIR analysis.

Declaration of interest

The authors report no declarations of interest.

References

1. Buys LM. Treatment options for atopic dermatitis. *Am Fam Physician* 2007;75:523–528.
2. Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis—part I: clinical and pathologic concepts. *J Allergy Clin Immunol* 2011;127:1110–1118.

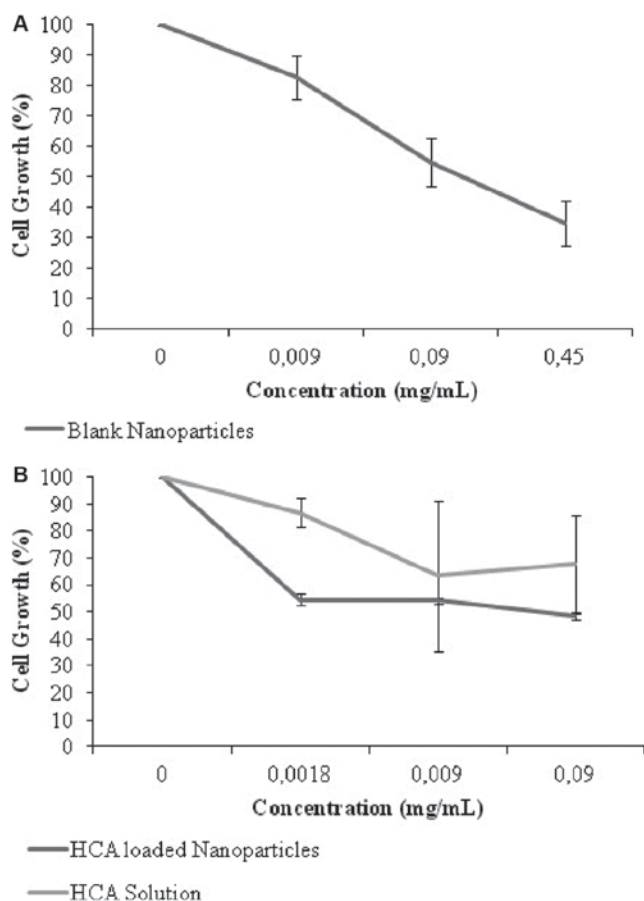


Figure 7. Growth of yeast cells when exposed to different formulations at different concentrations (mg/mL): (A) Blank NPs; (B) HCA-loaded nanoparticles and HCA solution (mean $\pm$ SD, $n = 8$). ** $p < 0.01$; *** $p < 0.001$, when comparing with the cell growth of nontreated control.

3. Del Rosso JQ, Bhambri S. Daily application of fluocinonide 0.1% cream for the treatment of atopic dermatitis. *J Clin Aesthet Dermatol* 2009;2:24–32.
4. Meireles C, Hergy F, Mousinho MC, Afonso S, Rosado C. Characteristics of infantile skin of the cosmetic products formulated for this age group. *Rev Lusófona de Ciências e Tecnologias da Saúde* 2007;4:73–80.
5. Novak N, Leung DY. Advances in atopic dermatitis. *Curr Opin Immunol* 2011;23:778–783.
6. Atherton DJ. Topical corticosteroids in atopic dermatitis. *BMJ* 2003;327:942–943.
7. Ference JD, Last AR. Choosing topical corticosteroids. *Am Fam Physician* 2009;79:135–140.
8. Pinto Reis C, Neufeld RJ, Ribeiro AJ, Veiga F. Nanoencapsulation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems. *Nanomedicine* 2006;2:53–65.
9. Bhowmik D, Chiranjib, Chandira RM, Jayakar B. Role of nanotechnology in novel drug delivery system. *JPST* 2009;1:20–35.
10. Hughes GA. Nanostructure-mediated drug delivery. *Nanomedicine* 2005;1:22–30.
11. Chen H, Chang X, Du D, Li J, Xu H, Yang X. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. *Int J Pharm* 2006;315:52–58.
12. Couvreur P, Puisieux F. Nano- and microparticles for the delivery of polypeptides and proteins. *Adv Drug Deliv Rev* 1993;10:141–162.
13. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. *Curr Opin Solid State Mater Sci* 2002;6:319–327.
14. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids Surf B Biointerfaces* 2010;75:1–18.
15. Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A. Poly-epsilon-caprolactone microspheres and nanospheres: an overview. *Int J Pharm* 2004;278:1–23.
16. Alvarez-Román R, Naik A, Kalia YN, Guy RH, Fessi H. Enhancement of topical delivery from biodegradable nanoparticles. *Pharm Res* 2004;21:1818–1825.
17. Ourique AF, Melero A, de Bona da Silva C, Schaefer UF, Pohlmann AR, Guterres SS et al. Improved photostability and reduced skin permeation of tretinoin: development of a semisolid nanomedicine. *Eur J Pharm Biopharm* 2011;79:95–101.
18. Pinto Reis C, Neufeld RJ, Ribeiro AJ, Veiga F. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. *Nanomedicine* 2006;2:8–21.
19. Nara S, Tripathi V, Singh H, Shrivastav TG. Colloidal gold probe based rapid immunochromatographic strip assay for cortisol. *Anal Chim Acta* 2010;682:66–71.
20. Boddu SH, Jwala J, Vaishya R, Earla R, Karla PK, Pal D et al. Novel nanoparticulate gel formulations of steroids for the treatment of macular edema. *J Ocul Pharmacol Ther* 2010;26:37–48.
21. Cavalli R, Peira E, Caputo O, Gasco MR. Solid lipid nanoparticles as carriers of hydrocortisone and progesterone complexes with beta-cyclodextrins. *Int J Pharm* 1999;182:59–69.
22. Jensen LB, Magnusson E, Gunnarsson L, Vermehren C, Nielsen HM, Petersson K. Corticosteroid solubility and lipid polarity control release from solid lipid nanoparticles. *Int J Pharm* 2010;390:53–60.
23. Vandervoort J, Ludwig A. Preparation and evaluation of drug-loaded gelatin nanoparticles for topical ophthalmic use. *Eur J Pharm Biopharm* 2004;57:251–261.
24. Lee CM, Lim S, Kim GY, Kim DW, Rhee JH, Lee KY. Rosin nanoparticles as a drug delivery carrier for the controlled release of hydrocortisone. *Biotechnol Lett* 2005;27:1487–1490.
25. Rosado C, Silva C, Reis CP. Hydrocortisone nanoparticles for atopic dermatitis treatment. *SOFW J* 2011;137:2–7.
26. Jennings V, Gysler A, Schäfer-Korting M, Gohla SH. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. *Eur J Pharm Biopharm* 2000;49:211–218.
27. Pople PV, Singh KK. Targeting tacrolimus to deeper layers of skin with improved safety for treatment of atopic dermatitis. *Int J Pharm* 2010;398:165–178.
28. Brown KK, Rehms WE, Kimball AB. Determining the relative importance of patient motivations for nonadherence to topical corticosteroid therapy in psoriasis. *J Am Acad Dermatol* 2006;55:607–613.
29. Brown KL, Krejci-Manwaring J, Tusa MG, Camacho F, Fleischer AB Jr, Balkrishnan R et al. Poor compliance with topical corticosteroids for atopic dermatitis despite severe disease. *Dermatol Online J* 2008;14:13.
30. Gelbard CM, Hebert AA. New and emerging trends in the treatment of atopic dermatitis. *Patient Prefer Adherence* 2008;2:387–392.
31. Jetzer WE, Huq AS, Ho NF, Flynn GL, Duraiswamy N, Condie L Jr. Permeation of mouse skin and silicone rubber membranes by phenols: relationship to *in vitro* partitioning. *J Pharm Sci* 1986;75:1098–1103.
32. Twist JN, Zatz JL. (1986). Influence of solvents on paraben permeation through idealised skin model membranes. *J Soc Cosm Chem* 37:429–444.
33. Gelotte KM, Lostritto RT. Solvent interaction with polydimethylsiloxane membranes and its effects on benzocaine solubility and diffusion. *Pharm Res* 1990;7:523–529.
34. Buschini A, Poli P, Rossi C. *Saccharomyces cerevisiae* as an eukaryotic cell model to assess cytotoxicity and genotoxicity of three anticancer anthraquinones. *Mutagenesis* 2003;18:25–36.
35. Marrot L, Labarussiat A, Perez P, Meunier JR. Use of the yeast *Saccharomyces cerevisiae* as a pre-screening approach for assessment of chemical-induced phototoxicity. *Toxicol In Vitro* 2006;20:1040–1050.
36. Roberto A, Caetano PP. A high-throughput screening method for general cytotoxicity. Part I: chemical toxicity. *Rev Lusófona de Ciências e Tecnologias da Saúde* 2005;2:95–100.
37. Al-Kassas RS, Al-Gohary OM, Al-Faadhel MM. Controlling of systemic absorption of gliclazide through incorporation into alginate beads. *Int J Pharm* 2007;341:230–237.
38. Törmä H, Lindberg M, Berne B. Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin *in vivo*. *J Invest Dermatol* 2008;128:1212–1219.
39. Rosado C, Lourenço A, Rodrigues LM. Assessment of the impact of a cream containing urea in skin barrier function. *Rev Lusófona de Ciências e Tecnologias da Saúde* 2008;2:166–173.
40. Hammady T, El-Gindy A, Lejmi E, Dhanikula RS, Moreau P, Hildgen P. Characteristics and properties of nanospheres co-loaded with lipophilic and hydrophilic drug models. *Int J Pharm* 2009;369:185–195.
41. Layre AM, Gref R, Richard J, Requier D, Chacun H, Appel M et al. Nanoencapsulation of a crystalline drug. *Int J Pharm* 2005;298:323–327.
42. Musumeci T, Ventura CA, Giannone I, Ruozi B, Montenegro L, Pignatello R et al. PLA/PLGA nanoparticles for sustained release of docetaxel. *Int J Pharm* 2006;325:172–179.
43. Raiman J, Hänninen K, Kontturi K, Murtomäki L, Hirvonen J. Drug adsorption in human skin: a streaming potential study. *J Pharm Sci* 2003;92:2366–2372.
44. Desai P, Patlolla RR, Singh M. Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. *Mol Membr Biol* 2010;27:247–259.
45. Baroli B. Penetration of nanoparticles and nanomaterials in the skin: fiction or reality? *J Pharm Sci* 2010;99:21–50.
46. Morykwas MJ, Thornton JW, Bartlett RH. Zeta potential of synthetic and biological skin substitutes: effects on initial adherence. *Plast Reconstr Surg* 1987;79:732–739.
47. Jung S, Otberg N, Thiede G, Richter H, Sterry W, Panzner S et al. Innovative liposomes as a transfollicular drug delivery system: penetration into porcine hair follicles. *J Invest Dermatol* 2006;126:1728–1732.
48. Kohli AK, Alpar HO. Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge. *Int J Pharm* 2004;275:13–17.

49. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W et al. Nanoparticles and microparticles for skin drug delivery. *Adv Drug Deliv Rev* 2011;63:470–491.
50. Liang CH, Chou TH. Effect of chain length on physicochemical properties and cytotoxicity of cationic vesicles composed of phosphatidylcholines and dialkyldimethylammonium bromides. *Chem Phys Lipids* 2009;158:81–90.
51. Okada S, Hiroshige R, Tanaka M, Murai M, Kimura T. [Hydrocortisone Acetate Reference Standard (Control 891) of National Institute of Hygienic Sciences]. *Eisei Shikenjo Hokoku* 1990;108:147–149.
52. Raghavan SL, Kiepfer B, Davis AF, Kazarian SG, Hadgraft J. Membrane transport of hydrocortisone acetate from supersaturated solutions; the role of polymers. *Int J Pharm* 2001;221:95–105.
53. Verma D, Katti K, Katti D. Experimental investigation of interfaces in hydroxyapatite/polyacrylic acid/polycaprolactone composites using photoacoustic FTIR spectroscopy. *J Biomed Mater Res A* 2006;77:59–66.
54. Vogel C, Wessel E, Siesler HW. FT-IR imaging spectroscopy of phase separation in blends of poly(3-hydroxybutyrate) with poly(L-lactic acid) and poly(epsilon-caprolactone). *Biomacromolecules* 2008;9:523–527.
55. Medina C, Santos-Martinez MJ, Radomski A, Corrigan OI, Radomski MW. Nanoparticles: pharmacological and toxicological significance. *Br J Pharmacol* 2007;150:552–558.
56. Hoetzenecker W, Meingassner JG, Ecker R, Stingl G, Stuetz A, Elbe-Bürger A. Corticosteroids but not pimecrolimus affect viability, maturation and immune function of murine epidermal Langerhans cells. *J Invest Dermatol* 2004;122:673–684.
57. Komine M. Analysis of the mechanism for the development of allergic skin inflammation and the application for its treatment: keratinocytes in atopic dermatitis - their pathogenic involvement. *J Pharmacol Sci* 2009;110:260–264.
58. Le Ray AM, Chiffolleau S, Iooss P, Grimandi G, Gouyette A, Daculsi G et al. Vancomycin encapsulation in biodegradable poly(epsilon-caprolactone) microparticles for bone implantation. Influence of the formulation process on size, drug loading, *in vitro* release and cytocompatibility. *Biomaterials* 2003;24:443–449.
59. Chawla JS, Amiji MM. Biodegradable poly(epsilon-caprolactone) nanoparticles for tumor-targeted delivery of tamoxifen. *Int J Pharm* 2002;249:127–138.
60. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. *Int J Nanomedicine* 2008;3:133–149.