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Evaluation of cationic polymer-coated nanocapsules as ocular drug carriers

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Abstract

New colloidal systems for ocular application were developed and their capacity for increasing the corneal penetration of drugs investigated. Chitosan (CS)-coated and poly-L-lysine (PLL)-coated poly-ϵ-caprolactone (PECL) nanocapsules, were designed based on a strategy that combines the features of PECL nanocapsules as ocular carriers with the advantages of a cationic mucoadhesive coating. Using this approach, an improved interaction of the carrier with the negatively charged corneal epithelium was attempted. The cationic polyaminoacid PLL was directly adsorbed onto preformed PECL nanocapsules whereas the cationic polysaccharide CS was included in the nanocapsules formation medium. The CS and PLL coatings conferred to nanocapsules a high positive surface charge, nevertheless, they did not modify the release profile of the model drug indomethacin from the colloidal system. In vivo studies showed that the systems investigated (uncoated, PLL-coated and CS-coated nanocapsules) increased significantly the concentration of indomethacin in the cornea and aqueous humor with respect of a commercial eye drops. Nevertheless, the ability of PLL-coated and CS-coated nanocapsules of enhancing the ocular penetration of indomethacin was substantially different: the CS coating increased twice, whereas the PLL coating failed to increase the ocular bioavailability of indomethacin when compared to the uncoated particles. Therefore, it is not the positive surface charge but the specific nature of CS that is responsible for the particularly enhanced uptake of the CS-coated nanocapsules. In addition, the PLL-coated and CS-coated nanocapsules displayed a good ocular tolerance. To summarize, the CS-coated nanocapsules represent a useful approach for increasing the ocular bioavailability of drugs.

Keywords: Ocular drug delivery; Nanocapsules; Chitosan; Corneal Penetration; Mucoadhesion

1. Introduction

The major problem in ocular therapeutics is to provide and maintain an adequate concentration

Abbreviations: CS, chitosan; PLL, poly-L-lysine; PECL, poly-ε-caprolactone; NC, nanocapsules.

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of the drug at the site of action. After instillation of a topical ophthalmic formulation, typically less than 5% of the applied drug penetrates the cornea and reaches intraocular tissues. This poor ocular bioavailability implies the necessity of frequent instillations in order to achieve the therapeutic effect; a situation that is frequently associated to undesirable side effects caused by systemic drug absorption. It is, consequently, clear that the increase of both, the retention of the drug in the precorneal area and the penetration of the drug through the cornea would be of a great benefit in ophthalmic therapy. One of the most successful approaches towards this aim has been the use of colloidal suspensions, such as liposomes and polymeric nanospheres (nanoparticles and nanocapsules). In particular, the effectiveness of liposomes in ocular drug delivery has been shown to depend on numerous factors but mainly on the liposomal surface charge, a property that has been reported as a major determinant on the precorneal vesicle retention (Fitzgerald et al., 1987; Guo et al., 1989). A common conclusion from these previous studies is that positively charged liposomes increase the corneal penetration of drugs when compared to neutral or negatively charged liposomes. This behavior was attributed to the mucoadhesion mediated by electrostatic interaction between the positive liposomes and the negatively charged mucin. More recently, other mucoadhesive polymers, such as Carbopol 934 P and Carbopol 1342, have also been found useful for prolonging the precorneal residence time of liposomes (Durrani et al., 1992; Davies et al., 1992). In the latter case, the interaction between the polymer and the mucin was explained by a hydrogen bonding mechanism. Despite the great value of these previous results, there are some limitations to the use of liposomes as ocular drug carriers, the most important being their instability and their limited drug loading capacity. As an alternative, biodegradable polymeric nanoparticles and nanocapsules have been proposed as ocular delivery systems. In this area, our group has showed that polyalkylcyanoacrylate (PACA) and poly-ϵ-caprolactone (PECL) nanoparticles have the H ability to improve the corneal penetration of hydrophilic, lipophilic drugs as well as some macromolecules, i.e. amikacin (Losa et al., 1991), metipranolol (Losa et al., 1993), indomethacin (Calvo et al., 1996a,b) cyclosporine A (Calvo et al., 1996c). Furthemmore, using confocal scanning microscopy, we have revealed that these colloidal systems are taken up by corneal epithelium cells without causing damage to the cells membrane (Calvo et al., 1994). This positive behavior could be considered as the key factor which explains the increased ocular penetration of drugs achieved with these carrier systems.

On the basis of these previous considerations it was thought plausible to combine the advantages of the PECL nanospheres as ocular delivery systems with the benefit of the presence of a cationic bioadhesive polymer on the surface of the particles. With this in mind, in the present study, we attempted to coat the PECL nanocapsules with two different bioadhesive cationic polymers, the polyaminoacid poly-L-lysine (PLL) and the biopolymer chitosan (CS), in order to investigate their ability of improving the ocular bioavailability of a model drug such as indomethacin. PLL was chosen because it is known to promote the cellular adhesion to surfaces in cell culture techniques (Ham and McKeehan, 1979). The cationic polysaccharide CS was selected because of its interesting properties in terms of mucoadhesion (Lehr et al., 1992), biocompatibility and not toxicity (Hirano et al., 1988).

2. Materials and methods

2.1. Chemicals and animals

The polymer poly-ϵ-caprolactone (PECL) (MOO: 40 000) was purchased from Aldrich Chemical Company (Steinheim, Germany) and used without further purification. The oil Migliol 840 was generously supplied by Lemmel (Barcelona, Spain). Poloxamer 188 (Synperonic F68) was a gift from ICI (Barcelona, Spain). The phospholipid mixture (soybean L-α-lecithin 40% phosphatidylcholine) and the poly-L-lysine (PLL) were supplied by Sigma Chemical Company (St. Louis, MO, USA). The polymer chitosan (CS) (Seacure 123) (viscosity 14 cps) was purchased

from Pronova Biopolymer A.S. (Norway). Indomethacin was a gift from Laboratorios Cusi S.A. (El Masnou, Spain). [2-14C] Indomethacin with a specific activity of 1.4 MBq/mmol (37.9 mCI/mmol) was it purchased from DuPont NEN (Bad Homburg, Germany). Tissue solubilizer (BTS-450) and liquid scintillation cocktails (Ready Organic and Ready Safe) were purchased from Beckman (Fullerton, CA, USA). Indocollyre was a gift from Laboratoires Chauvin (France). All other chemicals were reagent grade chemicals.

Male albino New Zealand rabbits weighing between 2.5–3.0 kg were used in the studies of the indomethacin ocular distribution. The rabbits were fed a regular diet with no restrictions on the amount of food or water consumed.

2.2. Preparation of the drug carrier systems

The uncoated PECL nanocapsules were prepared by the interfacial deposition technique with slight modifications (Fessi et al., 1989). Briefly, 100 mg of polymer and 100 mg of lecithin were first dissolved in 25 ml of acetone. Then, 0.5 ml of Migliol 840 oil containing 10 mg of indomethacin were added to the acetonic solution. This organic solution was poured, under moderate magnetic stirring, into 50 ml of aqueous phase containing 125 mg of poloxamer 188. The resulting mixed phase immediately turned milky as a result of the formation of nanocapsules due to the diffusion of the acetone towards the aqueous phase. The acetone was finally removed under reduced pressure and the colloidal suspension concentrated to the desired final volume (10 ml).

PLL-coated nanocapsules were obtained by adsorbing the polymer onto the preformed uncoated PECL nanocapsules. For the adsorption studies, known amounts of PLL were added to the suspension of nanocapsules and the system was maintained under magnetic stirring for 2 h at room temperature.

The preparation of CS-coated PECL nanocapsules was achieved by including the polysaccharide CS (100 mg) in the external aqueous phase during the nanocapsules manufacturing procedure (Calvo et al., 1997).

The [^{14}C]-indomethacin-loaded nanocapsules were used for the in vivo indomethacin ocular distribution studies. These drug carrier systems were prepared by adding [^{14}C]-indomethacin to the organic solution of indomethacin to attain an activity value of 50 μCi/ml. All suspensions were made isotonic with glucose (5% w/v).

2.3. Physicochemical characterization of the drug carrier systems

The morphological examination of CS-coated and PLL-coated PECL nanocapsules was performed using a transmission electron microscope (TEM), (Philips CM12), following negative staining with uracil acetate solution (0.2% w/v).

The mean particle size and size distribution of the colloidal systems were determined by photon correlation spectroscopy (PCS) using a Zetasizer III (Malvern W Instruments, Malvern, UK). The determination of the ζ potential was performed using the technique of laser Doppler anemometry (Zetasizer III). The colloidal suspensions were diluted properly with NaC1 10^{-3} M and placed in the electrophoretic cell where a potential of i 150 mV was established.

The amount of indomethacin encapsulated into nanocapsules was calculated by the difference between the total amount used and the amount of the free indomethacin determined in the aqueous medium following its separation by ultrafiltration-centrifugation (Ultrafree-MC 30 000 MW, Millipore, USA) at 3,000 $\times g$ for 15 min. Indomethacin was degraded in a basic medium (NaOH, 2.5 h) and the fluorescent degradation product assayed by spectrofluorimetry (λ excitation = 285, λ emission = 375)

2.4. In vitro release studies

The in vitro release studies of indomethacin from the nanocapsules was carried out by a bulk-equilibrium reverse dialysis technique at 37°C, as previously described (Levy and Benita, 1990). Briefly, a volume of 25 ml of the indomethacin loaded-colloidal suspensions was directly placed into 400 ml of a stirred sink solution (phosphate buffer 0.1 M, pH 7.4) where numerous dialysis

bags (cellulose membrane, Mw cut off 12 000 D, Sigma Chemical Company St. Louis, MO) containing 1.5 ml of the same sink solution were previously immersed. At given time intervals, a dialysis bag was withdrawn from the stirred release solution and the content of the dialysis bag was assayed for indomethacin.

2.5. In vivo studies ofindomethacin ocular distribution

A volume of 25 μl of [^{14}C]-indomethacin-loaded carrier systems or [^{14}C]-indomethacin control solution (Indocollyre) were administered to the cul-de-sac of the right eye of fully-awake New Zealand rabbits. After instillation animals were maintained in an upright position using restraining boxes. After 30 min, 1 h, 2 h, and 4 h rabbits were sacrificed with an intravenous injection of an overdose of sodium pentobarbital given. The eyes were proptosed, rinsed with normal saline, blotted dry in order to remove any adhering drug. Then, the aqueous humor was withdrawn from the anterior chamber. Cornea and iris-ciliary body were subsequently dissected in situ. Each tissue was rinsed with normal saline, blotted dry and transferred to preweighed counting vials. The vials were reweighed and the weight of the tissues was calculated. The tissues were digested in 1 ml of tissue solubilizer (BTS 450) at 35°C until completely dissolved and decolorized by adding 50 μl hydrogen peroxide. Ten ml of Ready Organic® scintillation cocktail and 30 μl acetic acid were added to each vial. The aqueous humor samples (100 μl) were dissolved directly in 10 ml of Ready Safe®. The samples were dark-adapted for at least 24 h in order to minimize chemiluminescence before counting in a liquid scintillation counter (LS 6000 LL, Beckman Instrument, Fullerton, CA USA).

2.6. Analysis of the data

Areas under the curves (AUC 0-4 h) of indomethacin concentration in cornea and aqueous humor were calculated using the trapezoidal method. The maximum indomethacin concentration (Cmax) in the cornea and aqueous humor was determined from actual data points. The statistical significance of the differences was tested by an analysis of the variance (ANOVA) and by a non parametric test (Kruskal-Wallis test).

2.7. Acute ocular tolerance studies and histological studies

New Zealand White rabbits in the weight range 2.5–3.5 kg were used to evaluate the acute ocular tolerance of the CS coated and uncoated nanocapsules. During experiments all rabbits were kept in restraining boxes in a normal upright posture. The animals were randomly divided into two groups of five rabbits. All rabbits of each group received in the right eye 30 μl of one formulation every 30 min for 6 h. The left eye remained untreated and served as a control. After each instillation a macroscopic evaluation was done to disclose possible lacrymation, blinking frequency, edema, conjunctival congestion, swelling and corneal opacification. At 3 h, 6.5 h and 24 h after the first instillation, a microscopic evaluation using a slit-lamp was done to study possible damage in the conjunctive, cornea and iris. These results were used to determine the irritant indices according to a scale of predetermined scores (Goldenthal, 1968). Finally, rabbits were sacrificed and eyes were enucleated and fixed with a formaldehyde solution. Complete cross-sections of the eye globe were obtained. The histological effect of the assayed formulations was determined by analysis of cross-sections randomly selected from each eye globe. The corneal epithelium was qualitatively compared under light microscopy.

3. Results and discussion

The main goal of the present work is to evaluate the potential of new positively charged PECL nanocapsules as ocular drug carriers. For the development of these novel systems our strategy was the incorporation, into the colloidal suspension, of the cationic polyaminoacid, poly-L-lysine (PLL) or the cationic polysaccharide chitosan (CS). The association of PLL to the nanocapsules

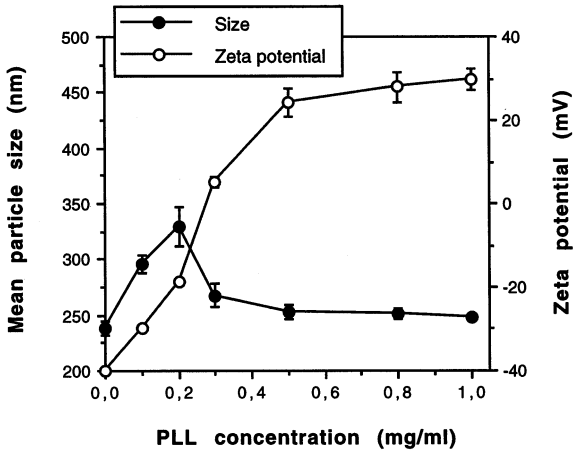

Fig. 1. Modification of the particle size and ζ potential of PECL nanocapsules caused by the adsorption of PLL.

was performed by adsorption onto the preformed nanocapsules whereas, the CS was incorporated in the nanocapsules formation medium.

In order to optimize the adsorption of PLL onto PECL nanocapsules, the colloidal system was incubated in the presence of different amounts of PLL. The efficiency of the coating process was evaluated by measuring the size and ζ potential of the nanocapsules before and after the adsorption of PLL. Results show that, by increasing the PLL concentration, it is possible to invert the ζ potential value from -40 to $+35$ mV (Fig. 1). This clearly indicates that the adsorption process is mediated by electrostatic interactions. On other hand, it is interesting to note that the size of the nanocapsules increased significantly with the concentration of PLL, reaching a maximum value when ζ potential was close to zero. This behavior could be attributed to a slight aggregation caused by the reduction of the negative surface charge and, thus, the repulsion forces between particles In fact, for PLL concentrations higher that 0.4 mg/mL, the ζ potential reaches important positive values and the particle size remains unaltered with respect to the initial value. Based on these results the formulation containing 0.8 mg/ml of PLL was selected for further studies.

The formation of the CS-coated nanocapsules was achieved by a novel single step procedure that involves the incorporation of the polysaccharide in the nanocapsules formation medium. (Calvo et al., 1997). The exact composition of the formulation for in vivo evaluation was described in the Section 2.

3.1. Physicochemical characterization of coated carrier systems

The physicochemical properties of colloidal systems containing indomethacin are shown in Table 1. Particle size analysis indicated that the mean size of the nanocapsules was affected by the nature of the coating varying between 200 and 400 nm. Statistical analysis of these data revealed significant differences (ANOVA, 95% level) between the coated and uncoated carriers. For the PLL-coated nanocapsules the slight increase in their size can be solely attributed to the adsorption of the PLL molecules onto the nanocapsules. On the other hand, the increased size of the CS-coated nanocapsules could be due to the presence of a CS coating around the particles and/or to an enlarged size of the oily globules dispersed in a CS-rich aqueous phase. In this sense, it should be mentioned that the CS enhances the viscosity of the aqueous medium and hence interferes with the interfacial hydrodynamic phenomena responsible for the spontaneous emulsification

Table 1
Particle size, ζ potential and indomethacin loading efficiency of CS-coated, PLL-coated and uncoated PECL nanocapsules

Formulation	Particle size (nm)	ζ Potential (mV)	Indomethacin loading efficiency (%)
Uncoated NC	238 ± 6	-39.9 ± 0.3	94.5 ± 4.3
PLL coated NC	251 ± 5	$+27.9 \pm 3.5$	93.1 ± 1.7
CS coated NC	384 ± 60	$+37.1 \pm 1.8$	91.8 ± 8.9

Data shown are the mean and S.D. ($n = 6$–10). NC: Nanocapsules.

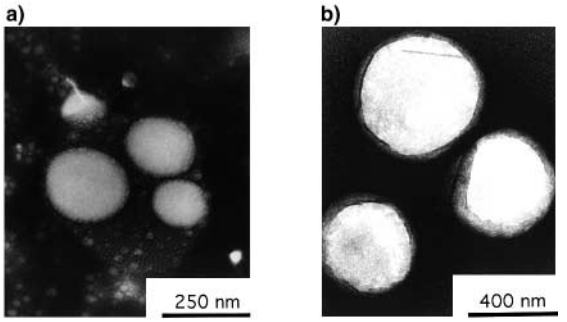

Fig. 2. Electron transmission microphotography of: (a) uncoated PECL nanocapsules; and (b) CS-coated PECL nanocapsules.

of the oily solution into the aqueous phase (Davis and Rideal, 1963). The formation of a coating around the nanocapsules was, however, identified by TEM analysis (Fig. 2).

The ζ potential values (Table 1) show the effect of the coating polymer on the surface charge. Uncoated nanocapsules exhibited a high negative charge, whereas PLL coated and CS-coated nanocapsules were positively charged. The higher positive charge observed for CS-coated nanocapsules could be explained by the higher molecular weight of CS (> 100 KD) with respect to the PLL (15 KD) and thus, the higher density of amine groups per mole of polymer (Fig. 3).

Table 1 also shows that the indomethacin loading efficiency (% of indomethacin encapsulated with respect to the total amount of drug used to prepare the particles), was very high irrespective of the carrier composition. This is logically explained by the lipophilic character of indomethacin.

3.2. In vitro release studies

Results of indomethacin release from CS-coated, PLL-coated and uncoated PECL nanocapsules are depicted in Fig. 4. It can be noted that, independent of the carrier composition, 85% of indomethacin diffused out from the colloidal systems within 2 h. The irrelevant influence of the PLL and CS coatings could be explained by the high solubility of indomethacin in

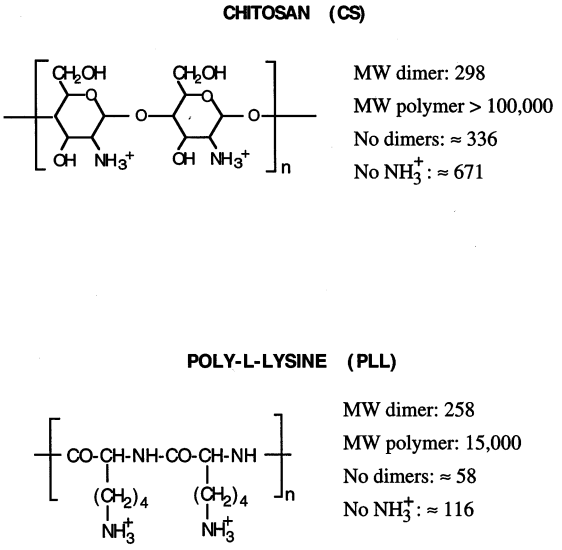

Fig. 3. Chemical structures and molecular weight of CS and PLL.

the release medium and, therefore, its great tendency to diffuse out of the oil core. This corroborates the previous observation that the release process is governed by the oil-water partition rather than by the drug diffusion through the polymer coating (Calvo et al., 1996a).

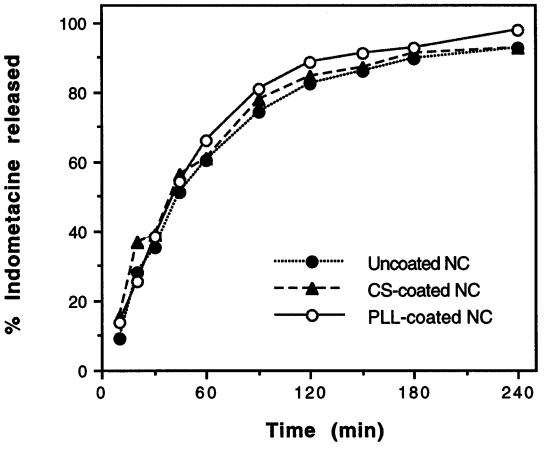

Fig. 4. Indomethacin release profiles from PLL-coated, CS-coated and uncoated PECL nanocapsules in phosphate buffer solution.

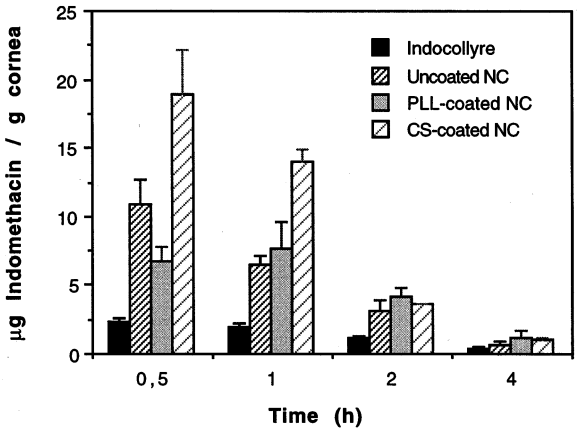

Fig. 5. Indomethacin concentrations attained in the cornea following the topical application, in rabbits, of the indomethacin-loaded carriers and the control solution. Mean values ± S.D. ($n = 3–4$) are shown. * Statistically significant different from Indocollyre (ANOVA 95% level), ** Statistically significant different from PLL coated and uncoated nanocapsules (ANOVA 95% level).

3.3. In vivo indomethacin distribution in rabbit eye: ocular bioavailability studies

The indomethacin concentrations attained in cornea and aqueous humor following instillation of the indomethacin carriers and the control solution (Indocollyre®), are displayed in Fig. 5 and Fig. 6, respectively. Results show that, at 30 and 60 min post instillation, the concentration of indomethacin in the cornea was approximately 4–6 times higher for the nanocapsules than for Indocollyre®. Similar results were obtained in the aqueous humor. Furthermore, it is interesting to note that the CS-coated nanocapsules increased the indomethacin concentration in the cornea and aqueous humor to a greater extent than the other colloidal systems. In fact, no significant differences were observed in the drug levels attained in cornea and aqueous humor following the administration of uncoated and PLL-coated nanocapsules.

The pharmacokinetic parameters computed from the indomethacin levels in the cornea and aqueous humor are summarized in Table 2. The ocular bioavailability of indomethacin is illustrated by the parameters: area-under-the-curve (AUC); the maximum indomethacin concentration (C_{max}), and the time at which the C_{max} is achieved (T_{max}). The AUC values were four times greater for PLL-coated and uncoated nanocapsules and eight times for CS-coated nanocapsules, and the C_{max} values up to seven-fold those corresponding to Indocollyre®. It is also important to note that, the elimination constants (k_{el}) of indomethacin from the cornea and aqueous humor (first order elimination kinetics) were similar for all the fommulations tested (Kruskal-Wallis, 95% level).

The improved ocular bioavailability of indomethacin achieved with the colloidal systems is consistent with previously reported data (Losa et al., 1993; Marchal-Heussler et al., 1992; Calvo et al., 1996b), from which it was concluded that PECL nanocapsules promote corneal penetration of encapsulated drugs. The originality of the present study is the development of two new ocular drug carriers which were expected to promote the ocular drug bioavailability. The coating of PECL nanocapsules with the cationic polymers CS and PLL was intentionally tailored in order to facilitate the electrostatic interaction between the positive charged particles and the negatively

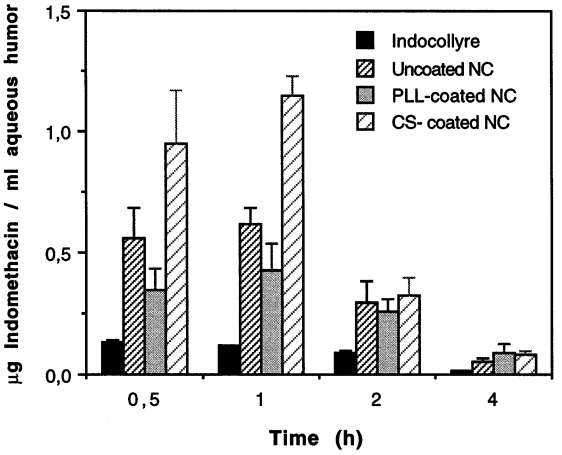

Fig. 6. Indomethacin concentrations attained in the aqueous humor following the topical application, in rabbits, of the indomethacin-loaded carriers and the control solutions. Mean values ± S.D. ($n = 3–4$) are shown. * Statistically significant different from Indocollyre (ANOVA 95% level), ** Statistically significant different from PLL coated and uncoated nanocapsules (ANOVA95% level).

Table 2
Pharmacokinetic parameters of indomethacin concentrations in cornea and aqueous humor after topical ocular instillation of the indomethacin-loaded carriers and the control solution

Tissue/formulation	AUC (μg/min per g)	Cmax (μper g)	t_{max} (min)	$k_{el}10^{-3}$ (min^{-1})	$t_{1/2}$(min)
Cornea					
Indocollyre	292 ± 22	2.4 ± 0.2	30	8.2 ± 1.4	86 ± 13
Uncoated NC	933 ± 177^{a}	10.9 ± 1.7^{a}	30	9.2 ± 1.4	77 ± 16
PLL coated NC	991 ± 272^{a}	7.6 ± 1.9^{a}	60	11.3 ± 6.0	61 ± 11
CS coated NC	1584 ± 254^{b}	18.9 ± 5.2^{a}	30	14.0 ± 1.6	49 ± 5
Aqueous Humor					
Indocollyre	17 ± 2^{d}	0.1 ± 0.1^{e}	30	8.7 ± 0.9	79 ± 8
Uncoated NC	73 ± 21^{a}	0.7 ± 0.1^{a}	30	10.7 ± 1.8	65 ± 10
PLL coated NC	70 ± 10^{a}	0.4 ± 0.1^{a}	60	9.4 ± 3.8	73 ± 10
CS coated NC	114 ± 8^{b}	$1.2 \pm$ O.l^{a}	30	$12.8 \pm$ O.4	53 ± 1

Data shown are the mean and S.D. ($n = 3$–4).
[a] Statistically significant differences from Indocollyre (ANOVA 95% level).
[b] Statistically significant differences from Indocollyre and colloidal suspensions (ANOVA 95% level).
[c] Statistically significant differences from colloidal suspensions (ANOVA 95% level).
[d] (μg/min per ml).
[e] ($\mu g/ml^{-1}$).

charged mucosa. Nevertheless, despite this common feature, their in vivo behavior was radically different. Both, PLL-coated and CS-coated nanocapsules displayed a similar positive surface charge, however, only CS-coated nanocapsules were successful in enhancing the corneal penetration of indomethacin with respect to uncoated nanocapsules.

In a previous study we showed that the capacity of PECL nanocapsules to increase the corneal penetration of indomethacin was common to other colloidal systems, such as PECL nanoparticles and submicron emulsions (Calvo et al., 1996b). Consequently, we deduced that it was the colloidal nature of these systems the key of their uptake by the corneal epithelium. This conclusion was also supported by confocal scanning microscopy studies which revealed that, independent of their composition, the three colloidal systems were able to penetrate the corneal epithelium cells. In this sense it should be added that all these colloidal systems previously investigated had a negative surface charge and thus, the role of the ζ potential was not taken into account. In the present study, we have found that it was not the positive charge but the specific nature of CS which was responsible for the particularly enhanced uptake of the CS-coated nanocapsules.

3.4. Acute ocular tolerance studies

Ocular tolerance results (Table 3) led us to conclude that CS nanocapsules are well tolerated (OLI values less than 10% of the limit value of acceptability, OLI max = 110) even though their OLI values were higher than those obtained for the uncoated nanocapsules. The only anomaly observed after the last instillation of CS-coated was a slight conjunctival hyperemia. On the other hand, the exposure of the corneal epithelium to the CS-coated nanocapsules failed to cause microscopically visible changes in the morphology of the epithelium. These results suggest that the CS did not cause appreciable disruptions in the epithelial cells which would lead to the enhancement

Table 3
Ocular lesion indices obtained for CS-coated and uncoated PECL nanocapsules

Formulation	Ocular lesion index (OLI)		
	OLI 3 h	OLI 6.5 h	OLI 24 h
Uncoated NC	0	3.2 ± 2.3	0
CS coated NC	3.6 ± 0.9	9.4 ± 5.4	2.0 ± 2.0

Data shown are the mean $\pm$ S.D. ($n = 5$).

of the penetration of indomethacin. Nevertheless, for the interpretation of the favorable behavior of the CS-coated nanocapsules it is important to take into account recent studies that showed that CS increases the permeability of the intestinal and nasal epithelia (Artursson et al., 1994; Kotze et al., 1996; Illum et al., 1994). The authors suggested a combined mechanism of mucoadhesion and ability to open the tight junctions of epithelial cells to allow for a paracellular transport pathway. Based on these observations we have proposed a mechanism that explains the increased ocular drug penetration achieved with CS-coated nanocapsules. This mechanism could be understood as a combination of various effects: (i) the penetration of the particle in the corneal epithelial cells as shown for other colloidal carriers; (ii) mucoadhesion to the corneal epithelium; and (iii) an effect on the tight junctions. The contribution of these effects will be studied in further investigations.

To summarize, we present in this paper two new colloidal systems, PLL-coated and CS-coated nanocapsules, which were successful in improving the ocular penetration of drugs. In particular, the presence of the bioadhesive polymer CS around the nanocapsules provided an optimal corneal penetration of the encapsulated drug. Furthermore, this colloidal system displayed a good ocular tolerance. Therefore, CS-coated colloidal drug carriers can be proposed as promising systems in order to overcome the corneal epithelium barrier.

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References

Artursson, P., Lindmark, T., Davis, S.S., Illum, L., 1994. Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2). Pharm. Res. 11, 1358–1361.

Calvo, P., Alonso, M.J., Vila-Jato, J.L., Robinson, J.R., 1996b. Improved ocular bioavailability of indomethacin by novel ocular drug carriers. J. Pharm. Pharmacol. 48, 1147–1152.

Calvo, P., Remunan-López, C., Vila-Jato, J.L., Alonso, M.J. 1997. Development of positively charged collodial drug carriers: chitosan-coated polyester nanocapsules and submicron emulsions. Colloid Polym. Sci. in press.

Calvo, P., Sanchez, A., Martinez, J., Lopez, M.I., Calonge, M., Pastor, C., Alonso, M.J., 1996c. Polyester nanocapsules as new topical ocular delivery systems for cyclosporin A. Pharm. Res. 13, 311–315.

Calvo, P., Thomas, C., Alonso, M.J., Vila-Jato, J.L., Robinson, J.R., 1994. Study of the mechanism of interation of poly(ϵ-caprolactone) nanocapsules with the cornea by confocal laser scanning microscopy. Int. J. Pharm. 103, 283–291.

Calvo, P., Vila-Jato, J.L., Alonso, M.J., 1996a. Comparative in vitro evaluation of several colloidal systems, nanoparticules, nanocapsules, and nanoemulsions, as ocular drug carriers. J. Pharm. Sci. 85, 530–536.

Davies, N.M., Farr, S.J., Hadgraft, J., Kellaway, I.W., 1992. Evaluation of mucoadhesive polymers in ocular drug delivery. II. Polymer-coated vesicles. Pharm. Res. 9, 1137–1144.

Davis, J.T., Rideal, E.K., 1963. Interfacial Phenomena, Academic Press, New York.

Durrani, A.M., Davies, N.M., Thomas, M., Kellaway, I.W., 1992. Pilocarpine bioavailability from a mucoadhesive liposomal ophthalmic drug delivery system. Int. J. Pharm. 88, 409–415.

Fessi, H., Puisieux, F., Devissaguet, J P. Ammoury, N., Benita, S., 1989. Nanocapsules formation by interfacial polymer deposition following solvent displacement. Int. J. Pharm., 55, R1–R4.

Fitzgerald, P., Hadgraft, J., Kreuter, J., Wilson, C.G., 1987. A γ-scintigraphic evaluation of microparticulate ophthalmic delivery systems: liposomes and nanoparticles. Int. J. Pharm. 40, 81–84.

Goldenthal, E.L., 1968. Current views on safety evaluation of drugs. FDA Pap. 2, 13–18.

Guo, L.S.S., Radhakrishnan, R., Redemann, C.T., 1989. Adhesion of positively charged liposomes to mucosal tissues. J. Liposomal. Res. 1, 319–337.

Hirano, S., Seino, H., Akiyama, Y., Nonaka, I., 1988. Biocompatibility of chitosan by oral and intravenous administration. Polym. Eng. Sci. 59, 897–901.

Ham, R.G., McKeehan, W.L., 1979. Media and growth requirements. In: Jakoby, W.B., Pastan, I.H. (Eds.), Methods in Emzymology, Vol. 58, Academic Press, San Diego, CA, pp. 44–93.

Illum, L., Farraj, N.F., Davis, S.S., 1994. Chitosan as a novel nasal delivery system for peptide drugs. Pharm. Res. 11, 1186–1189.

Kotze, A.F., van Wyk, C.J., Verhoef, J.C., de Boer, A.Gg., Junginger, H.E., 1996. The effect of chitosan on the paracellular transport pathway. Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 23, 425–426.

Lehr, C.M., Boustra, J.A., Schacht, E.H., Junginger, H.E., 1992. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. 78, 43–48.

Levy, M.Y., Benita, S. 1990. Drug release from submicronized O/W emulsion: a new in vitro kinetic evaluation model. Int. J. Pharm. 66, 29–37.

Losa, C., Calvo, P., Castro, E., Vila-Jato, J.L., Alonso, M.J., 1991. Improvement of ocular penetration of amikacin sulphate by association to poly(butylcyanoacrylate) nanoparticles. J. Pharm. Pharmacol. 43, 548–552.

Losa, C., Marchal-Heussler, L., Orallo, F., Vila-Jato, J.L., Alonso, M.J., 1993. Design of new formulations for topical ocular administration: polymeric nanocapsules containing metipranolol. Pharm. Res. 10, 80–87.

Marchal-Heussler, L., Fessi, H., Devissaguet, J.P., Hoffman, M., Maincent, P., 1992. Colloidal drug delivery systems for the eye. A comparison of the efficacy of three different polymers: polyisobutylcyanoacrylate, polylactic-co-glycolic acid, polyepsilon-caprolactone. S.T.P. Pharm. Sci. 2, 98–104.