Design of New Formulations for Topical Ocular Administration: Polymeric Nanocapsules Containing Metipranolol

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To investigate the potential of polymeric nanocapsules for ocular delivery of beta-blockers, several formulations of polyisobutylcyanoacrylate and polyepsiloncaprolactone nanocapsules containing metipranolol base were developed. These formulations differed in the polymer forming the coating and in the type and volume of the oil encapsulated. Analysis of particle-size distribution, electrophoretic mobility, and loading efficiency of the nanocapsules revealed that the type of oil is the most important factor influencing these properties. From the *in vitro* release studies, we concluded that drug diffusion through a dialysis membrane is delayed as a consequence of the encapsulation process. However, the release profiles were not influenced by the polymeric coating, suggesting that drug release from these systems is governed mainly by the partition of the drug between the oily core and the aqueous release medium. Nevertheless, despite the inability of the polymer coat to control the release of the drug, its contribution to the stabilization of the emulsion was noted. Finally, the suitability of these formulations for ophthalmic administration was investigated. Although the pharmacologic response was not affected by the encapsulated metipranolol compared with the commercial eye drops, a drastic reduction of the drug's systemic side effects was observed.

KEY WORDS: nanocapsules; polyepsiloncaprolactone; polyisobutylcyanoacrylate; metipranolol; ophthalmic administration.

INTRODUCTION

Colloidal polymeric particles, especially polyalkylcyanoacrylate nanoparticles, have been developed as drug-targeting delivery systems intended for intravenous administration (1). Currently, there has been interest in using these colloidal carriers not only to treat systemic processes (drug targeting), but also to increase the accessibility of the drug to the receptors localized in specific areas. In particular, they can serve as vehicles for use in treating ophthalmic pathologies, since increased corneal penetration (2) and prolonged therapeutic response (3) have been achieved for some specific drugs. The abilities to improve and prolong the corneal penetration were attributed to longer (4) and closer drug contact with the epithelial ocular surfaces. This initial hypothesis was confirmed by Zimmer *et al.* (5) in a recent study in which the authors visualized the uptake of polyalkylcyanoacrylate fluorescent nanoparticles by the corneal and conjunctival epithelial cells.

Because of the difficulty of incorporating lipophilic drugs in polyalkylcyanoacrylate nanoparticles, new colloidal systems comprised of an oily core surrounded by a polymeric coating were developed (6,7). These new structures, called nanocapsules, have been thought to increase the bioavailability of some orally administered drugs (8,9). Preliminary results also indicate that these colloidal structures are promising vehicles for the topical ocular administration of lipophilic drugs (10).

For the present study, metipranolol base, a beta-blocker used to treat glaucoma in the form of metipranolol sulfate, was selected as a model lipophilic drug. The major limitation of this glaucoma therapy is the systemic toxicity caused by the high conjunctival absorption (11). Since this is a common problem with all beta-blockers applied topically to the eye, several pharmaceutical approaches have been investigated, including the design of lipophilic prodrugs (12). However, most prodrugs cannot be used in practice because of their instability in aqueous solution and the need for a lipophilic vehicle. Consequently, the design of new formulations based on the encapsulation of the oily drug solution to form a colloidal aqueous dispersion is an interesting alternative.

The objectives of the present study were to design several nanocapsule formulations containing metipranolol base, to determine the formulation parameters that influence the physical properties of the capsules, to investigate the release mechanism of the model drug from the lipophilic nanocapsules, and to evaluate the ability of these drug-delivery systems to prevent the conjunctival absorption of metipranolol and subsequent systemic side effects.

MATERIALS AND METHODS

Materials

The polymer and monomer chosen for preparing the nanocapsules were polyepsiloncaprolactone (PECL) (Aldrich-Chemie, Steinheim, Germany) and isobutylcyanoacrylate (IBCA) (Sigma Quimica, Madrid, Spain), respectively. Metipranolol base and Betamann® eye drops were kindly provided by Boehringer Mannheim (Mannheim, Germany). The oils, Migliol 840 and Labrafil 1944 CS, were purchased from Lemmel (Barcelona, Spain) and Gattefosse (Madrid, Spain), respectively. Synperonic F 68, the surfactant agent, was a generous gift from ICI (Barcelona, Spain). Ethanol and acetone were purchased from Vorquimica (Vigo, Spain). All reagents were used as received.

Preparation of Nanocapsules

Two groups of formulations were prepared following a 2^2 factorial experimental design (13). Each group used a different polymer to form the coating and a different prepara-

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tion technique. The two independent variables investigated were the type and the volume of the oily phase (Table I).

Interfacial Polymerization of IBCA

Polyisobutylcyanoacrylate (PIBCA) nanocapsules containing metipranolol base were prepared according to the method described by Al-Khouri and co-workers (6). IBCA (0.1 ml) was dissolved in 15 ml ethanol. A solution of metipranolol base (34 mg/ml oil) in either 0.5 or 0.25 ml of Migliol 840 or Labrafil 1944 CS was added, and the final oil–ethanol solution was injected slowly, under stirring, into 50 ml of an aqueous solution of 0.4% (w/v) Synperonic F 68. The nanocapsules were formed by polymerization of the monomer at the water–oil interface.

Interfacial Deposition of PECL

PECL nanocapsules containing metipranolol were prepared following the technique described by Fessi and coworkers (7). The polymer (125 mg) was dissolved in 25 ml acetone. A solution of metipranolol base (34 mg/ml oil) in either 0.5 or 0.25 ml of Migliol 840 or Labrafil 1944 CS was added, and the oil–acetone solution was injected into a stirred aqueous solution of Synperonic F 68 (0.25%, w/v). The aqueous phase rapidly turned milky and opalescent because of the deposition of the polymer around the oily nanodroplets.

The ethanol and acetone used in each case were removed in a rotary evaporator, and the colloidal nanocapsule suspensions were concentrated to a final volume of 10 ml.

Reference metipranolol formulations (control emulsions) were prepared as the nanocapsules, using the procedures already described and the same formulation ingredients, except for the polymer that formed the coating.

Physicochemical Evaluation of Nanocapsules

The particle-size distribution and the electrophoretic mobility distribution of the nanocapsules and the corresponding control emulsions were determined by photon correlation spectroscopy and Laser doppler anemometry, respectively, using a Zetasizer III (Marlvern Instruments, Malvern, UK). For these measurements, formulation samples were diluted with 10^{-3} *M* NaCl to obtain an adequate particle concentration. Zeta potential values were calculated from the mean electrophoretic mobility values using the Helmholtz–Smoluchowski equation (14).

The molecular weight of the polymer forming the coating was determined by gel-permeation chromatography. Suspensions first were ultracentrifuged to isolate the nanocapsules, which were then freeze-dried. The freeze-dried product was dissolved in tetrahydrofuran and injected after filtration into a chromatograph equipped with a refractive index detector (Beckmann Instruments Inc., Palo Alto, CA).

Table I. The 2^2 Experimental Factorial Design Corresponding to the Nanocapsule Formulations of PIBCA (Group I) and PECL (Group II) Containing Metipranolol

		Volume phase ratio (o/w)	
	Type of oil	1/20	1/40
Group I	Migliol 840	A	B
	Labrafil 1944 CS	C	D
Group II	Migliol 840	E	F
	Labrafil 1944 CS	G	H

Determination of Drug Content

Free drug (non-encapsulated) was determined spectrophotometrically (wavelength, 220 nm) (15) in the clear supernatant following separation of the nanocapsules from the aqueous medium by an ultrafiltration-centrifugation technique (Ultrafree-MC 10,000 MW, Millipore, Spain) (12,718*g*, 30 min, Biofuge Sepatech, Heraeus, Germany). Metipranolol content in the nanocapsules was calculated according to the difference between the total theoretical amount in the nanocapsule suspension and the free amount in the supernatant.

***In Vitro* Release Experiments**

The *in vitro* release of metipranolol from the nanocapsules and the control emulsions was studied by determining the diffusion rate of the drug across a cellulose dialysis membrane (molecular weight cutoff, 12,000; Sigma Quimica, Spain). Three samples (10 ml) from different lots of each formulation were placed in dialysis bags that were hermetically sealed and placed into a receptor medium (200 ml phosphate buffer, pH 6). The system was thermostated at 37°C and stirred magnetically. Samples (3 ml) of the receptor medium were taken at various time intervals and assayed for metipranolol concentration spectrophotometrically. The volume of the receptor medium removed at each time point was replaced by the same quantity of fresh dialysis medium.

The physicochemical parameters (particle size, zeta potential, and polymer molecular weight) of the systems were determined before and after release of the active molecule from the nanocapsules to evaluate the degradation of the polymer forming the nanocapsules and the integrity of the nanocapsule structure during the release process. The determinations were performed as described previously.

Stability Studies

The formulations were stored at 4°C for 3 months, after which the particle size, surface charge, and drug content were determined according to the techniques already described.

***In Vivo* Determination of Intraocular Pressure (IOP)**

Commercial eye drops or nanocapsules (25 μl; formulation E) containing 0.1% (w/v) of metipranolol were administered in the cul-de-sac of one eye of 10 male pigmented rabbits. The untreated fellow eye of each rabbit served as a control to determine the local drug response. Three additional instillations were given at 2-min intervals to detect a significant pharmacologic response. The IOP was measured for 8 hr after the last instillation by pneumotonometer (Neumotonometer Digilab Modular One, Cambridge, MA).

In Vivo Determination of Cardiovascular Side Effects

Male albino rabbits were anesthetized with urethane (1.7 g/kg). Cannulas were inserted in the trachea to facilitate spontaneous respiration and in a common carotid artery to measure blood pressure. Systolic and diastolic pressures were monitored by a TRA Letica pressure transducer on a Letica Unigraph 1000-506 device (Letica, Barcelona, Spain). The polygraph was connected to a digital counter that transformed the arterial pulse waves into heart rate values. The animals were allowed to equilibrate for at least 30 min before drug administration. The selected formulations (25 μl; formulations and commercial eye drops, Betamann) were instilled in each eye by pulling the lower lid away from the eye. Three additional instillations were given at 2, 4, and 6 min after the first to observe the blood pressure and heart rate responses.

Statistical Analysis

The two groups of formulations, each corresponding to a type of polymer, were developed according to a 2^2 factorial experimental design (13) (Table I). The independent variables, the type and the quantity (expressed as volume phase ratio o/w) of the oil, were investigated at two levels (four formulations of PIBCA nanocapsules and four formulations of PECL nanocapsules). To evaluate the influence of the two variables on the physicochemical properties of the formulations (particle size and zeta potential) and the loading capacity, a two-way analysis of variance (ANOVA) was performed. The physicochemical parameters of the control formulations also were compared by two-way ANOVA. The release profiles were compared jointly for the two groups by three-way ANOVA.

Statistical analysis of the IOP data was performed using the Wilcoxon signed ranks nonpaired test.

The data from the *in vivo* evaluation of systemic side effects (heart rate) were analyzed statistically by the Kruskal–Wallis one-way test and multiple comparison between treatments.

RESULTS

Physicochemical Properties of Metipranolol Formulations: Influence of the Variables

Table II shows the mean values of the particle sizes, zeta potentials, molecular weight distributions, and percentages of the drug encapsulated in the formulations developed. For the two groups of formulations, statistical analysis revealed that the particle size was significantly affected by the type, but not the quantity, of oil used, meaning that by increasing the oil volume, more nanocapsules were formed. In contrast, for the data corresponding to the control emulsions (Table III), a significant influence of both the type and the ratio of the oil used was detected. Moreover, after comparing the data obtained from the two groups of control emulsions containing metipranolol, statistical differences were detected for the formulations prepared with the same kind and volume of oil, suggesting that the nature of the solvent had an important effect on the particle size. Finally, regarding the particle diameters (Tables II and III), we concluded that the presence of the polymer caused a reduction in size of the nanocapsules.

Regarding zeta potential, all formulations had a negative charge; for those prepared with Migliol, the charge was more negative. The nature of the oil also influenced the zeta potential of the control formulations. However, no differences were observed for this parameter when comparing the nanocapsules and the emulsion formulations prepared with the same oil.

The molecular weight of the polymer formed *in situ* around the oily droplets, during the preparation of PIBCA nanocapsules, was greatly affected by the nature of the oil. The more hydrophilic oil (Labrafil) leading the synthesis of a higher molecular weight polymer.

Finally, the type and quantity of oil used were shown to affect the drug-loading capacity of the nanocapsules. However, the nature of the polymer had no significant effect on this parameter.

Analysis of *in Vitro* Release Profiles and Release Mechanism

Figure 1 shows the *in vitro* diffusion profiles obtained when the drug in the form of aqueous solution of metipranolol sulfate (Betamann®) or in the form of a colloidal suspension of metipranolol base (formulation E) was placed into the dialysis tubing. A slower diffusion rate was detected for the encapsulated metipranolol. To investigate if the delay in release was due to the presence of the oily phase or whether the polymer had an effect, the release profiles obtained from the nanocapsule formulations were compared with those

Table II. Particle Size, Zeta Potential, Molecular Weight, and Percentage of Encapsulated Drug for PIBCA Nanocapsules (A, B, C, D) and PECL Nanocapsules (E, F, G, H)

Formulation	Particle size (nm)	Zeta potential (mV)	MW	% encapsulated drug
A	242.10 ± 06.02[a] (0.125)[b]	−17.04 ± 2.30[a]	1004 ± 0157[a]	49.88 ± 19.44[a]
B	250.33 ± 27.30 (0.214)	−22.30 ± 2.95	1049 ± 0071	40.65 ± 11.71
C	114.12 ± 08.24 (0.124)	−11.65 ± 2.35	51265 ± 0525	62.20 ± 09.61
D	110.12 ± 19.15 (0.120)	−11.31 ± 1.37	49725 ± 1109	52.69 ± 14.99
E	309.06 ± 34.90 (0.307)	−20.04 ± 2.83	40123 ± 0333	46.27 ± 07.25
F	293.10 ± 10.66 (0.125)	−19.06 ± 3.19	45570 ± 0962	34.61 ± 10.08
G	191.00 ± 42.23 (0.123)	−10.27 ± 3.23	42339 ± 3089	60.20 ± 09.66
H	194.21 ± 38.36 (0.096)	−11.80 ± 2.92	39185 ± 0375	48.64 ± 06.03

[a] Standard deviation, three determinations.
[b] Polydispersity.

Table III. Particle Size and Zeta Potential for Control Emulsions Corresponding to PIBCA Nanocapsules (a, b, c, d) and PECL Nanocapsules (e, f, g, h)

Formulation	Particle size (nm)	Zeta potential (mV)
a	378.83 ± 12.55[a] (0.410)[b]	−19.32 ± 1.27[a]
b	296.10 ± 05.96 (0.200)	−19.00 ± 0.28
c	195.10 ± 03.02 (0.280)	−10.85 ± 0.40
d	163.03 ± 07.29 (0.220)	−10.14 ± 0.92
e	652.15 ± 51.55 (0.320)	−16.62 ± 2.03
f	528.05 ± 08.84 (0.310)	−15.34 ± 0.31
g	318.85 ± 04.45 (0.240)	−10.84 ± 1.07
h	273.60 ± 19.37 (0.200)	−12.37 ± 3.05

[a] Standard deviation, three determinations.
[b] Polydispersity.

from the corresponding control emulsions. For example, Fig. 2 shows the release profiles obtained for formulations E and F and their control emulsions; similar release profiles (not shown) were found for all formulations. These *in vitro* data suggest that the polymer coating does not affect drug release from the colloidal suspension.

To find a more effective way to interpret and compare the release profiles, the release data were transformed according to the model proposed by Gupta and co-workers (16). This model was developed specifically to analyze the steps involved in the release process of a drug from a multiparticulate system and subsequent diffusion through a dialysis membrane. When applying this kinetic model, a linear plot with two slopes was found for each release profile. The values of the slopes, which represent the release rate constants, were calculated by plotting

$$\ln(C_o - C_i/V_i/V_t - Q_m/V_t) \text{ vs time}$$

where C_o is the drug concentration outside the dialysis membrane at time t, C_i is the drug concentration inside the membrane at time 0, Q_m is the total quantity of drug bound to the carrier at time 0, V_i is the volume of dissolution medium

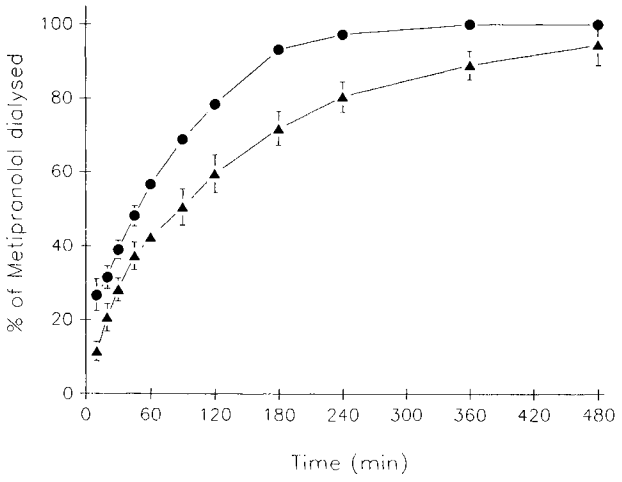

Fig. 1. *In vitro* diffusion profiles of metipranolol from a commercial formulation (Betamann®) (●) and formulation E (▲). Each point represents the average + SD from three experiments.

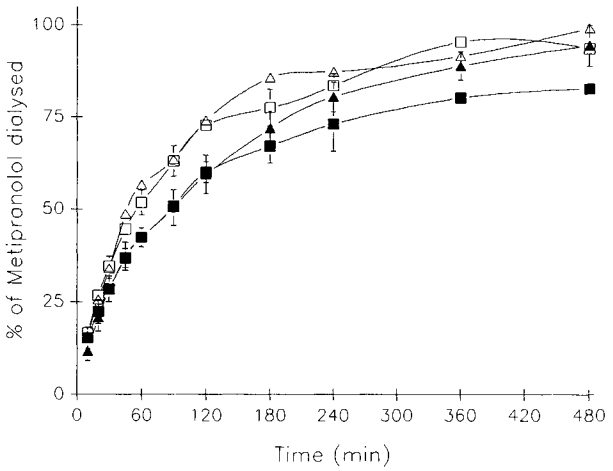

Fig. 2. Influence of the phase volume ratio (o/w) on the *in vitro* metipranolol released from the nanocapsules [formulations E (▲) and F (■)] and the corresponding control emulsions [e (△) and f (□)]. Each point represents the average + SD from three experiments.

inside the membrane, and V_t is the volume of liquid inside and outside the membrane.

The values obtained for these two diffusion rate constants and the corresponding correlation coefficients are shown in Table IV. Although the values obtained for the initial release rate constant are similar, those corresponding to the final release rate constant appear to be slightly different. From these values, we deduced that the nature of the oil affected the release kinetics of metipranolol from PIBCA nanocapsules. For PECL nanocapsules this influence was noticed only when the higher oil volume was used (formulation E).

Study of the Physicochemical Properties of the Formulation After Release

Changes in the nanocapsules during the release process and their possible consequences in the release mechanism of the active principle were assessed by comparing the physicochemical properties of the systems before and after release. When the values in Tables II and V were statistically compared, no significant differences were noted between parameters ($P < 0.05$).

Effect of Formulation Variables on Formulation Stability

Table VI shows the physicochemical parameters and drug loading of the nanocapsule formulations after 3 months of storage. In the formulations containing Labrafil (C, D, G, H), separation of the oil and aqueous phases was seen during storage. However, nanocapsules containing Migliol (A, B, E, F) were more stable. Comparing the data in Tables II and VI, no change in particle surface charge and only a small increase in the mean particle size were observed. In addition, a reduction in the quantity of drug encapsulated was seen after 3 months of storage. Regarding control emulsion stability, a separation of the phases was noticed after 1 day of storage. Therefore, these preparations should not be considered as realistic formulations for *in vivo* administration.

Table IV. *In Vitro* Release Constants for Metipranolol from PIBCA Nanocapsules (A, B, C, D) and PECL Nanocapsules (E, F, G, H)

Formulation	Initial release rate constant (k_i) (mg/min)	Final release rate constant (K_f) (mg/min)
A	$-2.81\ 10^{-2} \pm 0.78^{a}\ 10^{-2}$ (0.95)[b]	$-6.27\ 10^{-3} \pm 0.37^{a}\ 10^{-3}$ (0.99)[b]
B	$-1.20\ 10^{-2} \pm 0.10\ 10^{-2}$ (0.99)	$-6.25\ 10^{-3} \pm 1.30\ 10^{-3}$ (0.98)
C	$-1.18\ 10^{-2} \pm 0.24\ 10^{-2}$ (0.99)	$-4.20\ 10^{-3} \pm 0.84\ 10^{-3}$ (0.98)
D	$-1.07\ 10^{-2} \pm 0.05\ 10^{-2}$ (0.98)	$-3.67\ 10^{-3} \pm 0.57\ 10^{-3}$ (0.96)
E	$-1.61\ 10^{-2} \pm 0.25\ 10^{-2}$ (0.98)	$-6.61\ 10^{-3} \pm 1.72\ 10^{-3}$ (0.97)
F	$-1.30\ 10^{-2} \pm 0.09\ 10^{-2}$ (0.94)	$-3.55\ 10^{-3} \pm 0.99\ 10^{-3}$ (0.98)
G	$-1.84\ 10^{-2} \pm 0.48\ 10^{-2}$ (0.96)	$-3.20\ 10^{-3} \pm 0.51\ 10^{-3}$ (0.97)
H	$-1.05\ 10^{-2} \pm 0.08\ 10^{-2}$ (0.99)	$-2.98\ 10^{-3} \pm 0.61\ 10^{-3}$ (0.99)

[a] Standard deviation, three determinations.
[b] Correlation coefficient.

Effect of Encapsulation on the Pharmacologic Response: Reduction of IOP

Based on the stability data mentioned above, PECL nanocapsules containing Migliol (formulation E) were selected for *in vivo* administration to rabbits. The administration of the corresponding control emulsion was precluded because it was extremely unstable. Figure 3 shows the percentages of reduction of the IOP achieved after administration of formulation E and commercial eye drops. These values were calculated with the IOP values of untreated eyes as baseline. Statistical analysis of these data indicated that there were not significant differences in the pharmacologic response after administration of encapsulated metipranolol and the commercial eye drops.

Effect of Encapsulation on the Systemic Side Effects of Topically Applied Metipranolol

The heart rate decreases (bradycardia) detected in anesthetized rabbits after topical instillation of metipranolol nanocapsules (formulation E) and commercial eye drops are shown in Fig. 4. The bradycardia resulting from systemic absorption of metipranolol was greatly reduced when encapsulated metipranolol was administered. In fact, 1 hr postinstillation of metipranolol nanocapsules, the heart rates returned to the initial values; a pronounced bradycardia was observed for more than 2 hr when a standard drug solution was administered.

DISCUSSION

This study shows the importance of the effects of the formulation parameters on the physicochemical properties and stability of the metipranolol-loaded nanocapsules. In addition, the ability of these drug-delivery systems to reduce the conjunctival absorption of the beta-blocker was demonstrated.

Within the range of conditions investigated in this study, it was noted that the particle size of the nanocapsules depended mainly on the droplet size of the emulsion initially formed. Indeed, the particle size of the nanocapsules strictly correlated with the droplet size of the control emulsions, although the particle size was smaller for all nanocapsule formulations. The reduction of the particle size due to the presence of the polymer should be interpreted to be a consequence of its stabilizing effect. The formation (PIBCA) or precipitation (PECL) of the polymer around the oily nanodroplets prevents their coalescence during the solvent evaporation process. Consequently, the final particle size of the nanocapsules is smaller than the droplet size of the control emulsions. The influence of the oil is explainable in terms of its hydrophobic character: The more hydrophilic oil (Labrafil) is dispersed to a greater extent, and hence, a smaller

Table V. Particle Size, Zeta Potential, and Molecular Weight for PIBCA and PECL Nanocapsules After 8 hr of Incubation

Formulation	Particle size (nm)	Zeta potential (mV)	MW
A_I	240.41 ± 08.78^{a} (0.210)[b]	-21.21 ± 2.31^{a}	918 ± 0127^{a}
B_I	229.77 ± 06.37 (0.090)	-23.90 ± 1.92	1148 ± 0090
C_I	122.90 ± 08.63 (0.160)	-09.94 ± 1.24	49036 ± 1634
D_I	87.45 ± 03.34 (0.150)	-07.94 ± 2.97	50275 ± 1501
E_I	291.30 ± 30.87 (0.130)	-19.47 ± 2.17	42394 ± 0599
F_I	286.82 ± 39.96 (0.180)	-19.77 ± 2.40	44724 ± 0966
G_I	189.58 ± 40.79 (0.100)	-13.56 ± 1.66	41710 ± 0269
H_I	199.89 ± 35.87 (0.130)	-14.52 ± 2.17	40724 ± 1353

[a] Standard deviation, three determinations.
[b] Polydispersity.

Table VI. Particle Size, Zeta Potential, and Percentage of Encapsulated Drug for the Nanocapsules, After 3 Months of Storage

Formulation	Particle size (nm)	Zeta potential (mV)	% encapsulated drug
A	331.50 ± 15.81[a] (0.144)[b]	−18.45 ± 2.03[a]	34.38 ± 1.24[a]
B	273.10 ± 11.46 (0.205)	−23.49 ± 0.89	23.73 ± 1.80
C	NM	NM[c]	NM
D	NM	NM	NM
E	446.95 ± 09.55 (0.327)	−20.52 ± 1.49	22.07 ± 3.25
F	372.75 ± 58.48 (0.342)	−18.17 ± 1.91	15.97 ± 2.86
G	NM	NM	NM
H	NM	NM	NM

[a] Standard deviation, three determinations.
[b] Polydispersity.
[c] Nonmeasurable.

particle size is obtained. In addition, from the results shown in Tables II and III, we deduced that the oil volume does not affect the size of the nanocapsules, indicating that the degree of oil dispersion is not affected by its volume. Accordingly, more nanocapsules were formed when the oil volume was increased.

On the other hand, considering the similarities of the zeta potential values calculated for the nanocapsules and emulsions, independent of the nature of the polymer, we concluded that the polymeric coating formed around the oily droplets is not a continuous polymeric wall. Hence, the nature of the oil should be considered as the main factor determining the particle surface charge of the nanocapsules.

Regarding the molecular weight of the PIBCA nanocapsules, the large differences in this parameter can be attributed to the speed of the polymerization reaction (17). If the contact between the monomer molecules and the polymerization initiator (hydroxyl anions) is sudden, many polymerization nuclei are formed, resulting in many oligomeric chains. However, longer contact enables the monomeric units to order themselves, giving rise to long polymeric chains. Based on our results, we deduced that longer contact occurred when Labrafil was used.

The percentage of drug encapsulated is related to the solubility of metipranolol base in the oil. The solubility of metipranolol is higher with Labrafil than Migliol; consequently, formulations prepared with a high volume of Labrafil 1944 CS have greater loading efficiency.

Results from the release studies indicated a small but not significant contribution of the polymer coating to the release of the drug from these colloidal systems. The diffusion profiles in Fig. 2 display an overlap region during the first hour of the experiment that can be attributed solely to the diffusion of the free drug. This is confirmed in Fig. 1, where one of the profiles corresponds to the diffusion of the totally free drug (standard aqueous solution). It also should be noted that almost total drug release occurred from most of the formulations (nanocapsules and emulsion) during the experiment, proving that the lipophilic character of the drug is not a limiting step during the release process due to the experimental sink conditions. Other authors (18,19), using indomethacin as a drug model, found that not more than 60%

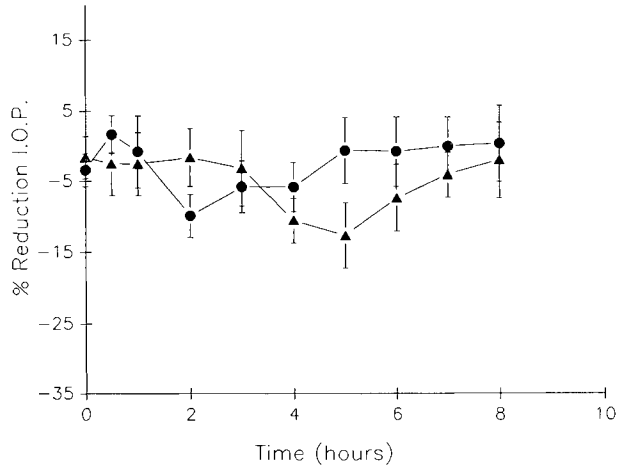

Fig. 3. Pharmacologic response (reduction of the intraocular pressure; IOP) of commercial eye drops and nanoencapsulated metipranolol. Each point represents the average + SEM from nine experiments. (●) Commercial eye drops; (▲) nanocapsules (formulation E).

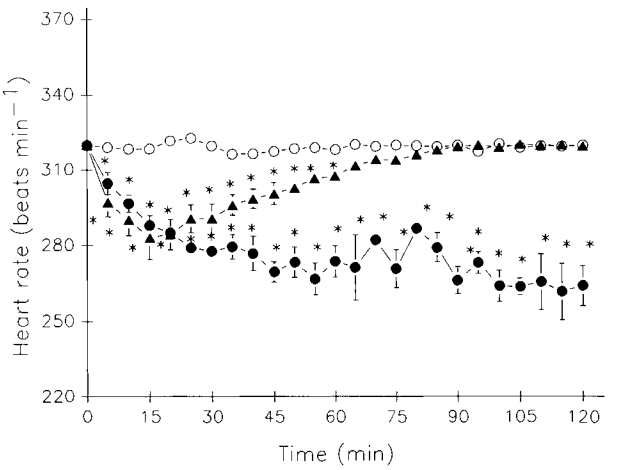

Fig. 4. Effects of commercial eye drops and nanoencapsulated metipranolol on the heart rates of anesthetized rabbits. Each point represents the average + SEM from five experiments. (○) Control; (●) commercial eye drops; (▲) nanocapsules (formulation E). (*) $P < 0.05$ with respect to controls.

of the drug was released, a finding they attributed to the high lipophilic character of the molecule and eventually its encapsulation in the micelles of surfactant present in the aqueous phase. The interpretation of the release constant values calculated from the diffusion rate data allows us to distinguish two periods: the first corresponding to the diffusion of the nonencapsulated drug (only the diffusion rate through a dialysis membrane was evaluated) and the second in which both processes (release and subsequent diffusion) were evaluated simultaneously. Considering the second part of the diffusion process, associated with K_t, the influence of the nature of the oil seems clear for PIBCA nanocapsules. In this case, a higher release rate from the nanocapsules containing Migliol is related to the lower solubility of the drug in this oil compared with Labrafil and, consequently, its favorable partition to the aqueous phase. In the case of PECL nanocapsules, this higher release rate was noticeable only with formulation E, not formulation F, possibly because of the small amount of drug encapsulated in the latter. Taking into account the small differences in the release pattern for the formulations prepared with and without polymer (nanocapsules and emulsions), we should accept as a general finding that the limiting step in the release process of metipranolol from the nanocapsules is the partition of the drug between the oily and the aqueous phases. Consequently, the drug solubility in the oily phase should be considered the most relevant factor determining the drug release from the colloidal system. Moreover, no changes in the nanocapsule structure or the polymer molecular weight were detected during the release process. Therefore, under the conditions of the *in vitro* release test, no destabilization of the systems contributed to the drug release.

To the best of our knowledge, no extensive work has been published on the relevance of formulation parameters to the properties of polymeric nanocapsules. Some authors have indicated the formation of a continuous polymeric wall theoretically able to control the release of the drug dissolved in the oily core (20). However, according to our observations, we concluded that even though the polymeric coating formed at the interface has an important stabilizing ability, it does not control the release of metipranolol from the oily core. It also is noteworthy that because the goal of this work was to develop formulations intended for ophthalmic applications, it is not desirable that release of the drug from the system be prolonged excessively with respect to the commercial eye drops, given the limited time the polymeric colloidal particles are in the eye (4,21).

Finally, based on the stability of the developed systems, formulation E was selected to check the efficacy of this new drug delivery system for the topical ocular administration of beta-blockers. The excellent results regarding the reduced bradycardia can be attributed to reduced conjunctival absorption, which theoretically could be related to greater corneal vs conjunctival absorption. However, due to the absence of statistically significant differences in the IOP after administration of formulation E and the commercial eye drops, an increase in the corneal drug penetration cannot be assured. Nevertheless, the lower systemic toxicity associated with metipranolol as a consequence of its encapsulation in PECL nanocapsules was confirmed.

In conclusion, although PIBCA and PECL nanocapsules are not able to control the release of metipranolol base, their stability (compared with that of an emulsion) and efficiency in reducing the systemic absorption of metipranolol show their potential as new drug delivery systems for ophthalmic use. More experiments must be carried out to obtain a better understanding of the *in vivo* behavior of these colloidal systems.

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