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Entrapment of β-lactams antibiotics in polyethylcyanoacrylate nanoparticles: Studies on the possible in vivo application of this colloidal delivery system

G. Cavallaro [a], M. Fresta [b], G. Giammona [a], G. Puglisi [c,*], A. Villari [d]

[a] *Dipartimento di Chimica e Tecnologie Farmaceutiche, Università di Palermo, via Archirafi 32, 90123 Palermo, Italy*
[b] *Institut für polymere, Eidgenössische Technische Hochschule, Universitätstrasse 6, CH-8092 Zürich, Switzerland*
[c] *Istituto di Chimica Farmaceutica e Tossicologica, Università di Catania, viale A. Doria 6, 95125 Catania, Italy*
[d] *Dipartimento Farmaco-Chimico, Università di Messina, V.le Annunziata 98010, Messina, Italy*

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Abstract

Polyethylcyanoacrylate (PECA) nanoparticles were prepared in the presence of three different non-ionic surfactants: Triton X-100, Tween 80 and Pluronic F68. The influence of the surfactant, employed during the nanoparticle preparation process, on drug loading, size and storage stability was evaluated. The amount of Cefaclor (CFR) and Cefsulodin (CFN) entrapped in the colloidal system was not influenced by the presence of the three different surfactants, achieving a mean loading value of about 1.6% (w/w). PECA nanoparticles prepared in the presence of Tween 80 and Pluronic F68 and entrapping the two drugs showed no dependence of the mean particle size on the various components present in the polymerization medium. Only the system obtained in the presence of Triton X-100 showed an increase in particle size compared to the nanoparticles prepared in the absence of the active compound. Storage at room temperature, as dried powder, caused a drastic increase in size and polydispersity index values of the PECA nanoparticles containing CFN or CFR. The PECA nanoparticle colloidal suspensions prepared in the presence of Pluronic F68 provided much greater values of the retention capability for CFN and CFR, followed by the systems prepared in the presence of Tween 80 and Triton X-100, respectively. For each nanoparticle formulation, the retention of CFR was higher than that of CFN. The permeability through a biological membrane model was greater for CFN loaded nanoparticles than for the free drug. In contrast, no noticeable difference was observed between free CFR and that loaded in PECA nanoparticles, prepared with Pluronic F68 or Triton X-100. Only the system prepared in the presence of Tween 80 showed a high diffusion rate of CFR compared to the free drug.

Key words: Polyethylcyanoacrylate; Nanoparticle; β-Lactam; Cefsulodin; Cefaclor; Release; Membrane permeability

1. Introduction

Several studies on controlled-release technology have been prompted over the past 20 years.

* Corresponding author.

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Much energy has been spent in pharmaceutical research to improve and to develop new therapeutic dosage forms: transdermal patches, modified-release oral dosage formulations, liposomes and microspheres. Of course, no single technology would suit every indication because of the diversity of the drugs, dosages, administration routes, and duration of action. The challenge, therefore, is to select the most suitable controlled-release technology for each particular drug and therapeutic application. In the meantime, it should be borne in mind that an ideal drug delivery device should imply selective distribution with minimal or zero uptake other than at the site of action, and this is extremely important when there is only a small margin between effective and toxic concentrations (Couvreur et al., 1980; Kreuter and Hartmann, 1983; Prescott, 1989).

Colloidal nanoparticles have attracted considerable attention. In fact, some degree of selectivity is possible (Davis et al., 1984; Kreuter 1985; Douglas et al., 1986), by the appropriate choice of particle size and surface characteristics. An important requisite in the realization of an injectable nanoparticle delivery system is the proper choice of the polymeric substrate. The polymeric bulk must fulfill many requirements as well as have suitable mechanical properties, biodegradability, high biocompatibility, drug compatibility and permeability. Suitable polymers that meet these requirements are polyalkylcyanoacrylates (PACA).

It is possible to obtain, by means of PACA, drug delivery systems with improved colloidal properties (100–200 nm in size) (Couvreur and Vauthier, 1991), which could have high tissue selectivity if coated with the appropriate non-ionic surfactant. In fact, it is possible to avoid uptake by the reticuloendothelial system, the major limiting factor in colloidal system therapy, by varying the physico-chemical characteristics of the particle surface, e.g., providing, by means of a coating with block copolymers of the Poloxamer series, a highly hydrophilic and sterically protected outer layer (Illum et al., 1982, 1986a,b; Illum and Davis, 1983, 1987). The possibility of retaining freely biocompatible colloidal particles in the blood circulation then opens up various therapeutic opportunities with anti-infective, thrombolytic, immunomodulator or antitumor drugs. Furthermore, by virtue of their improved colloidal dimension, the PACA nanoparticles could, in the presence of inflammation (the endothelial wall of the blood vessels could be disrupted), escape from the vascular compartment either via a passive process or be carried to the site of inflammation by white cells. In this way, highly colloidal nanoparticles containing anti-inflammatory drugs or antibacterial drugs could be employed for site-specific targeting to deep-seated sites of inflammation or infection.

The application of PACA nanoparticles in antimicrobial therapy through parenteral administration should not be the only route but also antibiotic local delivery seems quite promising. The topical administration of antibiotics to prevent infection in wounds is possible by means of a shaker-type dispenser or an aerosol spray. The capability of retaining antibiotic at the wound site for an extended period ensures efficacy. In fact, a single application may allow controlled release, whereby an initial burst is followed by prolonged release to maintain effective levels for prolonged periods (up 2 weeks). The treatment of ocular infection disease should be also possible considering the amenable adhesive properties of the PACA polymeric bulk (Marchal-Heussler et al., 1990).

Successful treatment of infection disease with β-lactam antibiotic-loaded liposomes has already been reported (Desiderio and Campbell, 1983a,b; Bakker-Woudenberg et al., 1985). In this study, the possibility of using polyethylcynoacrylate (PECA) nanoparticle as drug carriers for Cefaclor and Cefsulodin was evaluated. In fact, PECA nanoparticles could offer the advantage of a specific target site depending on their surface coating, and the possibility of prolonged stability during storage, which would render this system suitable for immediate application in clinical trials. Herein, the loading capacity, size, and release behaviour of PECA nanoparticles are reported, in addition to the influence of the non-ionic surfactant, used during preparation, on these parameters.

2. Materials and methods

2.1. Chemicals

Ethyl 2-cyanoacrylate (Sigma, St. Louis, MO, U.S.A.) was used as monomer during the polymerization process to obtain PECA nanoparticles. Polyethylene-polypropylene glycol block copolymer (Pluronic F68) was purchased from Fluka (Buchs, Switzerland). Polyoxyethylene sorbitan monooleate (Tween 80) and polyethylene glycol *tert*-octylphenyl ether (Triton X-100) were supplied by Merck (Darmstadt, Germany). These products were used as non-ionic surfactants in the nanoparticle preparation. Cefaclor monohydrate (CFR) was kindly provided by Eli Lilly s.p.a. (Sesto Fiorentino, Italy). Cefsulodin (CFN) was a gift from Laboratories Takeda (Puteaux Cedex, France). The purity of these two antibiotics was greater than 90% as assayed by HPLC analysis (Lee et al., 1982; Evora-Garcia et al. 1988).

Double-distilled water was used. All other chemicals were of analytical grade (Carlo Erba, Milano, Italy).

2.2. Preparation of CFR- and CFN-PECA nanoparticles

PECA nanoparticles were prepared by micellar polymerization (Kreuter, 1983; Vauthier-Holtzscherer et al., 1991) carrying out the incorporation method (Beck et al., 1993). Normally, 0.6 ml of ethyl 2-cyanoacrylate were poured at a flow rate of 60 μl/min into 50 ml of a filtered (0.2 μm membrane filter, Sartorius, Göttingen, Germany) aqueous solution of a non-ionic surfactant (Triton X-100, Tween 80 or Pluronic F68) at a concentration of 0.5% w/v. The addition of the monomer was carried out under mechanical stirring, at approx. 1000 rpm. The required amount of CFR and CFN (600 mg) was solubilized in the polymerization medium. The pH value of the aqueous solution was adjusted with 0.1 N HCl to 3.5 and 4, for CFR and CFN, respectively. These pH values were chosen based on the stability of these two drugs, which is high in the pH range 2.5–4.5 (Das Gupta and Stewart, 1984; Fujita and Koshiro, 1984; Budavari, 1989). Once the polymerization process had reached completion (usually 3 h), the nanoparticle suspension was brought to a pH value of 7.0 with 1 N sodium hydroxide solution. After buffering, stirring was continued for 2 h, from time to time monitoring the pH and adjusting it when necessary. Finally, the colloidal suspension was filtered through a sintered glass filter (grade 4, pore size 9–15) to remove contamination from particles and unwanted agglomerates whenever present. The whole preparation process was carried out at room temperature.

2.3. Drug entrapment evaluation

The unentrapped amount of the two antibiotics, CFR or CFN, was separated by centrifugation (model J2-21, Beckman, Fullerton, CA, U.S.A.) at 35 100 $\times g$ (Beckman JA-20.1 rotor, 16 500 rpm) for 1 h at a controlled temperature of 4°C. The nanoparticle pellet was resuspended in double-distilled water and centrifuged again to remove drug residue and any other soluble component from the interparticle spaces. The pellet obtained from the initial colloidal suspension was resuspended in double-distilled water (10 ml) and freeze-dried by connecting a round-bottomed flask to a lyophilizer (Edwards Freeze Dryer Modulyo, equipped with an Edwards high vacuum pump Serial E 2M8 42810). Usually, 30 mg of freeze-dried powder were solubilized in CH_3CN/CH_3OH (9:1 v/v), and filtered through 0.45 μm PTFE membrane filters (Sartorius, Göttingen, Germany). The organic solution was then injected into a Varian model 5000 liquid chromatograph in order to determine the amount of drug. The HPLC apparatus was equipped with a Hewlett Packard 3394 integrator and a Spherisorb ODS_2 C_{18} column (5 μm, 250 mm $\times$ 4.6 mm i.d., Kontron Analytical, Switzerland). The mobile phase, filtered and degassed by means of ultrasonication prior to use, was 0.02 M NH_4OCOCH_3 in $H_2O/CH_3OH/CH_3CN$ (95:3.5:1.5 v/v) and 1.5% (v/v) CH_3COOH aqueous solution/CH_3CN (97:3 v/v) for CFN and CFR, respectively. The elution flow rate was 1.5 and 2 ml/min for CFN and CFR, respectively. The chromatographic analysis was carried out at room

temperature. UV detection was conducted at 254 and 261 nm for CFR and CFN, respectively. The drug content was calculated by recording the relative peak height of the drug on an HPLC calibration curve constructed with drug solutions in methanol at known concentration. The CFN calibration curve yielded a linear regression value of 0.995 and a straight-line equation of $y = 0.558 + 666.2543x$, while for CFR $r = 0.972$ and $y = 4.4 + 97.38516x$ were obtained. The drug content of the formulation was expressed as the percentage of drug contained in the dried material resulting from lyophilization of the nanoparticle colloidal suspension.

2.4. CFN and CFR release from nanoparticles

An aliquot (40 mg) of the pellet of nanoparticles containing CFN or CFR was suspended in 6 ml of pH 7.4 phosphate buffer. This suspension was placed within a dialysis membrane (Spectrapor/Por 3 membrane Mol. Wt cut-off 3500; Spectrum, Los Angeles, CA, U.S.A.). The dialysis bag was put in a vessel containing 500 ml of pH 7.4 phosphate buffer. Dialysis was carried out at a temperature of 37 ± 0.5°C (I.S. Co. model BTU 6 thermostated water bath) under gentle mechanical stirring (200 rpm) (Variomag submersible stirrers, Multipoint HI, Munich, Germany). Samples of 2 ml of the receiver solution were removed and replaced immediately with an equal volume of the dialysis medium. The amount of drug released was determined spectrophotometrically at 263 nm reporting each absorbance value on a calibration curve. The linear regression was 0.9981 and 0.9975 for CFN ($y = 0.322 + 24.653x$) and CFR ($y = 0.095 + 18.982x$), respectively.

2.5. In vitro drug permeation through membrane

A Sartorius SM 16750 absorption simulating apparatus (Membrane Filter GmbH, Germany) was used to study the in vitro interaction and permeation of CFN- and CFR-loaded PECA nanoparticles through biological membrane and barrier. The apparatus was equipped with a Sartorius diffusion cell model SM 16754. Diffusion of the nanoparticles loaded with the two drugs took place from 100 ml of a particle colloidal suspension, containing 300 mg in dried powder, in pH 7.4 isotonic phosphate buffer (phase I) to 100 ml of the same isotonic buffer solution (phase II). The aqueous phases were thermostated at 39 ± 1°C during the permeation assay in order to maintain a temperature of 37 ± 1°C within the diffusion chambers. The permeation profiles of the two types of drug-loaded nanoparticles were compared with the permeation of the free drugs, in this case 7 and 5 mg of CFN and CFR, respectively, being dissolved in phase I. These amounts corresponded to the average aliquot of both drugs entrapped in the three different particle formulations.

The membrane separating the donor and acceptor chambers was a double-layer membrane (Sartorius SM 15703; effective diffusion area, 16 cm^2). This membrane barrier was prepared by laying a hydrophilic layer (Barrier Foil Sartorius, pre-soaked in pH 7.4 isotonic phosphate buffer for 1 h) on a hydrophobic layer consisting of a cellulose nitrate membrane filter (Sartorius), impregnated with a liquid paraffin-lauryl alcohol (2.1: 10 w/w) mixture until its weight had doubled. Under these conditions, a system simulating the biological fluids and membrane was obtained (Barrelet et al., 1975; Puglisi et al., 1989).

The time course of the in vitro permeation experiment was followed up to 250 min. At predetermined intervals, 3 ml samples were taken and assayed spectrophotometrically at 263 nm in order to determine the drug amount. No interference was evident for the materials constituting the particle colloidal suspension. Each result represents the average of three different experiments. The results of drug levels recovered in phase II were corrected in order to compensate for the dilution due to the repetitive sampling, and afterwards plotted as a function of time. The rate of permeation of the two drugs from the colloidal nanoparticle suspension through the membrane can be expressed by means of the diffusion rate constant (K_d = cm min^{-1}), according to the following equation:

$$K_d = \frac{\Delta C}{\Delta t} \times \frac{V_{II}^{o}}{C_0 A}$$

where ΔC is the increase in concentration (mg/100 ml) of drug in phase II during the time interval from zero to t, Δt denotes the time (min) during which the initial diffusion takes place, C_o is the initial drug concentration, V_{II}^o represents the initial volume of phase II (100 ml), and A is the effective diffusion area of the membrane (16 cm^2).

2.6. Particle size determination

Particle size was determined using light scattering techniques. For size analysis, the photon correlation spectroscopy (PCS) method was employed. The scattering angles were 50 and 90°. Scattering signals were correlated by a Malvern 4700 particle analyzer connected to an Olivetti 240 computer. For PCS analysis, the nanoparticle sample was diluted with filtered (0.2 μm Sartorius filter) double-distilled water until the optimal colloidal concentration had been reached. Each value was the average of three experiments.

Further details concerning the light-scattering apparatus and the technique employed in the present investigation have been extensively reported elsewhere (Fresta et al., 1993; Puglisi et al., 1993).

3. Results and discussion

PECA nanoparticles are solid, very porous colloidal delivery devices in which the drug is attached via a sorptive and/or incorporation process (El-Egakey and Speiser, 1982; Beck et al., 1993). An equilibrium exists between adsorbed/incorporated drug and drug in the surrounding solution. The in vivo release of the entrapped drug is due to a desorption process from the nanoparticle surface as well as degradation of the polymeric bulk when the drug is loaded during the incorporation method. For this reason, the rate of PACA biodegradation, due to the presence of enzymes such as esterases (Lenaerts et al., 1984), could be the regulating factor in the release of drug (degradation rate decreases with increasing length of the alkyl side-chain and molecular weight). Therefore, the incorporation method was chosen not only because it should offer more readily modulable in vivo drug release, but also because it usually leads to a large amount of drug entrapped compared to the adsorption method (Alonso et al. 1991). In fact, the incorporation method allows distribution of the drug in the PACA polymeric bulk in addition to adsorption along the particle surface. In a previous paper (Vandelli et al., 1994), we reported on the important role in drug disposition played by the type of non-ionic surfactant employed during the polymerization. In particular, the property of Pluronic F68 of entrapping a greater amount of drug than other surfactants, by positioning the major part of the compound along the shell of the PACA nanoparticles, was demonstrated.

Table 1
Drug loading capacity of PECA nanoparticles prepared in the presence of three different surfactants

Surfactant	Loading capacity (% w/w) [a]	
	Cefsulodin	Cefaclor
Triton X-100	1.68	1.08
Tween 80	1.7	1.78
Pluronic F68	2.28	1.52

[a] Entrapment efficiency was expressed as the amount of drug entrapped in 100 mg of dried material. Each value is the average of three experiments.

As shown in Table 1, no particular difference in drug loading capacity was observed among the different preparations of PECA nanoparticles. In this case, the amount of drug entrapped by PECA nanoparticles prepared in the presence of Pluronic F68 is almost the same as that achieved with the other two surfactants. Only in the case of CFN was a slightly higher loading capacity observed. Evidently, not only the surfactant, but also the physico-chemical properties of the entrapped molecules, play an important role in the loading capacity and drug disposition.

A good encapsulation capacity for the drug is not the only requisite for pharmaceutical formulations of colloidal systems, since the mean size of the nanoparticle suspension is also an important factor especially for parenteral administration. In fact, particle size could influence the body distribution (Illum et al., 1982) and hence the biologi-

Table 2
Light-scattering dimensional analysis of PECA nanoparticles prepared in the absence or in the presence of Cefaclor or Cefsulodin (the colloidal suspensions were obtained in the presence of different non-ionic surfactants [a]

Surfactant	Reference		Cefaclor		Cefsulodin	
	Size (nm)	PI [b]	Size (nm)	PI [b]	Size (nm)	PI [b]
Triton X-100	104	0.30	205	0.35	190	0.15
Tween 80	230	1.50	240	0.15	260	1.55
Pluronic F68	790	1.25	800	0.40	790	3.00

[a] Each value represents the average of three different experiments.
[b] Polydispersity index value.

cal response to drug-loaded PACA nanoparticles. In a previous paper (Puglisi et al., 1993), it was reported that the pH value and the kind of surfactant present in the polymerization medium are the two main factors influencing the PACA nanoparticle size. It should be taken into consideration that other factors, i.e., the presence of the drug in the medium, may influence the polymerization process and the polymeric nucleation resulting in an alteration of the nanoparticle size (Alonso et al., 1991).

As concerns the size of PECA nanoparticles prepared in the presence of the two drugs, no particular difference was observed between the drug-loaded systems and the reference PECA nanoparticles (prepared in the absence of the two drugs). Only the nanoparticle systems prepared in the presence of Triton X-100 showed an increase in mean size when CFR or CFN were present in the polymerization medium (Table 2). This behaviour could be due to the influence of the two drugs on the micellation process of the surfactant. In fact, the possibility of achieving larger micelles than those obtained in the absence of drug could lead, as a consequence, to the formation of PECA nanoparticles with a greater mean size.

As shown in Table 2, considering solely the mean size of the particles obtained in the presence of Tween 80 and Pluronic F68, no particular influence of the two drugs on the process of nanoparticle nucleation would appear to occur. The only effect, which may be ascribed to the presence of CFR, is the lowering of the polydispersity index (PI) values for every PECA nanoparticle formulation. In fact, as reported in Table 2, mean PI values of 0.3 were obtained for nanoparticles entrapping CFR, underlining the capability of this drug for achieving a colloidal suspension with a narrow size distribution range. Therefore, CFR was somehow able to influence the formation of nanoparticles (PI values). In contrast, the presence of CFN in the polymerization medium did not exert any influence on the PI values, an even higher PI value for Pluronic F68 being attained. In any case, it is noteworthy that, under the preparation conditions employed in this work, the nanoparticles prepared without CFR or CFN also demonstrated rather high PI values (except Triton X-100, PI = 0.3).

Table 3
Light-scattering dimensional analysis of PECA nanoparticle samples submitted to freeze-drying and storage for 3 months at room temperature as dry powder [a]

Surfactant	Reference		Cefaclor		Cefsulodin	
	Size (nm)	PI [b]	Size (nm)	PI [b]	Size (nm)	PI [b]
Triton X-100	110	0.4	1200	2.0	2100	4.0
Tween 80	360	3.0	2300	3.5	450	2.5
Pluronic F68	990	1.5	2800	3.0	790	5.0

[a] Before analysis, each sample was resuspended in the same starting volume of isotonic phosphate buffer.
[b] Polydispersity index value.

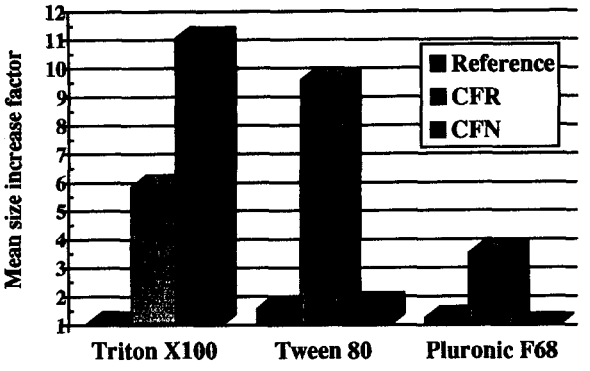

Fig. 1. Increase factor of mean PECA nanoparticle size. The various samples were submitted to freeze-drying and storage for 3 months at room temperature. Light-scattering analysis was carried out after resuspension of the dried powder.

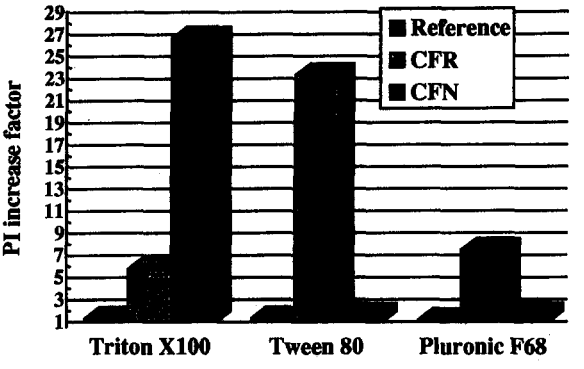

Fig. 2. Increase factor of polydispersity index values of the various PECA nanoparticles. The various samples were submitted to freeze-drying and storage for 3 months at room temperature. Light-scattering analysis was carried out after resuspension of the dried powder.

In order to evaluate the storage stability, the nanoparticle colloidal suspensions were freeze-dried and stored for 3 months at room temperature. The powder was then resuspended in the same starting volume with isotonic phosphate buffer, and submitted to light scattering analysis. The results, reported in Table 3, demonstrate that in the systems containing CFR and CFN obtained after resuspension, an appreciable increase in the mean size of the nanoparticles occurred. The increase factor (Fig. 1) was greater for PECA systems containing the two drugs. Among the surfactants employed during the preparation process, Pluronic F68 revealed the best protecting and stabilising properties. This surfactant was able to provide the lowest values of the increase factor as compared to the other two (Fig. 1). The PI values of the various nanoparticle preparations, as well as the size, showed a marked increase, indicative of the size heterogeneity of colloidal suspensions being achieved (Table 3). The increase factor of the PI values (Fig. 2) followed the same trend as reported for the size values.

Another important factor that should be taken into account in the evaluation of the drug carrier properties is the profile of release of the drug from the polymeric network of the delivery device. In fact, the retention capability characteristics are essential when particular release kinetics are desired in order to elicit a suitable biological action of the active substance, ensuring therapeutic blood or topical levels for a longer period than would be possible with the free drug. As reported in Fig. 3 and 4, the PECA nanoparticle system represents a suitable tool for achieving the modulated release of the entrapped drug. In fact, the presence of the surfactant employed during the preparation process, as well as of the monomer, was able to influence the release properties of the various PECA colloidal suspensions (Vandelli et al., 1994).

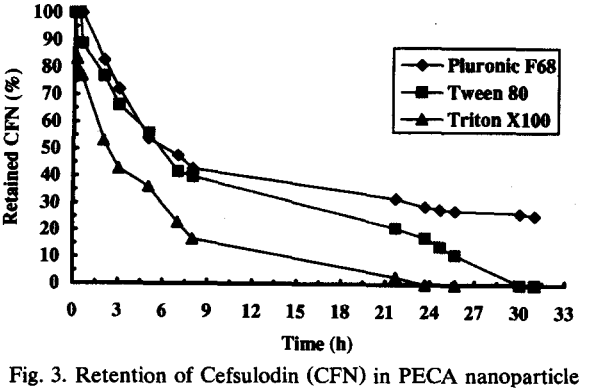

Fig. 3. Retention of Cefsulodin (CFN) in PECA nanoparticle colloidal suspensions prepared in the presence of three different non-ionic surfactants. Experiments were initiated after separation of the unentrapped amount of drug. Release was determined at a thermostated temperature of 37°C. Each value is the average of three different experiments.

Fig. 3 shows the release of CFN from PECA nanoparticles prepared in the presence of different non-ionic surfactants. As pointed out elsewhere (Vandelli et al., 1994), Pluronic F68 presented a much higher retention capability for CFN, followed in order by Tween 80 and Triton X-100. In fact, after 31 h almost 35% of CFN was still retained by the PECA nanoparticles prepared in the presence of Pluronic F68.

Concerning PECA nanoparticles containing CFR (Fig. 4), the same trend in drug retention as that reported for CFN was found. The only difference was the slower release of the CFR than that of CFN. After 31 h only the system prepared in the presence of Triton X-100 attained 100% release. In contrast, after 31 h, the PECA colloidal suspension prepared with Tween 80 and Pluronic F68 resulted in about 25 and 45% retention, respectively. Besides the influence of the surfactant and monomer type on the physicochemical characteristics of PACA nanoparticles, the chemical properties of the entrapped drug are also capable of influencing the pharmaceutical formulation parameters, as well as the release profile, dimension, size distribution and storage stability. In this case, the much stronger interaction of CFR with the PECA polymeric network ensured greater retention of the drug. This behaviour was probably due to the less hydrophilic

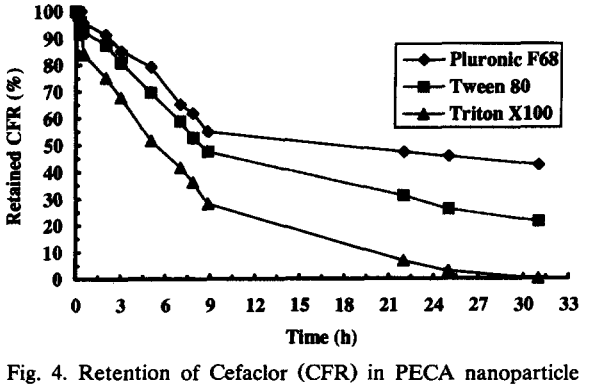

Fig. 4. Retention of Cefaclor (CFR) in PECA nanoparticle colloidal suspensions prepared in the presence of three different non-ionic surfactants. Experiments were initiated after separation of the unentrapped amount of drug. Release was determined at a thermostated temperature of 37°C. Each value is the average of three different experiments.

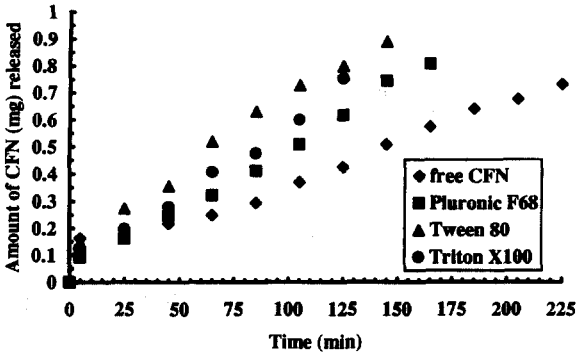

Fig. 5. Influence of the different non-ionic surfactants used during PECA nanoparticle preparation on in vitro CFN permeability through a biological membrane model. Results were compared with diffusion of the free drug. Experiments were carried out in pH 7.4 isotonic phosphate buffer at a thermostated temperature of 37°C. Each point represents the mean of three different experiments.

nature of CFR (uncharged molecule) than that of CFN, which presents a positive charge under the nanoparticle preparation conditions employed. Therefore, a better interaction between CFR and the lipophilic polymer matrix core might be possible.

In order to evaluate the interaction between the drug delivery system and the biological membrane, in vitro permeability studies through a model membrane were carried out. When the absorption of an active compound is considered to occur via a process of passive diffusion, in vitro methods can be used by mimicking the characteristics of a biological membrane with an artificial one. Therefore, the rate of release of the drug from the PECA nanoparticle colloidal carrier should be carefully evaluated in the preparation of systemic or topical delivery devices. Fig. 5 reports in vitro adsorption experiments of PECA nanoparticles containing CFN. The various PECA nanoparticle systems showed a slightly higher permeability of CFN through the membrane. The improved rate of diffusion of CFN (Table 4) from the PECA nanoparticles, compared to the free drug, was probably due to the facilitation of drug delivery in terms of permeation. In fact, the free drug, which is able to diffuse freely in the aqueous medium and to interact with the first hy-

Table 4
Diffusion rate constants for CFN- and CFR-loaded colloidal nanoparticle suspensions prepared in the presence of three different non-ionic surfactants

Formulation	K_d (cm min^{-1})	
	Cefsulodin	Cefaclor
Free drug	0.00284	0.00439
Triton X-100	0.00589	0.01260
Tween 80	0.00483	0.01170
Pluronic F68	0.00367	0.01010

drophilic layer of the membrane model, must pass through the lipophilic layer of the same membrane. This process could represent the limiting step in diffusion through the membrane model of a hydrophilic molecule. In the case of PECA nanoparticles, the outer hydrophilic shell of the particles (coated with non-ionic surfactant) should ensure interaction with the hydrophilic layer of the membrane, while the internal lipophilic core of the particle can lead to a closer interaction with the hydrophobic layer of the membrane, resulting in a greater extent of permeation of the drug.

No difference was observed in the permeation of CFR-loaded nanoparticles prepared in the presence of Pluronic F68 and Triton X-100, compared to the free drug. In contrast, the PECA system containing CFR and prepared with Tween 80 provided a high diffusion rate (Fig. 6).

Permeation through a biological membrane model is the result of various positively and negatively influencing factors, e.g., the physico-chemical properties of the drug and polymer molecules, the strength of interaction between the drug and the delivery device, capability of interaction between the drug carrier and the membrane, and diffusion processes. Therefore, the almost equal diffusion rates of CFR both free and entrapped in PECA nanoparticles (prepared with Pluronic F68 and Triton X-100) could be due to a tight interaction between the drug and the polymeric network (as demonstrated before), in addition to the physico-chemical properties that the drug displays within its environment.

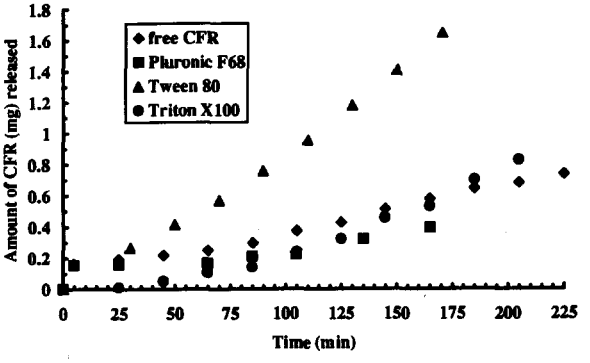

Fig. 6. Influence of the different non-ionic surfactants used during PECA nanoparticle preparation on in vitro CFR permeability through a biological membrane model. Results were compared with diffusion of the free drug. Experiments were carried out in pH 7.4 isotonic phosphate buffer at a thermostated temperature of 37°C. Each point represents the mean of three different experiments.

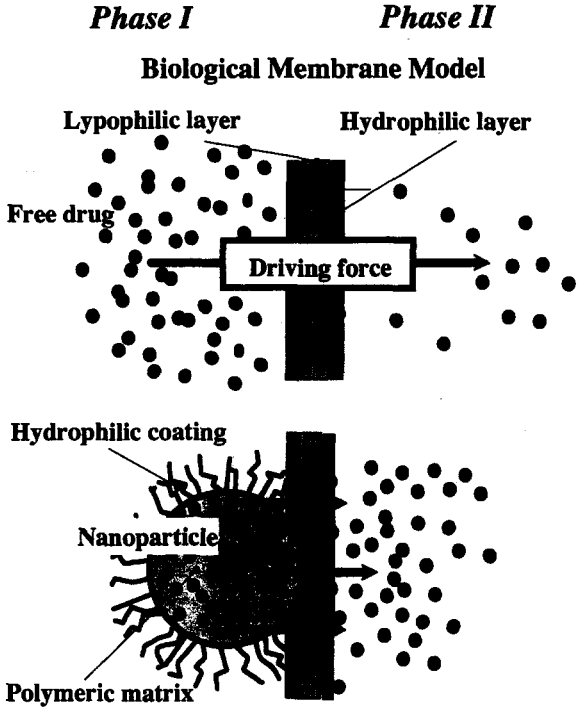

Fig. 7. Model of interaction between the aqueous phase containing free CFN or CFR, or the drug-loaded colloidal nanoparticle suspension and the biological membrane model. The permeation driving force is the different drug concentration between phase I and phase 2.

According to our model (Fig. 7), the driving force, which ensured a high diffusion rate of CFR from PECA nanoparticles, was the most favourable interaction of the polymeric drug delivery device with the biological membrane model. This result was probably due to the less dense coating of the PECA nanoparticle surface arising with Tween 80 (Carstensen et al., 1991), which led not only to an appreciable interaction with the hydrophilic membrane layer, but also to a suitable contact of the PECA polymeric matrix (hydrophobic part) with the lipophilic layer of the membrane, achieving a high rate of CFR diffusion. The systems prepared in the presence of Tween 80 provided considerable absorbability for both drugs (Fig. 5 and 6; Table 4).

Thus, the possibility of formulating the PECA nanoparticle suspensions in such a way as to fulfill the requirements for the various kinds of therapeutic application and administration route may represent an important achievement in modern drug therapy. With reference to the PECA systems studied herein, the possibility of prolonged controlled drug release and their bioadhesion properties make PECA nanoparticles containing CFN or CFR suitable candidates, and particularly the systems prepared with Pluronic F68, for topical treatment of wounds to provide a longer period of disinfection or for systemic administration, if long-circulating drug delivery devices are desired to achieve active antimicrobial blood levels for extended periods. Moreover, the systems prepared in the presence of Tween 80, which provided high permeability of the drugs through the membrane and a body distribution preferentially in the lungs (Illum et al., 1986b), could be useful in the treatment of microbial infection diseases of the respiratory apparatus, i.e., pneumonia, bronchitis and bronchial pneumonia. These systems deserve further investigation aimed at improving their long-term storage stability.

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