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Ciproflexacin-loaded polyisobutylcyanoacrylate nanoparticles: preparation and characterization

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Abstract

Experimental conditions for attachment of ciprofloxacin hydrochloride to poly(isobutylcyanoacrylate) (PIBCA) nanoparticles (NP) and release of drug were studied. Attachment of the drug was performed by the incorporative and adsorptive processes. The pH, and to a lesser degree the drug concentration in the reaction medium, were shown to be important factors in controlling the size of NP only in the incorporative process. The diameter of NP increased when the initial drug concentration was higher than 1.2 mg/ml. Ciproflaxacin content of NP was influenced by the drug concentration in the polymerization and incubation media. The binding capacity in the adsorptive process was not influenced by the pH. In contrast, the entrapment of ciprofloxacin in the NP by incorporation was greatly influenced by the pH in the range 1.5–4. Thin layer chromatography (TLC) of NP prepared by incorporation showed new products suggesting interactions between ciprofloxacin and isobutylcyanoacrylate. Ciprofloxacin release from nanoparticles prepared by the incorporating process was found to be slower as compared to nanoparticles produced by adsorptive process from which the drug release was practically complete within 1 h in absence of esterases in the release medium. Drug release from the two types of NP was accelerated in the presence of esterases in the release medium. © 1997 Elsevier Science B.V.

Keywords: Ciprofloxacin; Drug adsorption; Drug release; Isobutylcyanoacrylate; Nanoparticles

1. Introduction

Fluoroquinolones are synthetic antibacterial agents whose primary mechanism of action is inhibition of DNA gyrase. So, penetration in the

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bacterial cell is a major determinant of their antibacterial activity (Chapman and Georgopadakou, 1988). It has been proposed that quinolones can penetrate the outer membrane of Gram-negative bacteria through porin channels and phospholipid bilayers and that these routes are influenced by the hydrophobicity of the agent (Chapman and Georgopadakou, 1988). However, more recently it has been reported that there is no direct relationship between accumulation or activity and hydrophobicity for 15 quinolones (Asuquo and Piddock, 1993). Ciprofloxacin, a mono-fluorinated quinolone, is not considered as hydrophobic since its octanol/water partition coefficient (0.031) is less than one (Asuquo and Piddock, 1993). It has a good tissue and bacterial cell penetration, shows a wide spectrum of activity, and is effective against Gram-positive and Gram-negative bacterial species. The efficacy of ciprofloxacin has led to its use being proposed for the treatment of various bacterial diseases. However, ciprofloxacin is considered to be less effective than other fluoroquinolones like pefloxacin and ofloxacin in some intracellular infections. This has been attributed to its relatively short intracellular residence time (Dournon and Rajagopalan, 1987).

Concurrent to the search for new antibiotics with inherent intracellular efficiency, two other methods are explored which are the combination of different antibiotics and the association of antibiotics to colloidal drug carriers to achieve controlled release and targeting to specific sites. The first one permits an increase in the spectrum of activity, while the second allows intracellular targeting and so a better efficiency against intracellular infections. Thus, many antibiotics have been associated to colloidal drug carriers such as liposomes (Stevenson et al., 1983; Bakker-Woudenberg et al., 1985; Desiderio and Campbell, 1983a,b; Majumdar et al., 1992) and biodegradable polymeric nanoparticles (Henry-Micheland et al., 1987; Alonso et al., 1991; Fresta et al., 1995; Fresta and Puglisi, 1994; Cavallaro et al., 1994). The theoretical therapeutic advantages of antibiotic-loaded liposomes have been demonstrated in several investigations in which infected animal models with facultative intracellular pathogens were used (Desiderio and Campbell, 1983a,b; Fountain et al., 1985). Thus, ciprofloxacin-loaded liposomes have been prepared and their efficacy evaluated against *Mycobacterium avium–M. intracellulare* complex inside human macrophages (Majumdar et al., 1992) and more recently in the treatment of murine salmonellosis where it has been found that liposome-incorporated ciprofloxacin was more effective than free drug (Magallanes et al., 1993).

Nanoparticles are obtained from polymeric materials, therefore, they could be more stable than liposomes during storage and in the biological media. They are generally able to entrap antibiotics in a reproducible way (Henry-Micheland et al., 1987). However, polymeric materials must be biodegradable and biocompatible, compatible and permeable to drug and have suitable mechanical properties (Cavallaro et al., 1994). These requirements are met in polyalkylcyanoacrylates (PACA). Despite their acute in vitro cytotoxicity (Lherm et al., 1992), PACA have been recommended in the development of colloidal drug carriers for the chronic delivery of drug and successful treatments of infection diseases in animals with β-lactam antibiotic-loaded PACA nanoparticles have been reported (Youssef et al., 1988; Fattal et al., 1989).

In this study, the possibility of using polyisobutylcyanoacrylate (PIBCA) nanoparticles as colloidal drug carriers for ciprofloxacin was investigated. The influence of preparation conditions on the particle size and loading capacity of PIBCA was studied. The in vitro release profile was also evaluated.

2. Materials and methods

2.1. Chemicals

Ciproflaxacin hydrochloride was donated by Bayer Pharma (France), isobutylcyanoacrylate, D-glucose, dextran 70 and esterases from hog liver were obtained from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Prolabo (Paris, France).

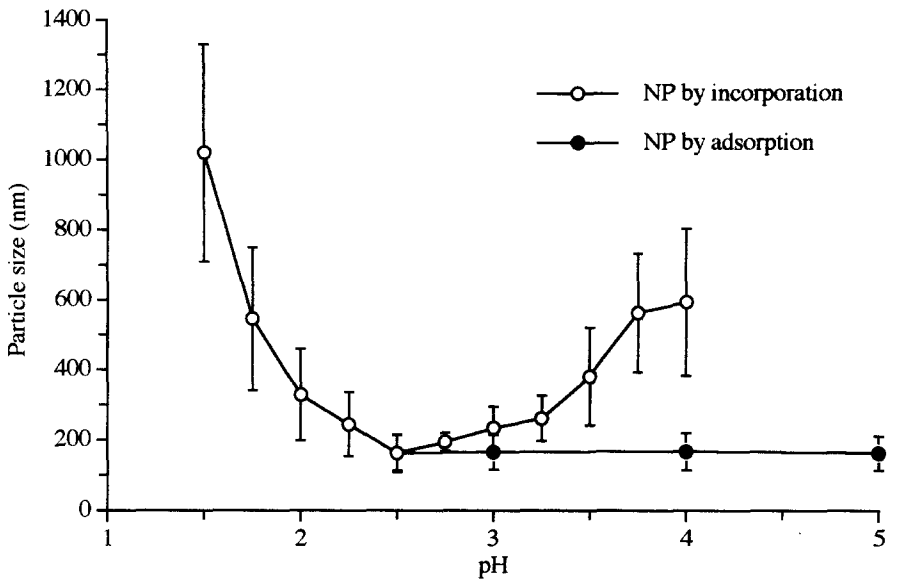

Fig. 1. Mean diameter (± S.D.) of PIBCA nanoparticles as a function of the pH of the reaction medium (1 mg of ciprofloxacin/ml).

2.2. Nanoparticle preparation

Ciprofloxacin loaded-nanoparticles were prepared by emulsion polymerization (Couvreur et al., 1982). Two different procedures were carried out for drug loading: the method by incorporating process and the method by adsorptive process.

In the method by incorporation, isobutylcyanoacrylate (100 μl) was added drop by drop during a period of 3 min under magnetic stirring to 10 ml of an aqueous polymerization medium containing D-glucose (0.5 g), dextran 70 (0.1 g) and amount of ciprofloxacin hydrochloride in the range of 0.4–3 mg of drug per ml of polymerization medium. The pH was adjusted with HCl 1 N to the desired value between 1.5 and 4. After polymerization was complete (3 h), the colloidal suspension obtained was brought to pH value of 5.5 by adding 0.5 N NaOH. The milky suspensions were then filtered through a sintered glass funnel (grade 4). Unloaded drug was eliminated by ultracentrifugation at $110\,000 \times g$ for 90 min (Beckman Model L 7-55 ultracentifuge, 7D I-Ti rotor, Beckman instruments, Gagny, France). Finally, suspensions were freeze-dried (Serail, France) for 24 h and stored at room temperature.

In the method by adsorption, unloaded polyisobutylcyanoacrylate (PIBCA) nanoparticles were prepared at pH 2.75 in the same way as in the procedure by incorporation but with no drug present in the polymerization medium. Unbound PIBCA nanoparticles were freeze-dried and then resuspended over a range of 0.4–3 mg/ml of ciprofloxacin hydrochloride in aqueous solution and adjusted to the desired pH value over the range 2.5–5. The mixture was stirred for 3 h and then unadsorbed drug was eliminated by ultracentrifugation under the same conditions as described above for nanoparticles prepared by the incorporating process. Nanoparticles were resuspended in the polymerization medium adjusted to pH 7.4 before being freeze-dried for 24 h and stored at room temperature.

For further processing, except the determination of the particle size, the two types of suspension were reconstituted just before use by addition of water by injection and handshaking.

2.3. Determination of the size of nanoparticles

The mean value and standard deviation for the overall particle size were determined by photon correlation spectroscopy (PCS). The instrument

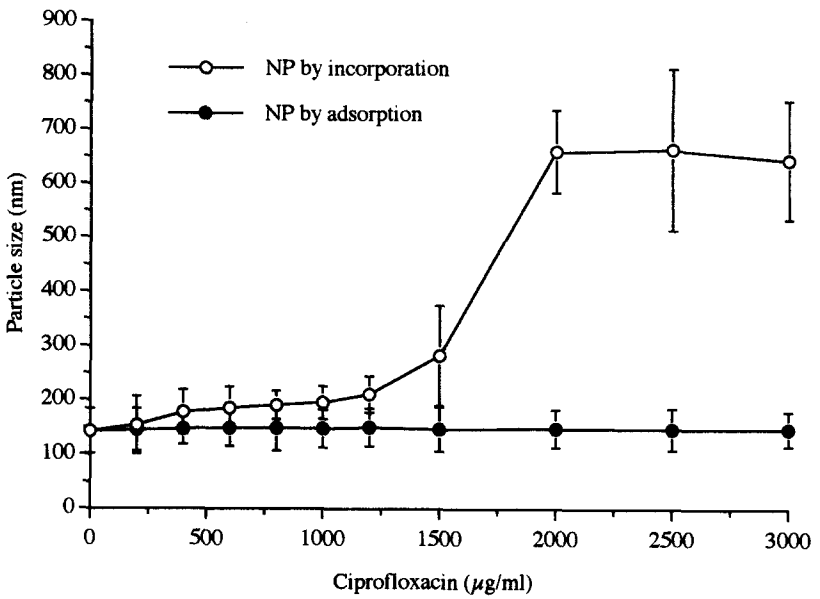

Fig. 2. Mean diameter (± S.D.) of PIBCA nanoparticles as a function of the ciprofloxacin concentration in the reaction medium (pH 2.75).

used was a Coulter N4MD Supernanosizer (4 mV, 632.8 nm helium–neon laser) from Coultronics Electronics (Margency, France). The resulting scattered light was detected at 20°C and 90° scattering angles. The measurements, for all types of nanoparticles, were carried out in triplicate on the same sample after freeze-drying. The freeze-dried nanoparticles were resuspended by handshaking in ultrafiltrated water as described above.

2.4. Determination of drug content

Ciprofloxacin associated with nanoparticles was calculated by difference between the total and the free estimated drug concentrations in the nanoparticle suspensions and the supernatant, respectively.

Free drug was separated by ultracentrifugation of suspensions as described above in Section 2.2. Fifty μl of the clear supernatant was then withdrawn and assayed for ciprofloxacin by the modified HPLC method of Awni et al. (1987) using sparfloxacin as internal standard. The HPLC equipment consisted of a model P100 pump (Thermo Separation Products, France), a Lambda 1000 UV detector (Bischoff, Germany), a model Wisp 712 auto-sampler and a Baseline 810 chromatography workstation (Waters, Saint-Quentin en Yvelines, France). Separation was achieved at room temperature on a 4.6 mm × 25 cm prepacked Microbondapack C18 column. The mobile phase was a mixture of methanol (14% v/v), acetonitrile (5% v/v), tetrabutyl ammonium hydroxide (0.3% w/v) in 0.02 M potassium dihydrogenphosphate, adjusted with phosphoric acid to pH 3 ± 0.03. The flow rate was 1.6 ml/min and detection was performed at 277 nm. In these conditions, the retention time was 4.5 min for ciprofloxacin and 7.6 min for sparfloxacin. Drug content was performed in triplicate on the same sample.

2.5. Thin-layer chromatography (TLC) experiments

TLC was carried out on glass plates precoated (0.25 mm) with silica gel 60 F 254 (Merck, Darmstadt, Germany). The mobile phase was a mixture of acetonitrile (150 ml), ammonia (7.5 ml) and distilled water (30 ml). Drug-loaded PIBCA nanoparticles were dissolved in methanol and ciprofloxacin was detected spectrophometrically

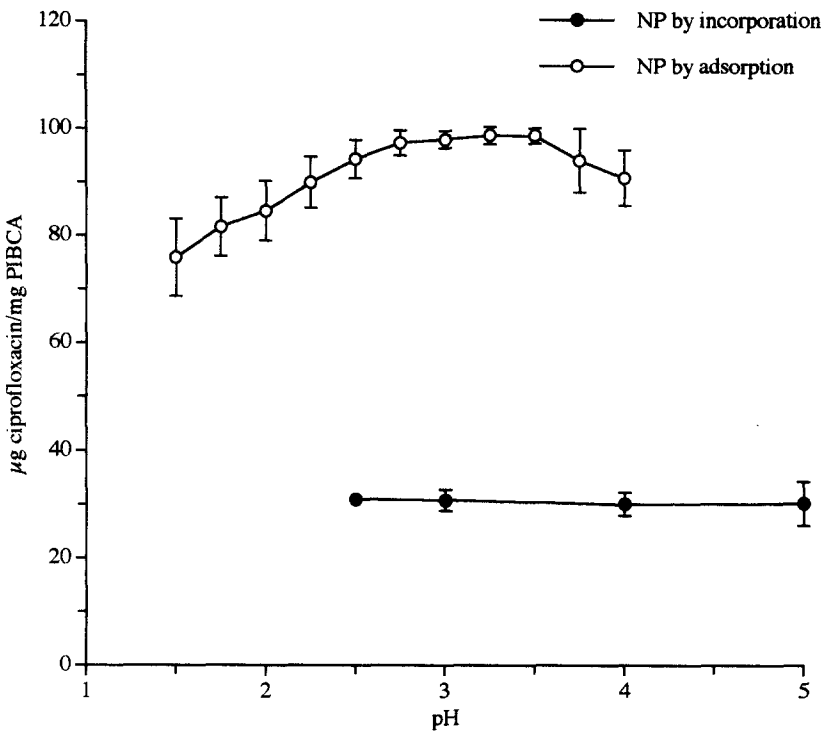

Fig. 3. Association of ciprofloxacin with PIBCA nanoparticles (mean ± S.D., $n = 3$) as a function of the pH of the polymerization and incubation media (1 mg of ciprofloxacin/ml).

at 254 nm. Pure drug, unloaded PIBCA nanoparticles and physical mixture of unloaded PIBCA nanoparticles and free ciprofloxacin hydrochloride were dissolved in methanol and used as references.

2.6. *In vitro release kinetics from nanoparticles*

In vitro release kinetics of ciprofloxacin from the nanoparticles was performed using the centrifugal ultrafiltration technique (Ammoury, 1990). It was carried out on nanoparticle suspensions obtained by the incorporation method at pH 2.75 and containing 1 mg of ciprofloxacin/ml and on nanoparticle suspensions prepared by the adsorptive process at pH 2.75 in the presence of 1 mg of ciprofloxacin/ml of the resuspension medium. The assay was performed in phosphate buffer (pH 7.0) at 37°C under continuous magnetic stirring (500 rpm) in the presence and in absence of esterases (100 µg/ml) in the release medium. The solubility of ciprofloxacin hydrochloride at 37°C being 0.15 mg/ml at pH 7.0 (Ross and Riley, 1990). The final concentration of ciprofloxacin in the dissolution medium was well below 10% of its solubility in the same medium at pH 7.0.

Practically, 1 ml of the ciprofloxacin-loaded nanoparticle suspension was directly placed in 100 ml of the release medium. At given time intervals, 0.4 ml of the release medium was withdrawn, deposited in an Ultra-free MC 10 000 NMWL filter (Millipore, Saint-Quentin en Yvelines, France) and then subjected to centrifugation at 6000 rpm for 15 min. After centrifugation, 50 µl of the ultrafiltrate were taken and ciprofloxacin content determined by the HPLC technique. The volume of the release medium remained constant since each withdrawn sample was replaced immediately with an equal volume of fresh release medium. Every kinetic experiment was repeated six times.

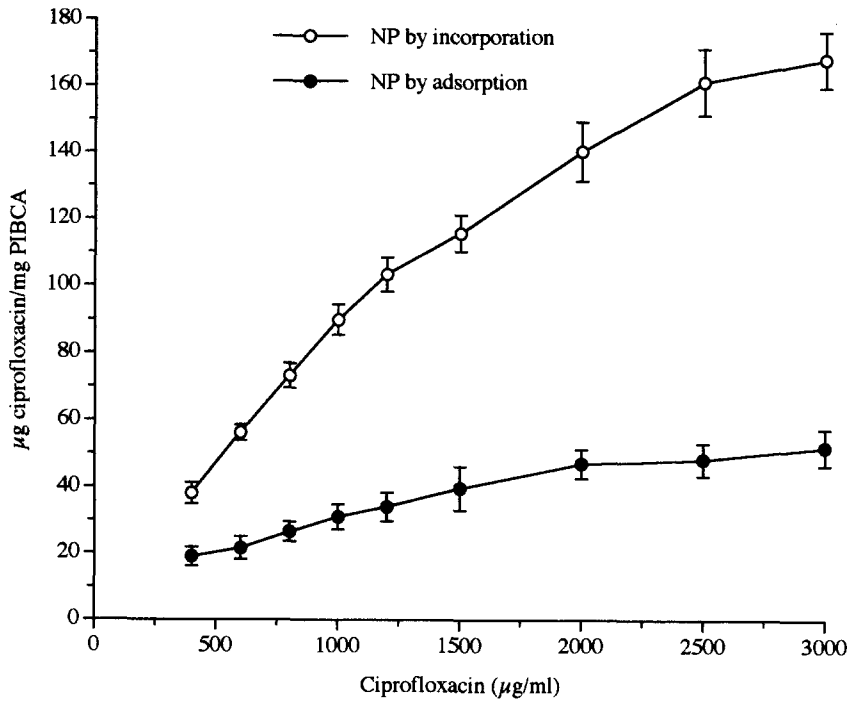

Fig. 4. Association of ciprofloxacin with PIBCA nanoparticles (mean ± S.D., $n = 3$) as a function of the drug concentration in the reaction medium (pH 2.75).

3. Results and discussion

3.1. Characterization of particle size

3.1.1. Influence of pH

The effect of pH on the size of nanoparticles prepared by both methods is seen in Fig. 1.

In the method by incorporation, the particle size is greatly influenced by the pH of the polymerization medium. Thus, in the presence of 1 mg/ml of drug in the medium, the mean diameter of nanoparticles varied from 1020 ± 310 nm (polydispersity index = 0.85) at pH 1.5 to 594 ± 210 nm (polydispersity index = 0.24) at pH 4.0. The smaller size was 163 ± 51 nm (polydispersity index = 0.16) and was obtained at pH 2.5. However, no linear relationship was found between nanoparticle size and pH of the polymerization medium. The best nanoparticle quality was obtained at pH 2.75 with a particle size of 195 ± 52 nm and a polydispersity index of 0.023. This result is in agreement with that reported by Douglas et al. (1984) for isobutylcyanoacrylate unloaded nanoparticles. These authors have found that particle size showed a minimum at pH 2 and that control of size by varying the pH could be achieved within the range 2–3.5 when polymerization is slow enough to give discrete particles but not too slow enough as to allow excessive particle coagulation.

In the method by adsorption, the particle size was nearly the same before and after incubation of nanoparticles in 1 mg/ml ciprofloxacin medium concentration at different pH values (2.5–5) (Fig. 1). Unloaded nanoparticles prepared at pH 2.75 had a mean diameter of 143 ± 41 nm. Similar results have been reported from ampicillin-loaded polyalkylcyanoacrylate nanoparticles within the pH range 1–3 (Seijo et al., 1990).

3.1.2. Influence of drug concentration in the medium

The size of the nanoparticles is also dependent of the drug concentration in the medium (Fig. 2).

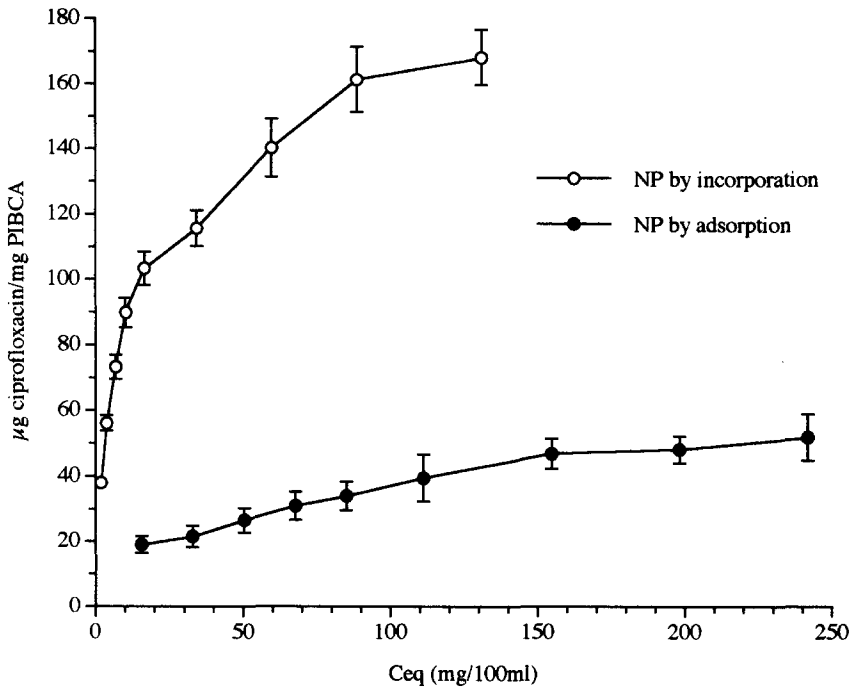

Fig. 5. Influence of the remaining ciprofloxacin concentration in the supernatant at equilibrium on the amount of drug associated to PIBCA nanoparticles (mean ± S.D., $n = 3$).

In the incorporating process, the size of nanoparticles increased with the concentration of ciprofloxacin in the polymerization medium in the range 0–3 mg/ml at pH 2.75. Relationship between particle size and drug concentration was nearly linear ($r = 0.987$) when drug concentrations were ranging from 0 to 1.2 mg/ml (Fig. 2). The particle size increased rapidly when the concentration ranged between 1.2 and 3 mg/ml. Fresta and Puglisi (1994) have also found that the particle size of PIBCA was greatly influenced by the concentration of netilmicin sulphate in the polymerization medium. The increase of particle size was attributed to interaction of the drug with chain nucleation and monomer polymerization leading to the increase in particle size and molecular weight (Fresta and Puglisi, 1994). However, Guzman et al. (1993) have found that the size of the PIBCA nanoparticles did not vary significantly when loaded with different amounts of cyclosporine.

As shown in Fig. 2, the size of ciprofloxacin-loaded PIBCA nanoparticles prepared by the adsorption method was not significantly influenced by the drug concentration in the incubation medium. Similar findings have been also reported from adsorption of primaquine onto PIBCA nanoparticles (Gaspar et al., 1991).

Comparative nanoparticle size determinations before and after freeze-drying showed a very little modification in the nanoparticle size distribution and mean nanoparticle diameter less than 5% (data not shown).

3.2. Nanoparticle drug loading and isotherms of adsorption

3.2.1. Effect of pH

The effect of the pH of the polymerization medium on the ciprofloxacin incorporation into PIBCA nanoparticles is presented in Fig. 3. When the concentration of ciprofloxacin in the polymerization medium was 1 mg/ml, the incorporation efficiency increased as the pH increased from 1.5 to 3.5 and then began to decrease. Such results lead us to think that a reduction of the ionic

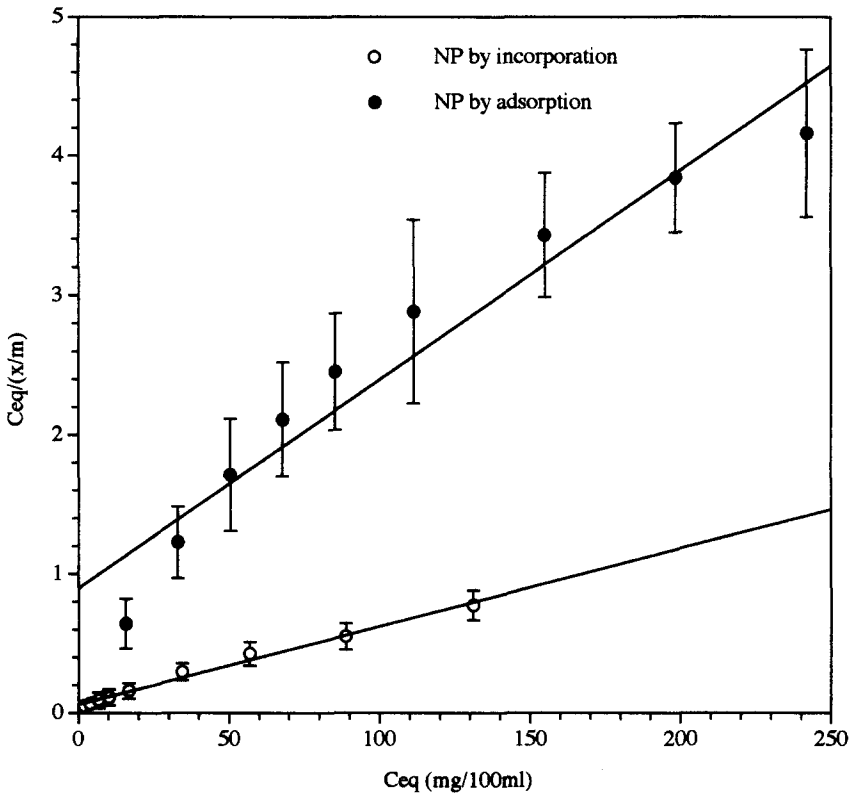

Fig. 6. Langmuir isotherms of ciprofloxacin adsorption onto PIBCA nanoparticles (pH 2.75) (mean ± S.D., $n = 3$).

character of ciprofloxacin does not increase its affinity to oligomeric chains in the matrix when the pH of the polymerization medium is higher than 3.5. Actually, we have no plausible explanation for this.

In the method by adsorption, whatever the duration of adsorption process (3, 6, 9 or 12 h, data not shown) and the pH value (2.5, 3, 4 or 5) of the incubation medium, the percentage of the adsorbed ciprofloxacin was nearly the same when the drug concentration in the incubation medium was 1 mg/ml (Fig. 3). These results suggest that the reduction of the ionic character of ciprofloxacin chloride at higher pH values does not increase its affinity to the surface of the preformed PIBCA nanoparticles. However, a great effect of the degree of ionization of the drug on its adsorption onto nanoparticles has been reported from hematoporphyrin (Brasseur et al., 1991) and primaquine (Gaspar et al., 1991) adsorption onto PIBCA nanoparticles.

Table 1
Calculated constants from Langmuir and Freundlich isotherms

Parameters	Method by incorporative process	Method by adsorptive process
Isotherms of Langmuir		
Affinity constant ($k_1 \cdot k_2$)	15.883	1.113
Maximum loading capacity (μg/ml) = k_2	179.24	66.711
Coefficient of correlation (r)	0.9961	0.9705
Isotherms of Freundlich		
Adsorptive capacity (K)	36.246	8.8348
Affinity constant (N)	0.3307	0.3240
Coefficient of correlation (r)	0.9767	0.9664

3.2.2. Effect of ciprofloxacin concentration in the polymerization medium and the incubation medium

The influence of the initial ciprofloxacin concentration in the polymerization and incubation media on the mean payload of drug is shown in Fig. 4. In both methods of nanoparticles preparation, the amount of drug associated with 1 mg of nanoparticles increased with the drug concentration up to 3 mg/ml in the reaction medium. Similar results have been reported from ampicillin- and dexamethasone-loaded IBCA nanoparticles (Seijo et al., 1990) and from adsorption of hematoporphyrin onto polyalkylcyanoacrylate nanoparticles (Brasseur et al., 1991). Bapat and Boroujerdi (1992) have also found that, whatever the preparation method, either by adsorption or by incorporation, the association of doxorubicin with PIBCA nanoparticles increased with the initial concentration of the drug in the reaction medium.

In both methods of preparation of nanoparticles, the amount of drug (x) associated with the unit weight of nanoparticles (m) increased with the drug concentration remaining in the supernatant at equilibrium (C_{eq}) (Fig. 5). Concurrently, when ciprofloxacin concentration in the reaction medium increased from 0.4 to 3 mg/ml, the percentage of the drug associated with nanoparticles

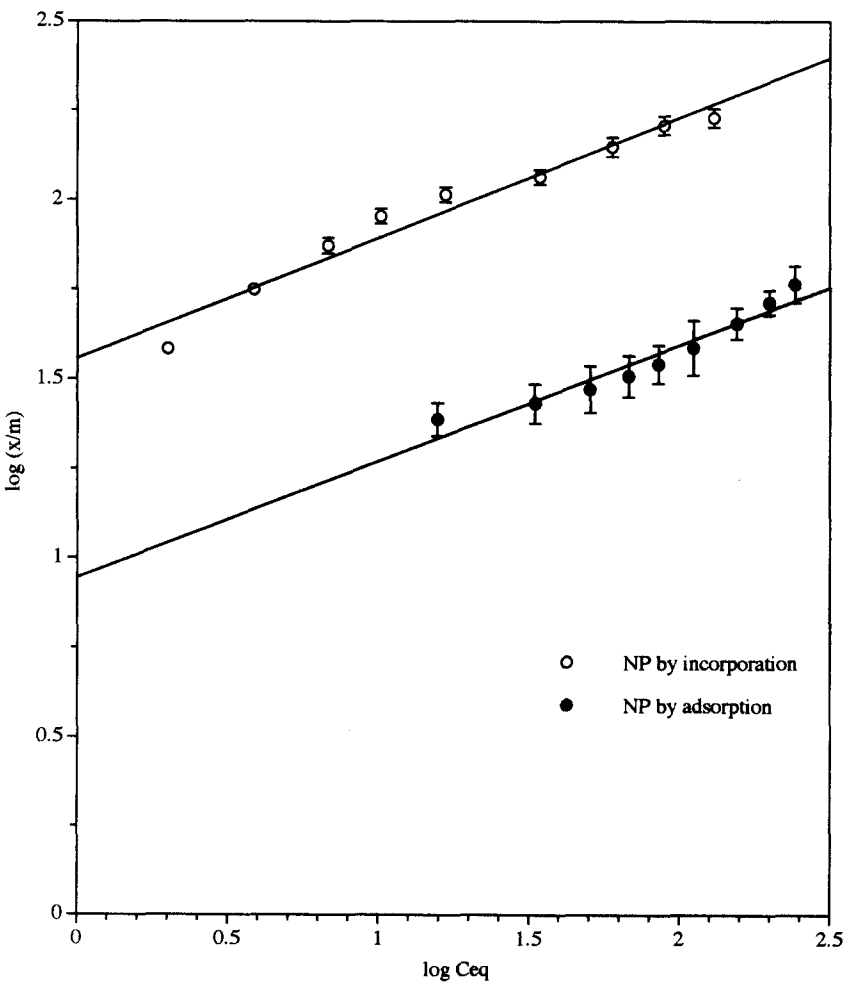

Fig. 7. Freundlich isotherms of ciprofloxacin adsorption onto PIBCA nanoparticles (pH 2.75) (mean ± S.D., $n = 3$).

dropped from 95 to 56% for the method by incorporation and from 47.7 to 17.3% for the method by adsorption (data not shown).

The data were fitted to the Langmuir isotherm equation in its linear form:

$$\frac{C_{eq}}{x/m} = \frac{1}{k_1 \cdot k_2} + \frac{C_{eq}}{k_2}$$

where $(k_1 \cdot k_2)$ is the constant of affinity and k_2 is the maximum amount of drug in (μg) that can be adsorbed per mg of nanoparticles. k_1 and k_2 are obtained from the intercept and slope of the plot of $C_{eq}/(x/m)$ against C_{eq} (Fig. 6). The maximum of loading capacity of PIBCA nanoparticles was 179.24 and 66.71 μg of ciprofloxacin/mg of polymeric material for incorporating and adsorptive processes respectively (Table 1). Using doxorubicin as a model, Van Snick et al. (1985) have demonstrated that the specific surface area of the adsorbent and adsorbate are determinant factors in the adsorbent efficiency. The difference in the uptake can then be explained by comparing these two factors (Bapat and Boroujerdi, 1992). So, as shown in Table 1, affinity constant and maximum loading capacity are respectively 14.27 and 2.7 times higher in the method by incorporation than in the method by adsorption. The molecules of drug would then interact first with the forming chains and later with external surface of nanoparticles. Thus, the interactive surface would be much greater in the incorporating process than in the adsorptive process (Bapat and Boroujerdi, 1992).

In order to explain the difference in the adsorptive capacity and the role of the affinity constant, data were also fitted to Freundlich's equation in its linear form:

$$\log \frac{x}{m} = \log K + N \cdot \log C_{eq}$$

where C_{eq} and (x/m) are as defined in the Langmuir equation, K is the adsorptive capacity and N is the affinity constant (Fig. 7). The coefficients of correlation were 0.9767 and 0.9664 for the methods by incorporation and method by adsorption, respectively (Table 1). The adsorptive capacity (K) was four times higher in the incorporating process than in the adsorptive process. Consequently, the difference in adsorptive capacity can be explained by the more important surface area available for adsorption in the method by incorporation. Similar results have been reported also by Bapat and Boroujerdi (1992) from adsorption of doxorubicin on polyalkylcyanoacrylate nanoparticles and attributed to adsorption of drug onto the surface of nanoparticles and onto the surface of the oligomeric chains in the nanoparticles.

3.3. Thin-layer chromatography (TLC)

As shown in Fig. 8, TLC yielded spots corresponding to intact ciprofloxacin ($R_f = 0.39$). However, two new products ($R_f = 0.31$ and 0.49) in small quantities, which were not seen neither on the TLC of pure drug nor on the TLC of physical mixture of ciprofloxacin-unloaded PIBCA nanoparticles, were detected in the nanoparticles prepared by incorporating process. Such results suggest an interaction between ciprofloxacin and isobutylcyaneacrylate during the polymerizing process. Similar findings have been reported from vidarabine-loaded PIBCA nanoparticles and at-

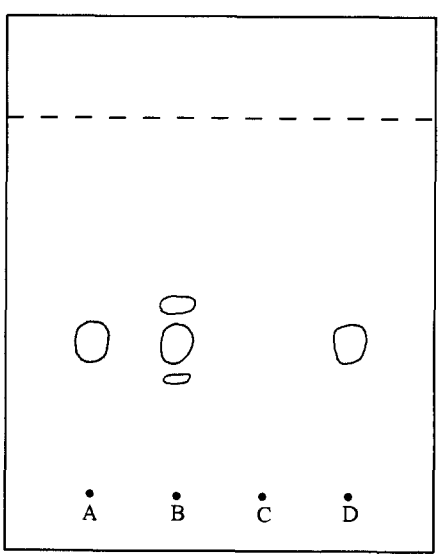

Fig. 8. TLC experiments: (A) pure ciprofloxacin; (B) ciprofloxacin-loaded PIBCA nanoparticles; (C) unloaded PIBCA nanoparticles; and (D) physical mixture of ciprofloxacin and unloaded PIBCA nanoparticles.

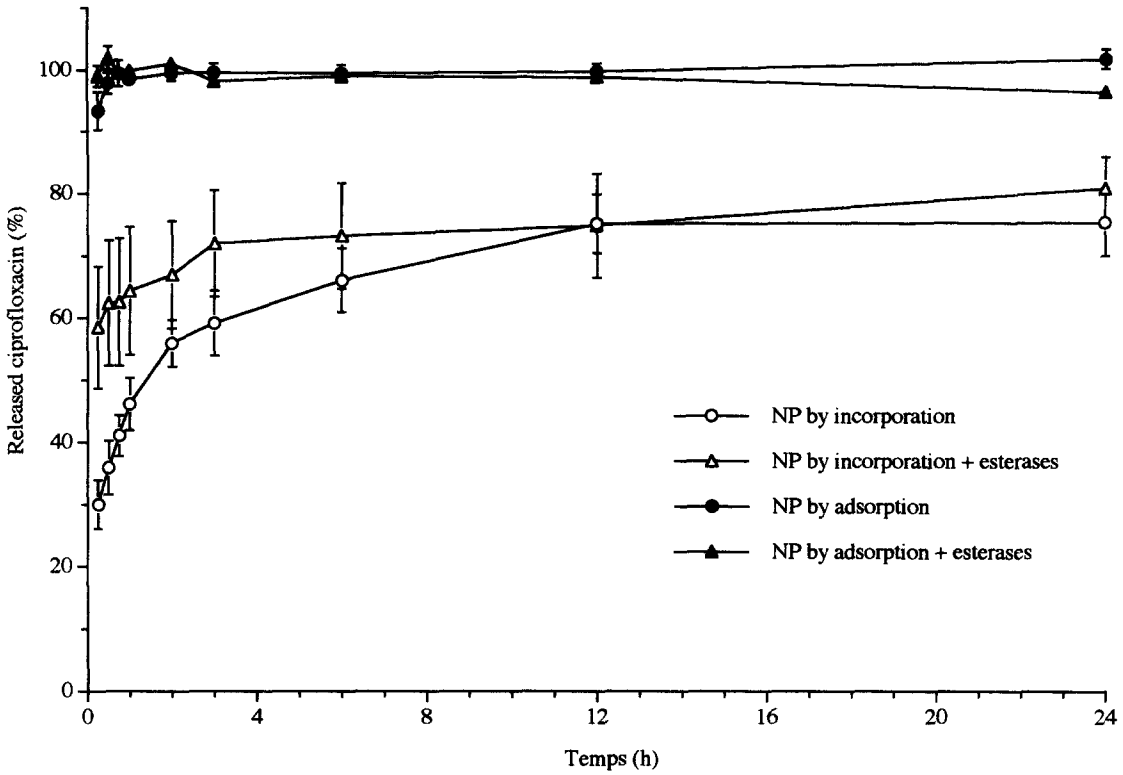

Fig. 9. Release of ciprofloxacin from PIBCA nanoparticles in absence and in presence of esterases in the release medium at pH 7 (mean ± S.D., $n = 6$).

tributed to induction of the monomer polymerization by the drug through a zwitterionic pathway (Guise et al., 1990). The induction of anionic polymerization of the monomer by the drug had already been evoked by Van Snick et al. (1985). Thus, the same interaction could likely have occured between ciprofloxacin and isobutylcyanoacrylate, while ciprofloxacin is an amphoteric fluoroquinolone and so it has a zwitterionic molecule (Ross and Riley, 1990).

3.4. Drug release from nanoparticles

Drug release profiles from PIBCA nanoparticles in the absence and in presence of esterases in the release medium are shown in Fig. 9. The mean total amount of ciprofloxacin released from nanoparticles obtained by incorporating process in absence and in presence of esterases was 75.5 ± 5.39% and 81 ± 5.1%, respectively, of their drug content. As expected, the rate of release was more rapid in the presence of esterases during the first 6 h. At 12 h after the beginning, the total amount released was nearly the same in presence and in absence of enzymes. So, it is interesting to emphasize that the release of ciprofloxacin was never complete while a maximum of 81 ± 5.1% only was released from nanoparticles in presence of esterases. These findings are in agreement with our TLC results where new products were seen on the plate from ciprofloxacin-loaded nanoparticles prepared by the incorporating process and attributed to interactions between ciprofloxacin and the isobutylcyanoacrylate. Gallardo et al. (1989) have also found that interactions between phenylbutazone and isobutylcyanoacrylate prevented completely the release of the drug from the particles.

Release of ciprofloxacin from nanoparticles prepared by adsorptive process was slightly more rapid in presence of esterases in the release

medium. Thus, the release of drug was practically complete within 0.25 h and 1 h in the presence and absence of esterases, respectively. The complete and rapid release of drug suggests that ciprofloxacin was likely adsorbed only on the external surface of nanoparticles. However, this does not explain the more rapid release of ciprofloxacin in the enzymes-containing release medium. Similar results have been reported from the release of adsorbed hematoporphyrin onto PACA nanoparticles (Brasseur et al., 1991). The proposed explanation was that adsorption of drug is not localized only at the external surface of the nanoparticles and that a part of drug could be adsorbed more deeply into superficial pores of nanoparticles (Brasseur et al., 1991). It was likely the case for ciprofloxacin-loaded PIBCA nanoparticles prepared by adsorptive process.

4. Conclusions

Ciprofloxacin-loaded PIBCA nanoparticles were prepared by adsorption onto the surface of the preformed nanoparticles and by the incorporating process. The adsorption of ciprofloxacin onto the external surface of particles was found to be inefficient as compared to the method by incorporation and allowed very rapid release of the drug in sink conditions. The incorporation of ciprofloxacin into the nanoparticles permitted a higher drug content and a slower release of the drug. So, ciprofloxacin-loaded PIBCA nanoparticles produced by the incorporating process would be useful for in vivo applications. In vivo evaluation of these colloids is actually in progress.

References

Alonso, M.J., Losa, C., Calvo, P., Vila-Jato, J.L., 1991. Approaches to improve the association of amikacin sulphate to poly(alkylcyaneacrylate)nanoparticles. Int. J. Pharm. 68, 69–76.

Ammoury, N., These de Doctorat de l'Universite de Paris XI, Chatenay Malabry, France. Etude physico-chimique et biologique de vecteurs colloidaux vesiculaires d'indometacine-acide polylactique, 1990.

Asuquo, A.E., Piddock, J.V., 1993. Accumulation and killing kinetics of fifteen quinolones for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. 31, 365–880.

Awni, W.M., Clarkson, J., Guay, D.R.P., 1987. Determination of ciprofloxacin and its 7-ethylenediamine metabolite in human serum and urine by high-performance liquid chromatography. J. Chromatogr. 419, 414–420.

Bakker-Woudenberg, I.A.J.M., Lokerse, A.F., Roerdink, F.H., Regts, D., Michel, M.F., 1985. Free versus liposome-entrapped ampicillin in treatment of infection due to *Listeria monocytogenes* in normal and athymic (nude mice). J. Infect. Dis. 151, 917–924.

Bapat, N., Boroujerdi, M., 1992. Uptake and adsorption isotherms of doxorubicin on polymeric nanoparticles: effect of methods of preparation. Drug Dev. Ind. Pharm. 18, 65–77.

Brasseur, N., Brault, D., Couvreur, P., 1991. Adsorption of hematoporphyrin onto polyalkylcyanoacrylate nanoparticles: carrier capacity and drug release. Int. J. Pharm. 70, 126–135.

Cavallaro, G., Fresta, M., Giammona, G., Puglisi, G., Villari, A., 1994. Entrapment of 13-lactams antibiotics in polyethylcyanoacrylate nanoparticles: studies on the possible in vivo application of this colloidal delivery system. Int. J. Pharm. 111, 31–41.

Chapman, J.S., Georgopadakou, N.H., 1988. Routes of quinolone permeation in *Escherichia coli*. Antimicrob. Agents Chemother. 32, 438–442.

Couvreur, P., Roland, M., Speiser, P., 1982. Biodegradable submicroscopic particles containing a biologically active substance and compositions containing them. US Patent No. 4, 329–332.

Desiderio, J.V., Campbell, S.G., 1983a. Intraphagocytic killing of Salmonella typhimurium by liposome-encapsulated cephalothin. J. Infect. Dis. 148, 563–570.

Desiderio, J.V., Campbell, S.G., 1983b. Liposome-encapsulated cephalothin in the treatment of experimental murine salmonellosis. J. Reticuloendothel. Soc. 34, 279–287.

Douglas, S.J., Illum, L., Davis, S.S., Kreuter, J., 1984. Particle size and size distribution of poly(butyl-2-cyanoacrylate) nanoparticles. I. Influence of physicochemical factors. J. Colloid. Interface Sci. 101, 149–163.

Dournon, E., Rajagopalan, P., Comparaison de l'activite de ciprofloxacine, ofloxacine et pefloxacine dans un modele animal d'infection a Legionella. In: Carbon C., Pocidalo, J.J., Cremieux, A.C. (Eds.), Acquisitions récentes sur l'ualisation des nouvelles quinolones, Arnette, Paris, 1987, pp. 39-43.

Fattal, E., Youssef, M., Couvreur, P., Andremont, A., 1989. Treatment of experimental salmonellosis in mice with ampicillin-bound nanoparticles. Antimicrob. Agents Chemother. 33, 1540–1543.

Fountain, M.W., Weiss, S.J., Fountain, A.G., Shen, A., Lenk, R.P., 1985. Treatment of *Brucella cants* and *Brucella abortus* in vitro and in vivo by stable plurilamellar vesicle encapsulated aminoglycosides. J. Infect. Dis. 152, 529–535.

Fresta, M., Puglisi, G., 1994. Association of netilmicin sulphate to poly(alkylcyanoacrylate) nanoparticles: factors influencing particle delivery behaviour. Drug Dev. Ind. Pharm. 20, 2227–2243.

Fresta, M., Puglisi, G., Giammona, G., Cavallaro, G., Micali, N., Furneri, P.M., 1995. Pefloxacine mesilate- and ofloxacin-loaded polyethylcyanoacrylate nanoparticles: characterization of the colloidal drug carrier formulation. J. Pharm. Sci. 84, 895–902.

Gallardo, M.M., Roblot-Treupel, L., Mahuteau, J., Genin, I., Couvreur, P., Plat, M., Puisieux, F., Nanocapsules et nanospheres d'alkylcyanoacrylate: interactions principe actif/polymere. Proc. APGI, 5th Int. Conf. Pharmaceutical Technology, Paris, vol IV, 1989, pp. 36-45.

Gaspar, R., Preat, V., Roland, M., 1991. Nanoparticles of isohexylcyanoacrylate (PIHCA) as carriers of primaquine: formulation, physico-chemical characterization and acute toxicity. Int. J. Pharm. 68, 111–119.

Guise, V., Drouin, J.-Y., Benoit, J., 1990. Vidarabine-loaded nanoparticles: a physico-chemical study. Pharm. Res. 7, 736–741.

Guzman, M., Molpeceres, J., Garcia, F., Aberturas, M.R., Rodriguez, M., 1993. Formation and characterization of eyclosporine-loaded nanoparticles. J. Pharm. Sci. 82, 498502.

Henry-Micheland, S., Alonso, M.J., Andremont, A., Maincent, P., Sauzieres, J., Couvreur, P., 1987. Attachment of antibiotics to nanoparticles: preparation, drug-release and antimicrobial activity in vitro. Int. J. Pharm. 35, 121–127.

Lherm, C., Muller, R.H., Puisieux, F., Couvreur, P., 1992. Alkylcyanoacrylate drug carriers: II. Cytotoxicity of cyanoacrylate nanoprticles with different alkyl chain lenght. Int. J. Pharm. 84, 13–22.

Magallanes, M., Dijkstra, J., Fierer, J., 1993. Liposome-incorporated ciprofloxacin in treatment of murine salmonellosis. Antimicrob. Agents Chemother. 37, 2293–2297.

Majumdar, S., Flasher, D., Friend, D.S., Nassos, P., Yalko, D., Hadley, W.K., Duzgunes, N., 1992. Efficacy of liposome-encapsulated streptomycin and ciprofloxacin against *Mycobacterium avium–M. intracellular* complex infections in human peripheral blood monocyte/macrophages. Antimicrob. Agents Chemother. 36, 2808–2815.

Ross, D.L., Riley, C.M., 1990. Aqueous solubilities of some variously substituted quinolone antimicrobials. Int. J. Pharm. 63, 237–250.

Seijo, B., Fattal, E., Roblot-Treupel, L., Couvreur, P., 1990. Design of nanoparticles of less than 50 nm: preparation, characterization and drug loading. Int. J. Pharm. 62, 1–7.

Stevenson, M., Baillie, A.J., Richards, R.M.I., 1983. Enhanced activity of streptomycin and chloramphenicol against intracellular *Escherichia coli* in the J774 macrophage cell line mediated by liposome delivery. Antimicrob. Agents Chemother. 24, 742–749.

Van Snick, L., Couvreur, P., Christiaens-Leyh, D., Roland, M., 1985. Molecular weights of free and drug loaded nanoparticles. Pharm. Res. 1, 36–41.

Youssef, M., Fattal, E., Alonso, M.-J., Roblot-Treupel, L., Sauzieres, J., Tancrede, C., Omnes, A., Couvreur, P., Andremont, A., 1988. Effectiveness of nanoparticle-bound ampicillin in the treatment of *Listeria monocytogenes* infection in athymic nude mice. Antimicrob. Agents Chemother. 32, 1204–1207.