J. Pharm. Pharmacol. 1991, 43: 548–552
Received January 14, 1991

Improvement of Ocular Penetration of Amikacin Sulphate by Association to Poly(butylcyanoacrylate) Nanoparticles

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Abstract—The main objective of this paper was to investigate the ability of polycyanoacrylate nanoparticles to improve the corneal penetration of hydrophilic drugs. Three different nanoparticle formulations were prepared by changing the nature of the stabilizer agent (Dextran 70000, Synperonic F 68 and sodium lauryl sulphate). The significant influence of the stabilizer type on the particle size, electrophoretic mobility and on the drug loading efficiency was proved. Moreover, the ocular disposition of amikacin was affected by its association to nanoparticles, displaying the most interesting results when Dextran 70000 was employed for preparation of nanoparticles. The increase of the amikacin concentration in cornea and aqueous humour was statistically significant for this nanoparticle formulation with respect to the other formulations and the control solution. The in-vitro release profiles obtained using a dialysis system were similar for all the nanoparticle formulations and for the control solution, indicating that drug molecules are desorbed from the nanoparticles quickly enough to maintain the equilibrium concentration in the dialysis system.

The efficacy of aminoglucoside antibiotics against Gram-negative microorganisms has led to them being proposed for the treatment and prophylaxis of eye infections, which often involve this kind of infective agent (Davis et al 1978). Unfortunately, conventional topical administration fails to provide an optimal supply of these drugs to intraocular tissues (Ipsler et al 1987). In particular, the polar molecules of amikacin sulphate do not penetrate the corneal barrier easily due to the lipophilic character of the corneal epithelium. Consequently, effective concentrations of the drug are not attained in the anterior chamber (Eiferman & Stagner 1982). Therapeutic concentrations of aminoglucosides in the aqueous humour have been achieved by intravenous or intramuscular injections, but these concentrations are not maintained for a sufficient period of time and repeated administration of large doses involves a risk of toxicity at the systemic level.

Among the new drug release systems that have been put forward in recent years to improve the poor bioavailability of ophthalmic drugs, most attention has been paid to low viscosity colloidal suspensions of liposomes or biocompatible polymer nanoparticles (Fitzgerald et al 1987). Wood et al (1985) found that hexylcyanoacrylate nanoparticles remained longer in the tear film than ophthalmic solutions, which they attributed to adhesion to the mucin/epithelial surface of the cornea and conjunctiva. It has also been shown that the efficacy of nanoparticle formulations depends on the way in which the active principle is incorporated. For example, Harmia et al (1986a, b) reported that pilocarpine is more effective when adsorbed onto nanoparticles than when incorporated into the particle matrix. More recently Marchal-Heussler et al (1990) have demonstrated that the superficial charge of the particles and the binding type of the drug onto nanoparticles were the most important factors regarding the improvement of the therapeutic response of betaxolol chlorhydrate.

The aim of this research was to evaluate the influence of the polymerization conditions on the physicochemical characteristics and on the drug loading efficiency onto nanoparticles. In addition, the ocular disposition of a model hydrophilic drug, amikacin, was studied in order to investigate the ability of these carrier systems to improve the ocular disposition of this drug.

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Materials and Methods

Preparation of amikacin nanoparticle formulations

Nanoparticles were synthesized following the method of Couvreur et al (1982) and several procedures were carried out to improve the amikacin sulphate (AKS) payload of the polybutylcyanoacrylate (PBCA) nanoparticles. Briefly, 80 mg butylcyanoacrylate was added dropwise to 10 mL of an acidic aqueous solution of three different stabilizers: Formulation A: 0·001 M HCl containing Dextran 70000 (1%). Formulation B: 0·001 M HCl containing a mixture of Dextran 70000 (1%) and sodium lauryl sulphate (SLS) (0·1%). Formulation C: 0·001 M HCl containing Synperonic F 68 (2%).

Once polymerization was complete the nanoparticle suspensions were filtered and brought to pH 7 with 0·1 M NaOH. The suspensions were then freeze-dried using glucose 2% as a cryoprotective agent and 50 mg batches of polymer particles were rehydrated with 5 mL of AKS solutions of various concentrations.

Physicochemical characteristics of the particles

The mean particle size and the mean electrophoretic mobility of the particles with and without AKS, were determined by photonic correlation spectroscopy (PCS) and by laser Doppler anemometry (LDA), respectively. Both analyses were performed using a Zetasizer III (Malvern Instruments, UK). For electrophoretic measures, particles were diluted properly with an electrolyte, NaCl, and placed in the electrophoretic cell, where a potential of −150 mV was established. The zeta potential values were calculated by means of the electrophoretic mobility distributions using the

Smoluchovsky equation (Hunter 1981; James 1979). Each formulation was prepared 3 times and for each sample 4 determinations of particle size, zeta potential and AKS loading efficiency were performed.

Determination of amikacin content

Samples of nanoparticles were ultracentrifuged and the concentration of amikacin in the supernatant was measured by polarization fluoro-immunoanalysis (PFIA) (Abbott Cientifica Instruments S.A.).

In-vitro release characteristics

The release of amikacin by the nanoparticles was studied using a dialysis system comprising two chambers separated by a cellulose membrane (Sigma Chemical Co., USA). The donor chamber was loaded with 0·5 mL of 10 mg mL^{-1} nanoparticle suspension (containing a total of 20 mg mL^{-1} AKS) or with 20 mg mL^{-1} amikacin solution; the receptor chamber was loaded with 10 mL of phosphate buffer pH 7·4. The whole apparatus was placed in a water bath thermostated at 37°C, the contents of both chambers were stirred at 100 rev min^{-1} and the concentration of amikacin in the receptor chamber was periodically determined by PFIA.

In-vivo disposition studies

Amikacin (500 μg) was administered to the eyes of New Zealand White rabbits by separating the lower eyelid from the eyeball, applying 25 μL of amikacin solution or nanoparticle suspension of the appropriate concentration, and immediately letting the eye lid resume its normal position. At various times after administration, rabbits were killed with intravenous injections of sodium pentobarbitone, immediately after which, 100 μL samples of aqueous humour were extracted from the anterior chamber. The cornea was extirpated with a trepan, frozen in liquid nitrogen at −189°C, weighed and ground in a mortar. The ground corneas were homogenized at 10000 rev min^{-1} in 1 mL of phosphate buffer in a glass homogenizer, the homogenate was centrifuged and the resulting supernatant was drawn off. The concentration of amikacin in the corneal supernatant and in the aqueous humour samples was determined by PFIA.

Results and Discussion

Particle size, zeta potential and carrier capacity of PBCA nanoparticles

According to the design established in a previous work (Alonso et al 1991) three different adsorbate formulations were developed. Tables 1 and 2 show particle size, AKS loading efficiency obtained for these particulate systems, and zeta potential values before and after the process of amikacin sulphate adsorption. The three systems bear a small negative charge. This charge probably arises from the adsorption of anions from the aqueous phase. Cations, which are generally more hydrated than anions, have a greater tendency to remain in the bulk aqueous phase compared with the large, more polarizing and less hydrated anions which consequently are adsorbed more easily. Given that AKS is positively charged (pKa = 8·1) at the pH of the incubation medium (pH = 7·4) (Monteleone et al 1983) and that the drug

Table 1. Particle size and drug loading efficiency of nanoparticle formulations.

Formulation	Particle size (nm)	Drug loading μg AKS (mg polymer)$^{-1}$
A	210·1 ± 3·77*	453·3 ± 94·5*
B	235·0 ± 1·31	112·5 ± 17·68
C	80·0 ± 1·13	83·75 ± 12·37

* s.d., n = 3.

Table 2. Zeta potential values before and after the association of AKS onto nanoparticle formulations.

	Zeta potential (mV)	
Formulation	Non-loaded	Loaded
A	−10·36 ± 2·19*	−0·26 ± 0·08*
B	−6·07 ± 1·63	−1·67 ± 0·34
C	−4·65 ± 1·33	−2·75 ± 0·37

* s.d., n = 3.

adsorption process induced a reduction of a zeta potential of the particles, it can be assumed that AKS was adsorbed onto the particles essentially by a mechanism of electrostatic interaction between drug and adsorbent polymer particles. Moreover, this mechanism explains the lower adsorption capacity of Synperonic stabilized nanoparticles. Actually, this type of surfactant agent has been proposed to reduce the particle surface charge due to its adsorption onto the surface of the particles (Müller et al 1986), consequently, the number of active sites of adsorption must be reduced. In the case of SLS stabilized nanoparticles, the situation became more complicated, due to the complex formation between drug and surfactant (Alonso et al 1991); we assume that the association of the complex to nanoparticles, allows for the reduction of the particle surface charge with a limited number of drug molecules compared with the adsorption of the drug alone.

To elucidate the role of the stabilizer on the drug adsorption pattern, two mathematical models were used: the Langmuir isotherm and Freundlich isotherm. As shown in Fig. 1, adsorption data obtained experimentally provide a good fit to the Freundlich isotherm for both formulations. Moreover the adsorption pattern corresponding to formula-

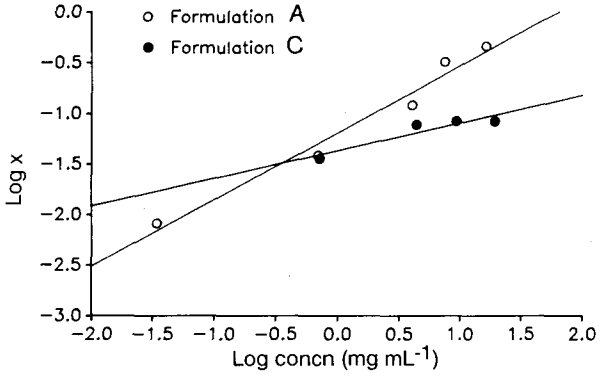

FIG. 1. Freundlinch adsorption isotherms obtained for nanoparticles prepared using Dextran 70000 (formulation A) and Synperonic F 68 (formulation C).

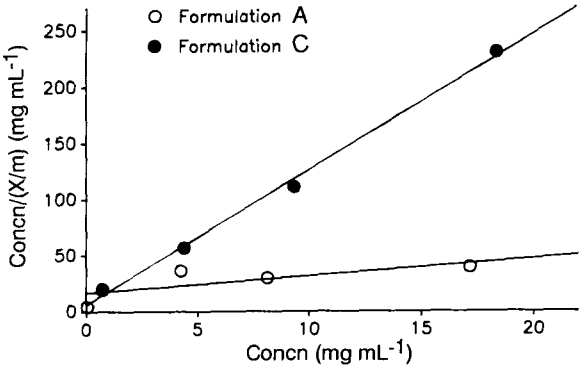

FIG. 2. Langmuir adsorption isotherms obtained for nanoparticles prepared using Dextran 70000 (formulation A) and Synperonic F 68 (formulation C).

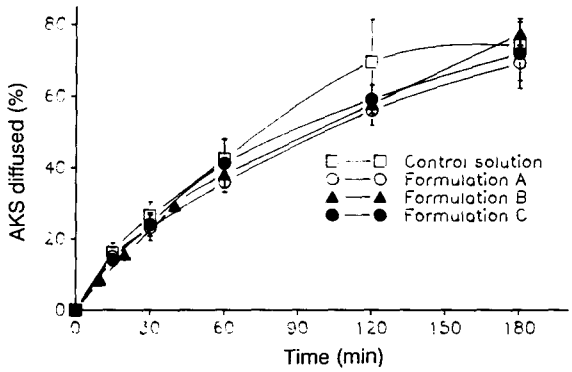

FIG. 3. In-vitro drug release profiles obtained using dialysis systems. Each point and vertical bar represent the mean and s.d. of three determinations.

Table 3. The Freundlich and Langmuir constants of formulations A and C.

	Freundlich constants			Langmuir constants		
Formulation	r	n	k 10^3	r	a	b
A	0·98	0·070	0·424	0·74	0·694	0·097
C	0·93	0·043	0·274	0·99	0·088	1·333

Freundlich equation: $\log(x) = \log k + n \log c$.
Langmuir equation: $c/x = 1/ab + 1/ac$.
x: The amount of AKS adsorbed (mg (mg polymer)$^{-1}$). c: the equilibrium AKS concentration. k and n: the Freundlich constants. a and b: the Langmuir constants.

tion C is in agreement with the Langmuir linearization (Fig. 2, Table 3). The adsorption constants and correlation coefficients were estimated by linear regression analysis. Taking into account that the Freundlich constant k (Table 3) represents the amount of drug adsorbed per unit weight of nanoparticles at the unit drug concentration (Ganjian et al 1980; Al-Achi & Boroujerdi 1990) the dextran stabilized nanoparticles are about twice as efficient for AKS adsorption than for Symperonic stabilized nanoparticles, indicating that the number of active sites available are smaller for the latter formulation. These results support the proposed theory of electrostatic interaction between the anionic adsorbent and the positively charged drug molecules. In addition, the Freundlich constant n represents the drug amount adsorbed for a given concentration change, reflecting the ratio of the rate of adsorption to desorption and the relative strength of the binding between the drug and the binding site. The n value obtained for formulation A is higher than the corresponding value for formulation C, which appears to indicate a stronger drug-polymer interaction in formulation A. Although these conclusions must be considered with caution, the Freundlich and Langmuir equations indicate that the AKS does not penetrate into the polymeric matrix, but rather is adsorbed onto the surface forming a drug monolayer.

Release of amikacin sulphate in-vitro

The in-vitro drug release results (Fig. 3) are expressed as percentages of drug diffused across a dialysis membrane. The experimental data obtained in this study were similar for the nanoparticle formulations and for a control solution. These results are understandable if we keep in mind that, in the nanoparticle formulations, most of the drug is in a free form (80% of AKS is not adsorbed), consequently the driving force for diffusion through the membrane should not be noticeably different compared with the control solution. Moreover, the proximity of the release profiles could be interpreted by an equilibrium being established between free and adsorbed drug, which means that drug molecules are desorbed at the same rate that drug molecules diffuse through the dialysis membrane. Consequently, the concentration gradient is maintained, until the drug is totally desorbed from the polymeric support. On the other hand, the presence of surfactants, SLS or Synperonic F 68, does not modify the diffusion profiles, which suggest that if some interaction exists between the active principle and the surfactant, it must be so slight as not to affect the quantity of free drug that can normally diffuse using this kind of dialysis system. Diepold et al (1989) have found similar results for pilocarpine adsorbates, using corneal perfusion cells instead of a dialysis membrane. In this study, a faster, although not statistically significant pilocarpine flux was found after binding to nanoparticles. This has been interpreted as increased membrane transport of pilocarpine by binding to colloidal carriers. Information from this kind of experiment is limited because we cannot determine the desorption rate of the drug but only the diffusion rate of the free drug through the dialysis membrane. However, this information is useful in the sense that we know the liberation rate is as quick as desired when an ophthalmic application of this system is intended.

Ocular disposition of amikacin sulphate

The ocular disposition of AKS after its administration in an aqueous solution and in the nanoparticle formulations is presented in Figs 4 and 5, respectively. ANOVA of these results indicates that there is a significant statistical difference ($P < 0.05$) between the amikacin concentration in the cornea after administration of formulation A and after administration of the control solution or formulation C. In the same way, 2 h after administration of formulation B, the corneal concentration of amikacin was significantly greater

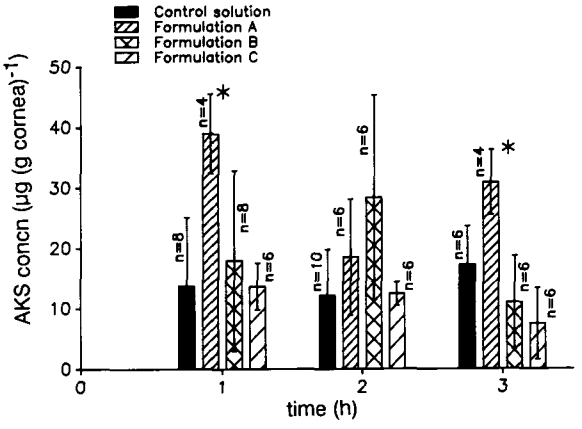

FIG. 4. Concentration of amikacin sulphate achieved in the cornea following topical administration of control solution and formulations A, B and C. The vertical bar represents the s.d. $*P<0.05$ compared with control solution.

than the corresponding result after administration of the control solution or formulation C. In contrast, the differences in corneal concentration of amikacin, were not statistically significant ($P>0.1$) at 1 and 3 h after topical instillation of the control solution and formulation B or C. Moreover, we have verified that the improved corneal penetration of amikacin 2 h after administration of formulation B, 28·37(±16·92) µg amikacin (g cornea)$^{-1}$ was mainly due to the presence of SLS in this formulation. Indeed the amikacin concentration 2 h after administration of the complex AKS-SLS was 28·48(±20·66) µg (g cornea)$^{-1}$.

Although there were no significant differences ($P>0.1$) at any time among the concentrations in aqueous humour after instillation of formulations B or C and the control solution, the differences were statistically significant with respect to formulation A ($P<0.001$). Fig. 5 indicates that concentrations of AKS in aqueous humour after administration of formulation A is at least three times greater than the concentration achieved after instillation of the control solution.

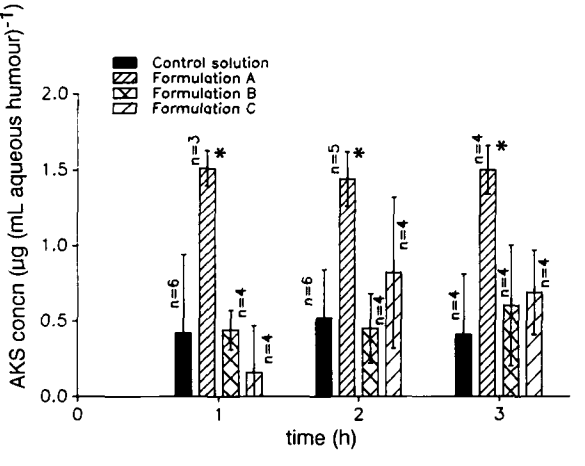

FIG. 5. Concentration of amikacin sulphate achieved in aqueous humour following topical administration of control solution and formulations A, B and C. The vertical bar represents the s.d. $*P<0.05$ compared with control solution.

The improved results concerning the ocular penetration of AKS by adsorption onto polymeric nanoparticles could be interpreted as an improved contact, in terms of intensity, quantity and time, of AKS with the corneal epithelial surface. It is also assumed that nanoparticles are retained in the mucin layer and facilitate the entrance of the positively charged drug molecules most probably by a paracellular pathway. Although the binding capacity of nanoparticles for AKS was rather low, due to the adhesive properties of these particles (Wood et al 1985) even the delivery of tiny amounts of drug release from the system that adheres to the adsorbing tissue, will significantly improve the efficiency of the delivery system. Moreover, as has been shown for other colloidal systems (Fitzgerald et al 1987), the reduction of the particle surface charge by the association of AKS, allows for the prolongation of the residence time of the adsorbate on the epithelial surface. Accordingly, the higher levels of amikacin in aqueous humour can be explained by increased membrane transport of AKS by binding to colloidal carriers.

The ineffectiveness of formulations B and C should be attributed to the surfactants used in these formulations. The influence of the surfactants could be interpreted in two different ways. On the one hand, the drug association efficiency is decreased by the presence of the surfactants. On the other hand, complex formation of cationic compounds is typical with non-ionic and anionic surfactants and we can assume that there is an interaction between the drug, which bears a positive charge at pH 7, and the surfactants (Smolka 1967). We believe that this interaction may lead to reduced drug absorption. Although there is a difference between the performance of formulation B and the control solution as regards corneal amikacin concentration 2 h after administration, it also seems likely that SLS may have altered the permeability of the cornea as do many surfactants (Reddy et al 1976).

The results of this study emphasize the potential of the polymeric colloidal carriers as new ocular drug delivery systems. It could be predicted that extremely water-soluble drugs would not benefit from incorporation into this delivery system, since they would be discharged from the nanoparticles more rapidly than the nanoparticles themselves would be cleared from the cornea (Lee & Robinson 1986); nevertheless, from our experimental data, we suggest that, assuming close contact between nanoparticles and the epithelial surface, all the drug molecules which desorb from the carrier, diffuse across the cornea more easily than free molecules which remain in the lacrimal fluid. We also believe that an equilibrium between the desorbed and adsorbed drug could be established, which means that the desorbed drug passing across the cornea is replaced by the previously free drug molecules, which eventually occupy active sites on the colloidal carrier. These results agree with the conclusions of Marchal-Heussler et al (1990), who indicated a relative low amount of drug fixed onto the nanoparticles is enough for a therapeutic effect. Moreover, this work emphasizes the crucial role played by surfactants in the drug loading of these carriers and on the surface charge of the particles, and finally, the need for further research on the poorly understood relationships between carrier, drug and surfactant in this and other nanoparticle formulations.

Acknowledgements

This research was supported by a grant from "Ministerio de Industria y Energía" and "Laboratorios Cusí", S.A.

References

Al-Achi, A., Boroujerdi, M. (1990) Adsorption isotherm for doxorubicin on erythrocyte-membrane. Drug Dev. Ind. Pharm. 16: 1325–1338

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

Couvreur, P., Roland, M., Speiser, P. (1982) U.S. Patent No 4: 329–332

Davis, S. S., Darff, L., Hyndiuk, R. (1978) Topical tobramycin therapy of experimental pseudomonas keratitis: an evaluation of some factors that potentially enhance efficacy. Arch. Ophthalmol. 96: 123–125

Diepold, R., Kreuter, J., Himber, J., Gurny, R., Lee, V. H. L., Robinson, J. R., Saettone, M. F., Schnaudigel, O. E. (1989) Comparison of different models for the testing of pilocarpine eyedrops using conventional eyedrops and a novel depot formulation (nanoparticles). Graefe's Arch. Clin. Exp. Ophthalmol. 227: 188–193

Eiferman, R. A., Stagner, J. I. (1982) Intraocular penetration of amikacin: iris binding and bioavailability. Arch. Ophthalmol. 100: 1817–1819

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

Ganjian, F., Cutie, A. J., Jochsberger, T. (1980) In-vitro adsorption studies of cimetidine. J. Pharm. Sci. 69: 352–353

Harmia, T., Kreuter, J., Speiser, P., Boye, T., Gurny, R., Kubis, A. (1986a) Enhancement of the miotic response of rabbits with pilocarpine loaded polybutylcyanoacrylate nanoparticles. Int. J. Pharm. 33: 187–193

Harmia, T., Speiser, P., Kreuter, J. (1986b) Optimization of pilocarpine loading onto nanoparticles by sorption procedures. Ibid. 33: 45–54

Hunter, R. J. (1981) Zeta Potential in Colloid Science. Principles and Applications, Academic Press, London

Ipsler, M. S., Helm, C. J., George, W. J. (1987) Topical vs systemic gentamicin penetration into the human cornea and aqueous humor. Arch. Ophthalmol. 105: 922–924

James, A. M. (1979) Electrophoresis of particles in suspensions. In: Good R. J., Stromberg R. R. (eds) Surface and Colloid Science. Vol 11, Plenum Press, New York

Lee, V. H. L., Robinson, J. R. (1986) Review: topical ocular delivery: recent developments and future challenges. J. Ocular Pharmacol. 2: 67–108

Marchal-Heussler, L., Maincent, P., Hoffman, M., Spittler, J., Couvreur, P. (1990) Antiglaucomatous activity of betaxolol chlorhydrate sorbed onto different isobutylcyanoacrylate nanoparticles preparations. Int. J. Pharm. 58: 115–122

Monteleone, P. M., Muhammad, N., Brown, R. D., Mogrory, J., Hanna, A. (1983) Amikacin sulphate. In: Florey, K. (ed.) Analytical Profiles of Drug Substances. Academic Press, New York, pp 37–77

Müller, R. H., Davis, S. S., Illum, L., Mak, E. (1986) Colloidal carriers for drug targeting—charge reduction by coating with polymers. Abstr. 32. Annual Meeting of the Inter. Pharm. Assoc. (APV) 16–19 April Leiden, The Netherlands

Reddy, R. K., Khalil, S. A., Goude, M. W. (1976) Effect of dioctyl sodium sulfosuccinate and poloxamer 188 on dissolution and intestinal adsorption of sulfadiazine and sulfisoxazole in rats. J. Pharm. Sci. 65: 115–118

Smolka, I. R. (1967) Pluronic polyoles. In: Schick, J. (ed.) Nonionic Surfactans. Marcel Dekker Inc. New York. pp 309–340

Wood, R. W., Vicent, H. K. L., Kreuter, J., Robinson, J. R. (1985) Ocular disposition of poly-hexyl-2-cyano(3-14C)acrylate nanoparticles in the albino rabbit. Int. J. Pharm. 23: 175–183