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Research Papers

Interaction of amikacin loaded nanoparticles with phosphatidylcholine monolayers as membrane models

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Summary

Using monolayers as a membrane model, the interaction of free amikacin and amikacin-loaded polybutylcyanoacrylate nanoparticles with phosphatidylcholine (PC) has been investigated. The kinetics of penetration of this drug into the monolayers at 2.5, 5, 10 and 20 mN m^{-1} were measured and compared with those of free nanoparticles. The stability of amikacin sulphate (AMK)-loaded poly(butyl-2-cyanoacrylate) (PBCA) nanoparticles over a period of 98 days at 25 °C has been studied. The kinetics of their penetration into the lipid monolayer as a membrane model was analysed periodically. Their size and polydispersity index were determined at the same time by photon correlation spectroscopy. A decrease in the penetration of AMK-loaded PBCA nanoparticles on phosphatidylcholine monolayers was observed, this effect being most pronounced during the final weeks, when the loss of surface pressure increases reached 31.59% of the initial value. At the same time, the particle size and polydispersity index increased during the final stages. Neither effect is significant in the first 2 months of the assays.

Introduction

Polyalkylcyanoacrylate nanoparticles are produced by the emulsion-polymerisation technique involving the anionic polymerisation of an alkylcyanoacrylate in the presence of a stabilising agent to yield discrete colloidal particles in the nanometer size range (Couvreur et al., 1979). Owing to their biocompatible and biodegradable nature, these particles have been proposed to carry biologically active materials in order to improve the pattern of drug distribution after intravenous administration (Verdun et al., 1986) and to improve the bioavailability of some drugs for ophthalmic use (Harmia et al., 1986).

Recently, Diepold et al. (1989) demonstrated the ability of this kind of particles to adhere to ocular tissues for several hours and to prolong the residence time of the drug.

The chemical structure of amikacin, an aminoglucoside antibiotic, proposed for the treatment of eye infections, tends to reduce corneal barrier penetration and consequently only a small amount of this drug penetrates the cornea and reaches the

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anterior or posterior chamber. Again, most of this drug is rapidly eliminated from the eye through the lacrimal system (Eiferman and Stagner, 1982).

Several molecules have been efficiently associated to polyalkylcyanoacrylate (PACA) nanoparticles. However, the drug payload achieved per unit weight of nanoparticles depends on the physicochemical characteristics of the model drug. In particular, highly polar compounds such as aminoglycoside antibiotics show slight affinity for this type of hydrophobic polymer (Henry-Micheland et al., 1987). Consequently, many efforts have been made to improve the drug loading capacity of polybutylcyanoacrylate (PBCA) nanoparticles (Losa et al., 1988). However, there is still insufficient information concerning the physicochemical characteristics of the polymer itself and about the interaction between the polymer, the drug associated and the additives used in the polymerization process (Alonso et al., 1989).

Monolayer measurements can give valuable information about the interfacial behaviour of membrane compounds and also on interactions at interfaces, which can well be correlated with phenomena in biological membranes.

The present study was undertaken in order to determine the nature of the interaction between free nanoparticles and amikacin-loaded polyalkylcyanoacrylate nanoparticles, with phosphatidylcholine (PC) monolayers, used as a membrane model. The possible interactions of the surfactant used in the nanoparticle formulation with the lipids mentioned above was also investigated. Stability of amikacin loaded nanoparticles has been determined by means of two different procedures. On the one hand, an analysis of the penetration kinetics of this drug carrier in lipid monolayers was peformed once every 2 weeks. On the other, AMK nanoparticle sizes were measured using photon correlation spectroscopy (PCS).

The amikacin-loaded nanoparticles were chosen because of the interest in improving the ocular bioavailability of this product in this new colloidal drug delivery system (Eiferman and Stagner, 1982). Sodium lauryl sulphate was introduced in the formulation to increase the drug payload of the particles.

Materials and Methods

Egg yolk phosphatidylcholine (PC) was supplied by Merck and purified by repeated column chromatography process on silica gel using chloroform/methanol (9:1) as eluent (Barlett, 1959; Singleton et al., 1965). Solutions of pure lipid were prepared by weighing a dry lipid sample, to which was added a known weight of chloroform. Butyl-2-cyanoacrylate was purchased from Brawn Melsungen AG, F.R.G. Amikacin sulphate (AMK) from Bristol-Meyers SA and sodium lauryl sulphate (LSS) from Sigma.

All reagents used were Merck analytical grade. Water was twice distilled from a permanganate solution using a Pyrex apparatus.

Preparation of nanoparticles

Polybutyl-2-cyanoacrylate (PBCA) nanoparticles were prepared using the emulsion polymerization procedure described by Couvreur et al. (1979). The composition of the polymerization medium was: HCl (10^{-3} N), containing 1% Dextran 70 and 0.1% sodium lauryl sulphate. For the formulation of Amikacin-loaded nanoparticles 10 mg of the drug were added to 10 ml of the polymerization medium prior to incorporation of the monomer; at this moment the process of insolubilisation of the drug was developed due to the formation of a complex between the amikacin and the surfactant sodium lauryl sulphate. Afterwards, the monomer (80 μl) was added dropwise and the mixture was stirred for 3 h at room temperature. Once polymerization was complete, the nanoparticle suspensions were filtered brought to pH 7 with 0.1 NaOH and then lyophilized.

A suspension of the amikacin-sodium lauryl sulphate complex was also prepared using the same composition of the polymerization medium but without addition of the monomer.

In a previous work, we demonstrated that the presence of surfactant contributes to insolubilization of AMK in the polymerization medium, by formation of a complex between both products which were finally converted by poly-*n*-butyl-2-cyanoacrylate, without changing the antimicrobial activity of drug. Certainly, the bacteriological assays carried out have shown that the minimal

inhibitory concentration of this antibiotic against *E. coli* is not changed after association with sodium lauryl sulphate and with the polymer (nanoparticle formulation) (Losa et al., 1989).

Surface pressure measurements: penetration kinetics and compression isotherms

The analyses of the penetration kinetics in these lipids were performed on a Langmuir film balance equipped with a Wilhelmy platinum plate, designed in our laboratory, similar to that described by Verger and De Haas (1973). The output of the pressure pickup (Sartorius A 120 S microbalance) was calibrated by recording the well-known isotherm of stearic acid. This isotherm is characterized by a sharp phase transition at 25 mN m^{-1} for pure water at 20 °C. The Teflon trough (surface area 495 cm, volume 309.73 ml) and the platinum plate were regularly cleaned with chromate mixture and rinsed with twice-distilled water before each experiment. All measurements were made at a subphase temperature of 21 ± 1 °C.

The penetration ability of the formulation design was measured following the method described by Schacht et al. (1978). A given amount of lipid was spread on the air-water interface.to obtain the desired initial surface pressure, and thereafter, increasing amounts of the formulation to be tested were successively injected into the subphase, and the increases of surface pressure were plotted. Successive drug injections were made at intervals of 60 min in order to ensure that the system had reached equilibrium after each addition. The values reported are averages of triplicate runs. Reproducibility was ± 0.5 mN m^{-1} for surface pressures.

In the isotherm study, lipid films were spread on the interface from a microsyringe, and at least 10 min was allowed for solvent evaporation. Films were compressed as a rate of 4.2 cm/min: a change in the compression rate did not alter the shape of the isotherms. All isotherms were run at least four times in the direction of increasing pressure with freshly prepared films. The accuracy of the system under the conditions in which the bulk of the reported measurements were made was ± 0.5 mN m^{-1} for surface pressure.

Before spreading the films, the absence of surface-active impurities from the solvents on the subphase was measured by the changes in the surface pressure that occurred on decreasing the interface area to 10% of its initial value. These changes were less than 0.1 mN m^{-1}.

Particle size analysis

Photon correlation spectroscopy was carried out in a Malvern Autosizer II C (Malvern Instruments, Malvern, U.K.) consisting of a Spectrometer Optics unit, 5 mW, 632.8 nm, He-Ne laser, irradiating the scattering cell held in a thermostatted water bath at 25 ± 0.05 °C. The resulting scattered light was detected at 90° by a photon detection system which transmitted the signal to a Malvern Multi-8 (type 7032 CE), 72-channel multibit correlator. This was interfaced with an Olivetti computer (type 12 A/4009) (Gulari et al., 1979; McConnell, 1981).

Samples were diluted before measurement with twice-distilled water freshly filtered through a membrane filter (Millipore®).

Results and Discussion

The penetration of AMK-LSS loaded polybutyl-2-cyanoacrylate nanoparticles, free AMK, AMK-LSS and free nanoparticles in PC monolayers was carried out measuring the surface pressure increases ($\Delta\Pi$), produced in lipid monolayers spread at initial surface pressures of 0, 2.5, 5, 10 and 20 mN m^{-1}.

Experiments carried out when the lipid monolayer had not been spread on the surface (0 mN m^{-1} of initial surface pressure) are shown in Fig. 1. Variations of surface pressure were only recorded for AMK/LSS loaded nanoparticles ($\Delta\Pi < 1$ mN m^{-1}) and, to a lesser extent, for free nanoparticles ($\Delta\Pi < 0.5$ mN m^{-1}).

Contrary to what may be expected, according to the surface activity of LSS, the free AMK/LSS complex did not produce variation of surface pressure when lipid was not spread on the interface ($\Delta\Pi = 0$ mN m^{-1}). This fact may be attributed to the formation of an insoluble complex between

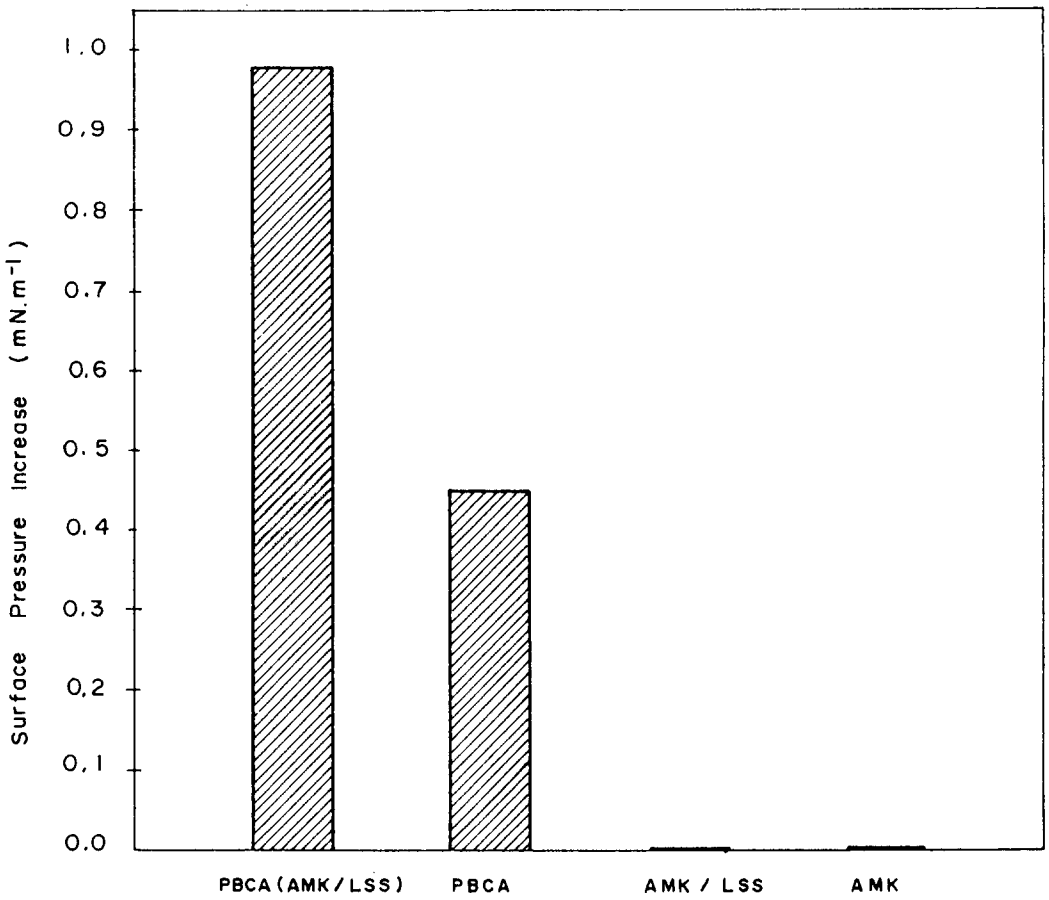

Fig. 1. Surface pressure increases when 50 μl of AMK/LSS nanoparticles (30.25 mg of AMK/ml), free nanoparticles, AMK/LSS and free AMK were injected into the aqueous subphase in the absence of lipid monolayer (0 mN m^{-1} of initial surface pressure).

AMK and surfactant, which did not show surface activity, as follows:

$$2(C_{12}H_{25}NaSO_4) + AMK \cdot 2H_2SO_4 \rightarrow AMK \cdot 2(C_{12}H_{25}SO_4H) + 2NaSO_4H \quad (1)$$

Fig. 2 shows the surface pressure increases produced in lipid monolayers spread at initial pressures of 2.5, 5, 10 and 20 mN m^{-1} when different concentrations of AMK associated to nanoparticles were added to the aqueous subphase. In this figure the penetration of AMK/LSS nanoparticles in the absence of the lipid monolayer (0 mN m^{-1}) is also plotted. A maximum increment value was attained at a surface pressure of 2.5 mN m^{-1} for an AMK concentration of 24.20×10^{-3} mg/ml. Higher concentrations of AMK associated to nanoparticles did not produce a significant change in surface pressure increases.

The surface pressure increases attained when free AMK, AMK/LSS complex, AMK/LSS nanoparticles and free nanoparticles were added to the subphase at 2.5 mN m^{-1} of initial surface pressure of lipid monolayers are shown in Fig. 3. As can be seen in Fig. 3, the penetration of AMK/LSS nanoparticles was higher than the penetration of AMK, AMK/LSS and free nanoparticles. Nevertheless, a higher penetration was recorded for free nanoparticles containing dextran and LSS than for free nanoparticles containing only dextran.

The presence of LSS in the nanoparticles could suggest that this product might be responsible for

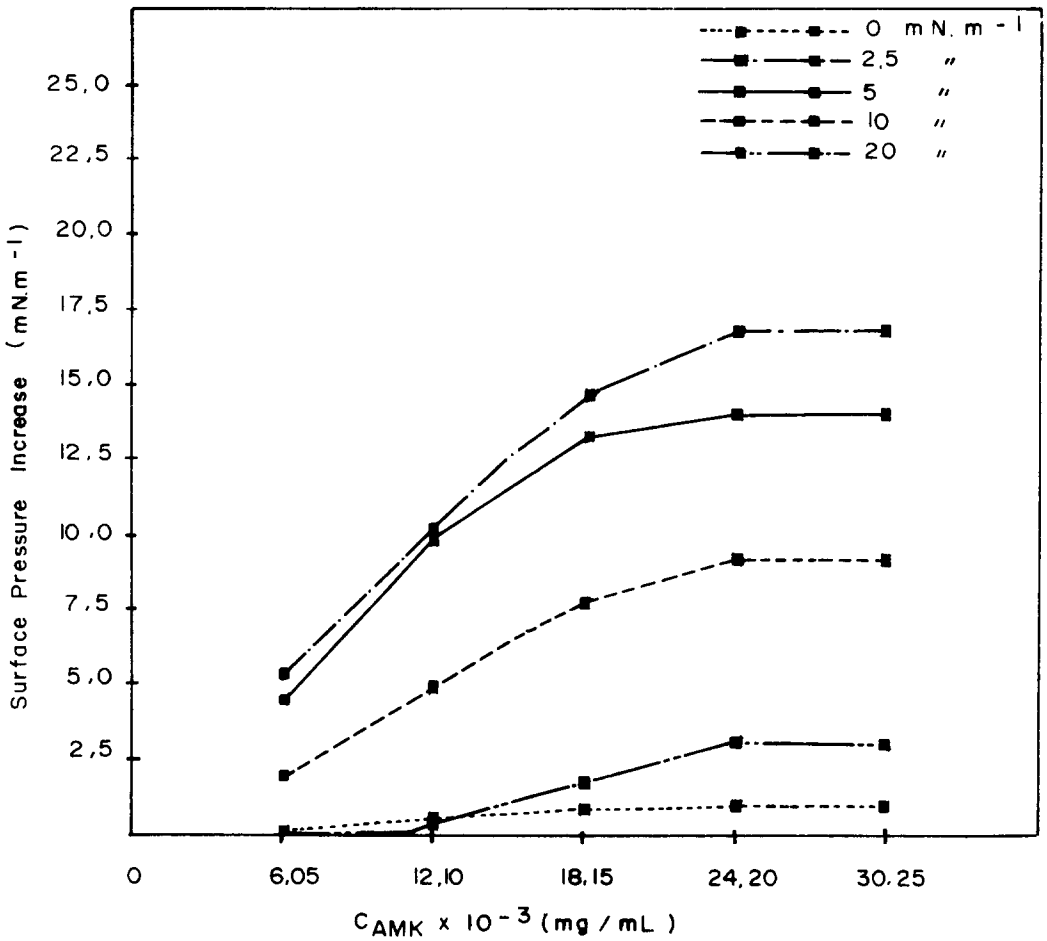

Fig. 2. Surface pressure increases of PC monolayers as a function of the concentration of the AMK/LSS nanoparticles in the subphase at different initial surface pressures (0–20 mN m^{-1}).

the nanoparticle penetration into the lipid monolayer, due to its surface activity, reported in some papers (Gibaldi and Feldman, 1970). However, this is not the only factor involved in this process because results obtained here provide evidence that the penetration effect in PC films, measured through $\Delta\Pi$ values, was most pronounced when AMK-LSS loaded nanoparticles were injected into the subphase in relation with LSS or free nanoparticles.

Compression isotherms obtained when PC or PC with various amounts of free AMK added were spread on a twice-distilled water subphase are reported in Fig. 4. As can be seen, the presence of AMK did not produce appreciable variation in the shape of the isotherm of the lipid monolayer, but did cause expansion of the film. This effect was most pronounced for maximum amounts of AMK added. Initial surface pressures of lipid monolayer were increased in the presence of AMK, but the final values at collapse pressure were the same in all cases. Comparing these results with others obtained (Fig. 3), it may be suggested that nanoparticles contribute to carrying the complex of AMK-LSS to the surface where free AMK interacts with the lipid monolayer.

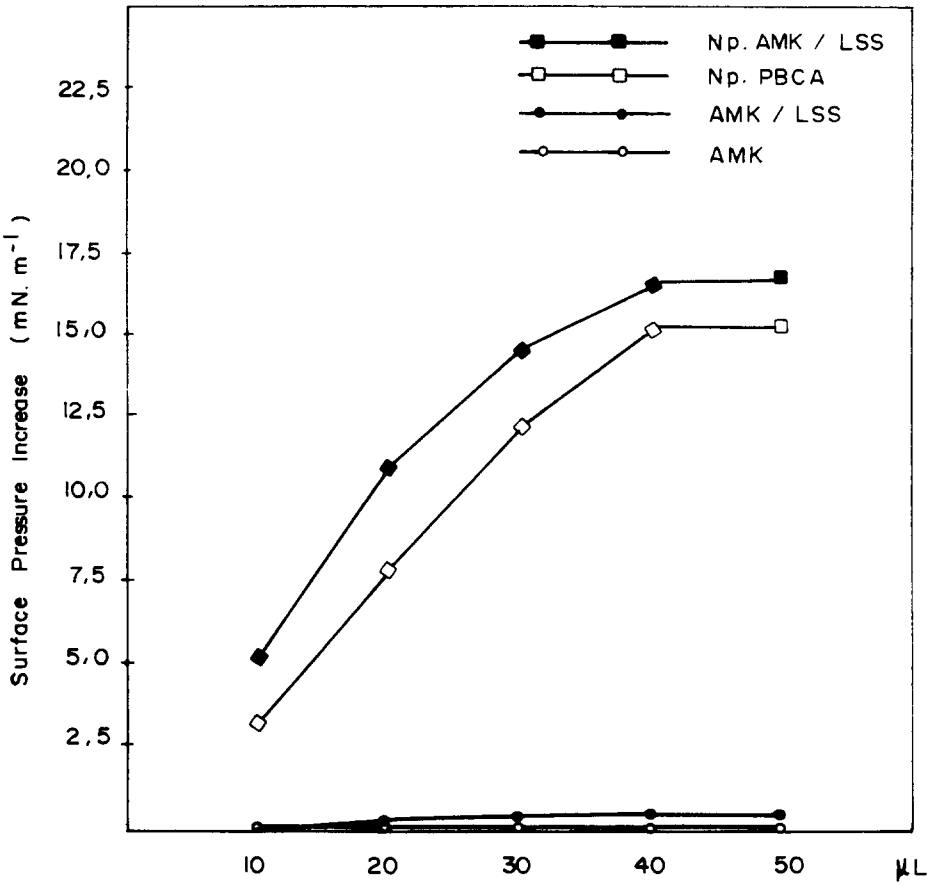

Fig. 3. Penetration of AMK, AMK/LSS solution, AMK/LSS nanoparticles and free nanoparticles in PC monolayers at an initial surface pressure of 2.5 mN m^{-1} (10 μl of drug solution injected into subphase = 6.05×10^{-3} mg/ml of AMK or 0.08 mg/ml of PBCA).

In order to determine the stability of the system, AMK/LSS-PBCA nanoparticles, penetration kinetics in phosphatidylcholine have been analysed, measuring the surface pressure increases produced in lipid monolayers, spread at 2.5 mN m^{-1}, every 14 days for 14 weeks. This initial surface pressure of lipid monolayer was chosen because it is the pressure at which this drug achieves maximum penetration, as described above (see Fig. 2).

The surface pressure increases obtained at different times when different quantities of AMK/LSS nanoparticles were injected into the subphase, are listed in Table 1. As can be observed, penetration increases when amounts of this drug increase, the maximum being for 24.20×10^{-3} mg/ml of AMK injected (volume of solution added, 40 μl). Concentrations higher than this do not produce significant variation in surface pressure increases. On the other hand, comparing results obtained in the first analysis with those recorded subsequently, the penetration of this drug in the lipid monolayer decreases with time.

Fig. 5 shows the surface pressure increases ob-

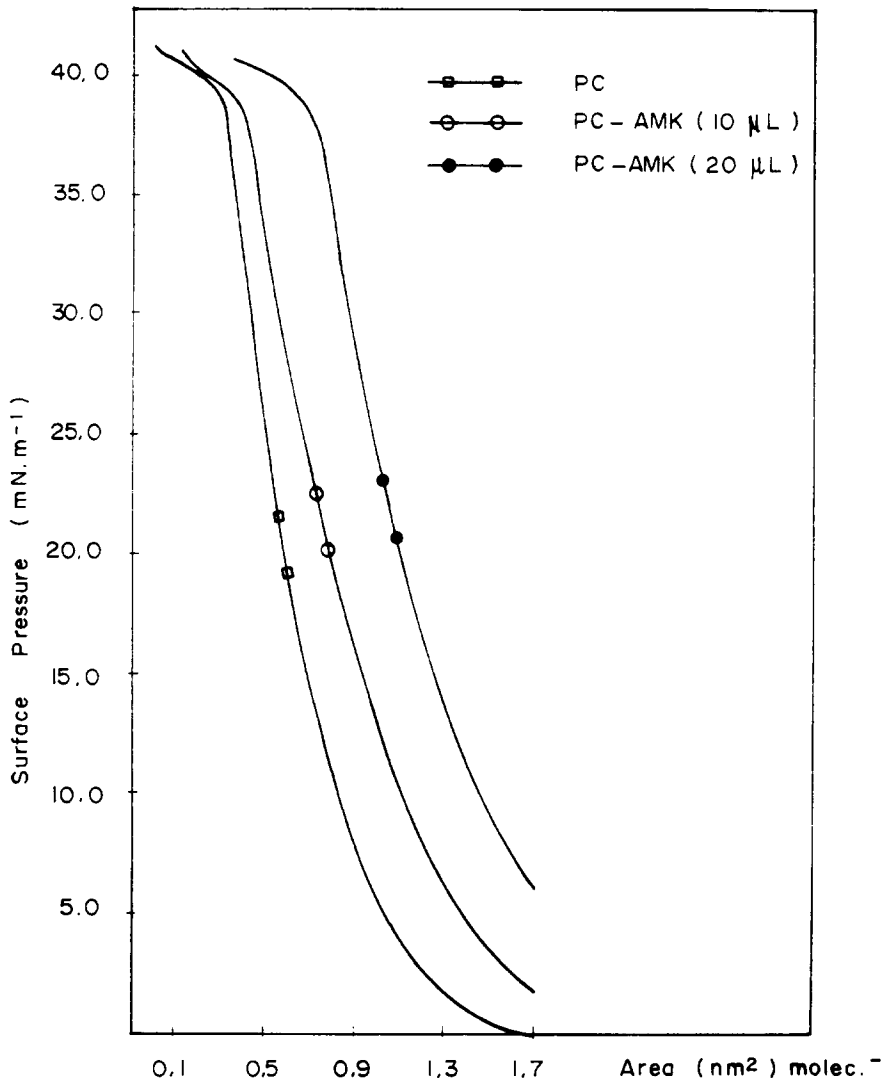

Fig. 4. Compression isotherms of PC and PC with different amounts of free AMK added.

TABLE 1

Increase in surface pressures of lipid monolayer for different concentrations of amikacin loaded poly-n-butyl-2-cyanoacrylate nanoparticles (AMK-PBCA), injected into the subphase, at different times

AMK-PBCA [(C_{AMK} mg/ml) × 10^{-3}]	Increase in surface pressure (Π) (mN m^{-1}) at time (days)							
	0	14	28	42	56	70	84	98
6.05	5.09	4.61	3.64	3.15	2.18	1.94	1.21	0.73
12.10	11.88	11.16	9.70	9.22	8.00	7.28	6.79	5.72
18.15	14.55	15.76	15.04	13.34	13.82	13.10	11.64	9.70
24.20	17.22	17.46	17.46	16.49	16.25	15.52	13.10	11.78

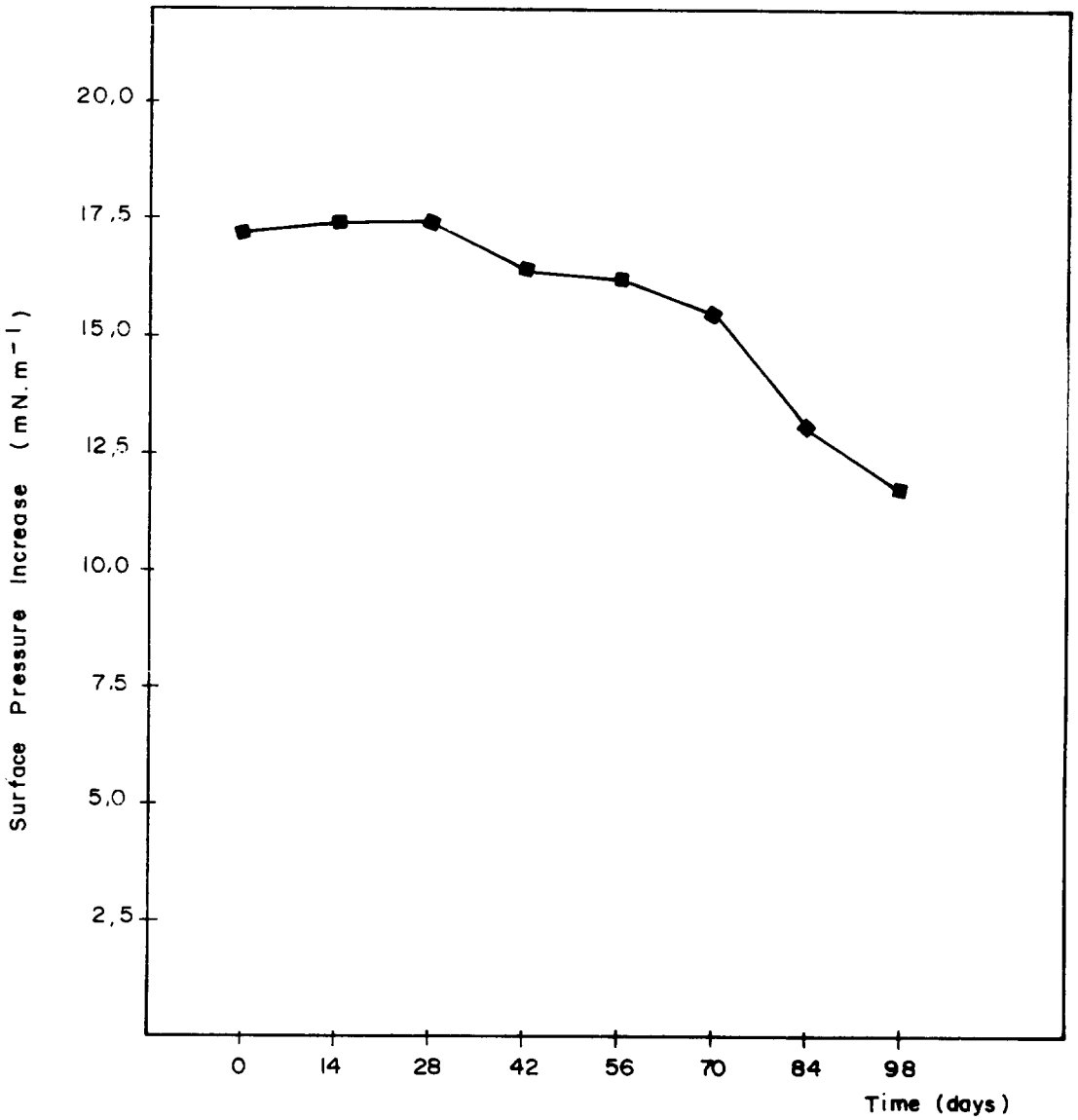

Fig. 5. Increase surface pressures of lipid monolayer (PC) at different times. Concentration of AMK nanoparticles injected into the subphase 24.20×10^{-3} mg/ml.

tained for maximum penetration of AMK/LSS nanoparticles (24.20×10^{-3} mg/ml). The surface pressure increases diminish with time but this effect is not significant for the first few weeks.

Measurement of particle size by photon correlation spectroscopy (PCS) is based on the theory that the observed time dependence of the fluctuations in intensity of scattered light from a colloidal dispersion is a function of the size of the scattering particles.

The autocorrelation function, $g(\tau)$, of the scattering intensity can be expressed, for a monodisperse system, as a function of the decay rate (Γ) and the correlation delay time (τ).

$$g(\tau) = \exp(-\Gamma\tau) \tag{2}$$

TABLE 2

Nanoparticle size z-average mean of size and polydispersity index of amikacin loaded poly-n-butyl-2-cyanoacrylate nanoparticles (AMK-PBCA), at different times

AMK-PBCA	Time intervals (days)							
	0	14	28	42	56	70	84	98
z-av. mean	188.20	194.80	194.95	205.06	206.72	215.20	242.60	450.22
Polydispersity	0.030	0.040	0.042	0.063	0.071	0.095	0.147	0.190

The decay rate can be expressed as $\Gamma = DK^2$, where D is the macromolecular translational diffusion coefficient, $K = (4\pi/\lambda)n \cdot \sin(\theta/2)$, is the magnitude of the scattering vector, n is the refractive index of the solution, and θ, the scattering angle. For polydisperse systems, $g(\tau)$ consists of a sum or distribution of single exponentials:

$$g(\tau) = \int_0^\infty G(\Gamma) \exp(-\Gamma\tau)\, d\Gamma \qquad (3)$$

The z average diffusion coefficient and the equivalent z-average particle diameter (dz) may be calculated from the mean decay rate (Γ), obtained by expansion of Eqn. 3 (Douglas et al., 1984), and the variance of the distribution is given by:

$$Q = \mu_2/\Gamma^2 \qquad (4)$$

where Q is known as the quality parameter or polydispersity index (Pusey et al., 1974).

Results of the analysis of AMK/LSS nanoparticle sizes are listed in Table 2, in which the z average mean of size and polydispersity are shown. As can be observed the size of this nanoparticle increases with time. An increase in polydispersity was also noted.

Conclusions

The present results provide evidence that amikacin-sodium lauryl sulphate complex loaded polybutylcyanoacrylate nanoparticles have a greater interaction with molecular films of PC than the free complex (AMK/LSS). The free drug alone does not demonstrate any significant penetration into the lipid monolayers studied.

The interaction of PBCA nanoparticles without AMK, produced by a similar technique in the presence of dextran (1%) and LSS (0.1%), with lipid monolayers was significant but less than that of AMK/LSS nanoparticles.

As usual, penetration decreases in both rate and magnitude when the monolayer pressure increases, being maximal in monolayers spread at 2.5 mN m^{-1}.

The results of this study suggest that nanoparticles should easily be able to attain the air-water interface, but when lipid monolayers have not been spread, this effect cannot be observed. On the other hand, in the presence of a spread lipid monolayer, a partially liposoluble drug associated with nanoparticles (such as the AMK/LSS complex) could readily be liberated until a distribution equilibrium between nanoparticles and lipid monolayer is reached. Probably due to these facts, the penetration of AMK loaded nanoparticles in lipid monolayers is significantly higher than that of free AMK or the AMK/LSS complex. This drug loaded with nanoparticles probably acts in a more efficient way than the free drug.

When amikacin was included in PBCA nanoparticles, stored at 4°C, penetration on lipid monomolecular films (PC) showed an average loss in the penetration capacity of 31.59% after 98 days. On the other hand, the z average mean of size and polydispersity of these nanoparticles shows an increase with time, being higher during the final period of the analysis. Besides it would appear reasonable to suggest that this fact can be attributed to changes in the chemical interactions

between AMK/LSS and between this complex and dextran and PBCA.

Acknowledgments

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References

Alonso, M.J., Losa, C., Seijo, B., Torres, D. and Vila-Jato, J.L., New ophthalmic drug release systems; Formulation and ocular disposition of amikacin-loaded nanoparticles. *5th International Conference on Pharmaceutical Technology*, Paris, 1989.

Barlett, G.R., Phosphorus assay in column chromatography. *J. Biol. Chem.*, 234 (1959) 466–468.

Couvreur, P., Kante, B., Roland, M., Guiot, P., Baudin, P. and Speiser, P., Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties. *J. Pharm. Pharmacol.*, 31 (1979) 331–332.

Diepold, R., Kreuter, J., Gruggenbuhl, P. and Robinson, J.R., Distribution of poly-hexyl-2-cyanoacrylate nanoparticles in healthy and chronically inflamed eyes. *Int. J. Pharm.*, 54 (1989) 149–153.

Douglas, S.J., Illum, L., Davis, S.S. and Kreuter, J., Particle size and size distribution of poly(butyl-2-cyanoacrylate) nanoparticles. *J. Colloid Interface Sci.*, 101 (1984) 149–158.

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

Gibaldi, M. and Feldman, S., Mechanisms of surfactant effects on drug absorption. *J. Pharm. Sci.*, 59 (1970) 579–588.

Gulari, E., Gulari, E., Tsunashima, Y. and Chu, B., Photon correlation spectroscopy of particle distributions. *J. Chem. Phys.*, 70 (1979) 3965–3972.

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

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

Losa, C., Criado, T., Vila-Jato, J.L., and Alonso, M.J., Mise au point de plusieures formulations de nanoparticules polymériques d'amikacine. Evaluation de l'activité antimicrobienne. *3èmes Journées Scientifiques G.T.R.V.*, Paris, December, 1988.

McConnell, M.L., Particle size determination by quasielastic light scattering. *Anal. Chem.*, 53 (1981) 1007A–1018A.

Pusey, P.N., Koppel, D.E., Schaefer, D.W., Camerini-Otero, R.D. and Koening, S.H., Intensity fluctuation spectroscopy of laser light scattered by solutions of spherical viruses: R17, Q_β, BSV, and T7.1. Light-scattering technique. *Biochemistry*, 13 (1974) 952–960.

Schacht, J., Weiner, N.D. and Shahid, L., Interaction of aminocyclitol antibiotics with polyphosphoinositides in mammalian tissues and artificial membranes. Wells, W.W. and Eisenberg, F. (Eds.), *Cyclitols Phosphoinositides (Proc. Symp.)*, Academic Press, New York, 1977, pp. 153–165.

Singleton, W.S., Gray, M.S., Brown, M.L. and White, J.L., Chromatographically homogeneous lecithin from egg phospholipids. *J. Am. Oil. Chem. Soc.*, 42 (1965) 53–56.

Verdun, C., Couvreur, P., Vranckx, H., Lenaerts, V. and Roland, M., Development of a nanoparticle controlled-release formulation for human use. *J. Controlled Release*, 3 (1986) 205–210.

Verger, R. and De Haas, G.H., Enzyme reactions in a membrane model. I. New technique to study enzyme reactions in monolayers. *Chem. Phys. Lipids*, 10 (1973) 127–136.