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Approaches to improve the association of amikacin sulphate to poly(alkylcyanoacrylate) nanoparticles

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Summary

Owing to the low rate of association of polar drugs, such as amikacin sulphate, to hydrophobic carriers, e.g. poly(alkylcyanoacrylate) nanoparticles, several procedures have been investigated to improve the drug carrier capacity. The greatest improvement was noted with the covalent reaction between the drug and the suspension agent dextran 70, attached to the particle surface. The antibacterial activity in this case was dramatically reduced, however. The addition of sodium lauryl sulphate to the polymerisation medium reduced the hydrosolubility of the drug, improving its incorporation into the nanoparticles. The study of the sorption behaviour of amikacin sulphate onto freeze-dried nanoparticles, prepared either using a surfactant (Synperonic F 68) or a suspension agent (dextran 70), shows preferrable results in the former case. The presence of drug in the polymerisation medium drastically increased the mean particle size, destabilising the nanoparticles at the highest concentration assayed (20 mg/ml). Less drug was adsorbed onto these particles at a later stage, which seems to be due to their larger size compared to non-loaded nanoparticles. Moreover, the drug-polymer association mechanism, investigated by zeta-potential studies, appears to be an electrostatic interaction, in keeping with the observed reduction of particle surface charge after adsorption of amikacin. Finally, the modification of the polymer molecular weight in the formulations developed was investigated.

Introduction

The efficacy of aminoglucoside antibiotics against certain microorganisms had led to their use being proposed for the treatment and prophylaxis of different kinds of infections, and the aminoglucoside amikacin, in particular, has been shown to be active against infection from the *M. avium-M. intracellulare* complex which is diagnosed in most AIDS patients (Armstrong et al., 1985). In addition, the efficacy of this drug against gram-negative microorganisms involved in eye infections has been probed (Knothe, 1976).

Intravenously administered aminoglucosides are unable to achieve bactericidal levels at infection sites without producing unacceptable toxic reactions at the level of the system, thus targeting these drugs is very important if in vivo efficacy is to be enhanced. Moreover, association of aminoglucoside antibiotics to polymeric carriers to improve the poor bioavailability of these drugs after ocular administration (Stagner, 1982) could be of therapeutic interest.

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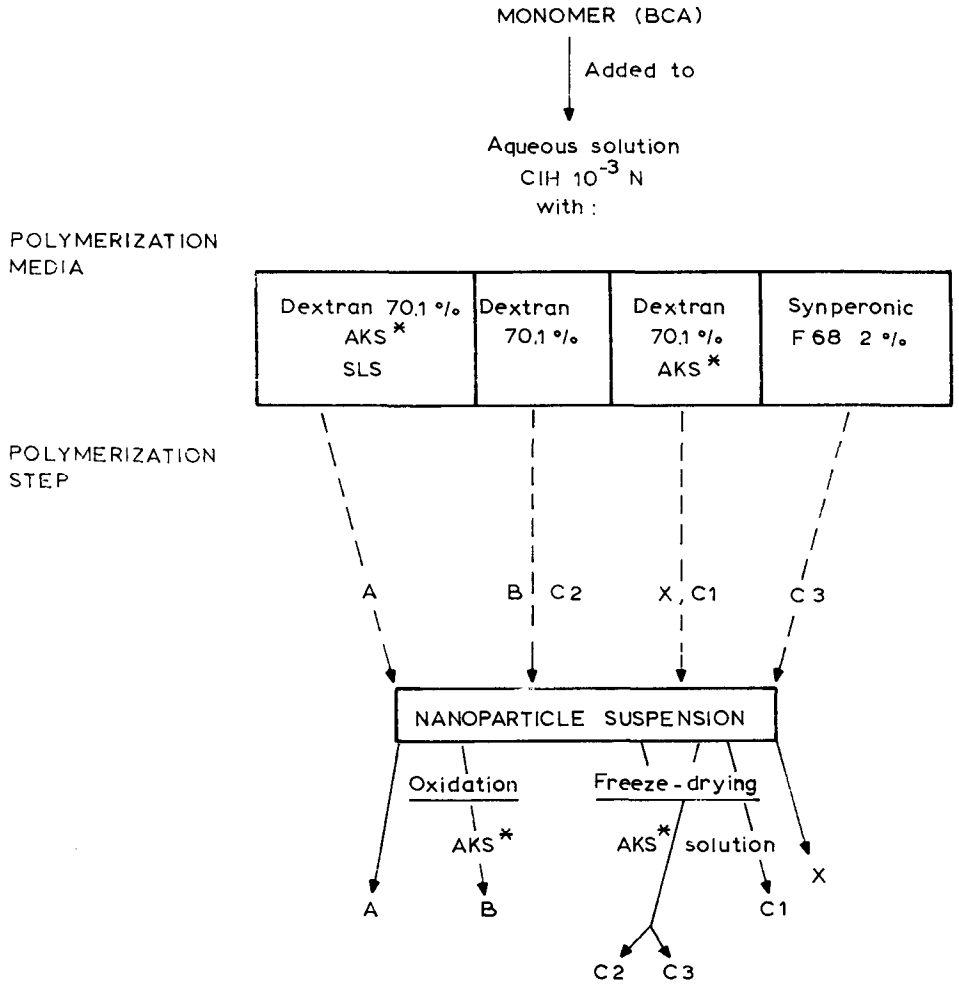
Among the polymeric carriers that have been put forward in recent years, polyalkylcyanoacrylate (PACA) nanoparticles have been examined further because of their biodegradability and efficiency at including certain drugs (Couvreur et al., 1986). Enhancement of the therapeutic efficacy of antibiotics (Youssef et al., 1988) and anticancer drugs (Kreuter and Hartmann, 1983) has been reported in the literature but, whereas most were administered intravenously, Maincent et al. (1986) found considerable improvement in the bioavailability of one such drug, vincamin, after its inclusion into polybutylcyanoacrylate (PBCA) nanoparticles. This kind of particles has been proposed for ophthalmic applications due to their adhesive properties (Refojo et al., 1971; Wood et al., 1985); the therapeutic activity of pilocarpin and betaxolol has accordingly been improved after association to nanoparticles (Harmia et al., 1986; Marchal-Heussler et al., 1990).

From a pharmaceutical viewpoint the acceptability of polymeric carriers is limited by the degree



* The ASK concentration was variable for each preparation

Scheme 1. Flow diagram of nanoparticles preparation and drug-loading procedures.

of drug-polymer association attainable (association efficiency). Association of a drug to PACA nanoparticles commonly requires a certain hydrophilic nature of the drug, however, the process of association appears to be difficult for very polar molecules, such as amikacin sulphate, due to the hydrophobicity of the carrier (Henry-Micheland et al., 1987).

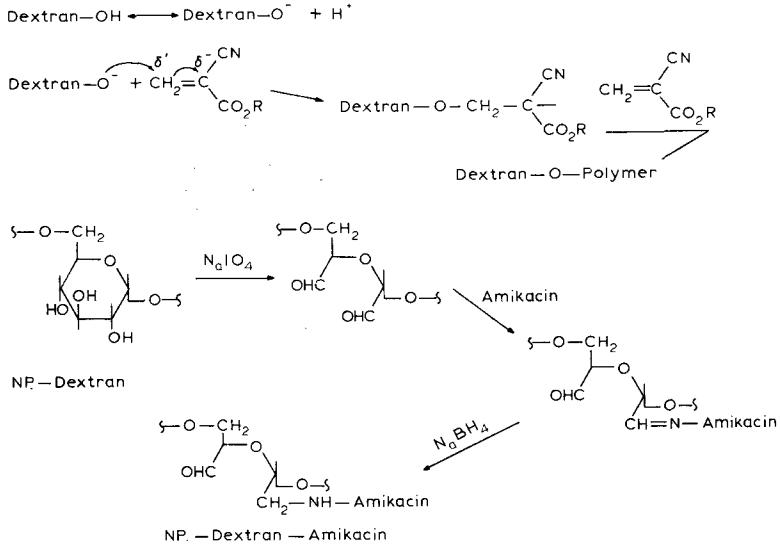
The aim of the present work was to modify the physico-chemical properties of either the chosen drug (amikacin sulphate) or carrier (polybutylcyanoacrylate nanoparticles) in order to improve drug loading into and/or onto the particles, for the same initial concentration of the drug in the suspension. The particle size, zeta potential and polymer molecular weight distribution were determined for each formulation in order to clarify the drug-carrier interaction mechanisms. The in vitro antimicrobial activity of the liberated drug was also assayed to check the possible reduction of its activity caused by the modifications introduced.

Materials and Methods

Butylcyanoacrylate (BCA from Braun Melsungen AG, Germany) was used for the monomer. Amikacin sulphate (AKS) (from Bristol-Meyers S.A., Spain), sodium lauryl sulphate (SLS) (from Sigma Quimica, Spain) and Synperonic F 68 (kindly provided by ICI, Spain) were used as surfactants and dextran 70 (Sigma Química, Spain) as a suspension stabilizer. For freeze-drying purposes glucose (Sigma Química, Spain) was used as a cryoprotectant. All other reagents were of analytical grade.

Preparation of amikacin-PBCA nanoparticles

Six polybutylcyanoacrylate (PBCA) nanoparticle formulations, prepared from butylcyanoacrylate monomer using the emulsion polymerization method described by Couvreur et al. (1979), were made as follows (Scheme 1). 80 μl of monomer were added dropwise to 10 ml of an acidic aqueous solution (10^{-3} N HCl) containing the following stabilisers: 1% Dextran 70 and 0.1% SLS, (formulation A); 1% Dextran 70 (formulations B, X, C1 and C2); 2% Synperonic F 68 (formulation C3). Different concentrations (100–20000 μg/ml) of AKS were added at the polymerization stage to formulations A, X and C1. The suspensions were stirred for 3 h at room temperature. Once polymerisation had reached completion, the nanoparticle suspensions were filtered and brought to pH 7 by adding 0.1 N NaOH.



Scheme 2. Covalent binding of amikacin sulphate to nanoparticles.

Batches of nanoparticle formulations C2 and C3 were freeze-dried, and then resuspended over a range of concentrations (100–20 000 μg/ml) of AKS in solution for 1 h in order to determine the adsorption isotherms. For formulation C1 AKS was included at the polymerization stage and the adsorption isotherm was similarly determined after freeze-drying.

Formulation B was prepared as follows: sodium periodate (0.6 mg/mg particle solids) was added to a sample of dextran-stabilized nanoparticles to oxidise dextran molecules attached on the outside of the oligomeric chains. Then 8 mg of oxidised nanoparticles were incubated with 1 mg of AKS for 24 h to promote the reaction between the dextran aldehyde groups and the amine groups in AKS, followed by reduction of the Schiff's bases formed with equimolar quantities of sodium cyanoborohydride. This reaction is shown in Scheme 2.

Physico-chemical characteristics of the particles

Mean particle size and electrophoretic mobility were determined for the particles by photon correlation spectroscopy (PCS) and laser Doppler anemometry (LDA), respectively. Both analyses were performed using a Zetasizer III apparatus (Malvern Instruments, U.K.). For this purpose each sample of suspension was diluted with distilled water until an optimal particle concentration was obtained. The zeta potential values were calculated from the means of the electrophoretic mobility distributions using the Smoluchovsky equation.

For determination of polymer molecular weight distributions, freeze-dried nanoparticles were dissolved in tetrahydrofuran (THF) and analysed using a polystyrene-calibrated GPC system (Beckman Instruments, U.S.A.) with THF as eluent. Data from a refractive index detector were analysed using a data handling system (Spectra-Physics, U.S.A.). Molecular weight distributions were calculated from the refractive index data. Results are quoted as the number average molecular weights of total and main-peak populations.

Determination of drug-polymer association

The nanoparticles were ultracentrifuged (3000 rpm, 120 min.) (Kontron Instruments, U.K.), and the concentration of AKS in the supernatant and degraded sediment was determined by polarization fluoro-immunoanalysis (PFIA) (Abbot Scientific Instruments, U.S.A.).

Determination of in antimicrobial activity in vitro

To check whether reaction with the polymer and/or the other compounds incorporated in the polymerisation medium affected the antimicrobial activity of the active principle once released, nanoparticle suspensions were made alkaline to release all amikacin and the minimum inhibitory concentrations of these suspensions, amikacin solution and unloaded nanoparticles for *E. coli* P109 were determined by incubation of dilution series in liquid culture medium.

Results and Discussion

The standard procedure for loading nanoparticles (formulation X) gave a very low value of drug-polymer percentage association (4.76%) (Table 1) due to the highly polar AKS molecules. In an attempt to improve the association rate, the experimental conditions for nanoparticle formation and drug loading were changed as described (Scheme 1):

(1) Modification of the polymerization conditions;

(2) Modification of the non-loaded preformed nanoparticles by: (a) oxidation of dextran surface

TABLE 1

Average particle size, drug loading and % association data for the formulations prepared (amikacin concentration in the polymerization or incubation media: 1 mg/ml)

Formulation	Particle size (nm)	Association (%)	Drug loading (μg/mg PBCA)
X	201 (1.6) [a]	4.76 (0.1) [a]	5.95 (0.19) [a]
A	236 (3.0)	60.47 (4.3)	75.59 (5.39)
B	235 (2.1)	30.82 (1.3)	41.00 (1.65)
C1	265 (3.0)	52.90 (0.3)	66.12 (8.40)
C2	210 (2.9)	30.8 (6.26)	38.50 (6.30)
C3	69 (1.2)	28.5 (0.92)	35.69 (1.15)

[a] (S.D.) 4 determinations.

molecules and subsequent covalent linkage with amikacin; (b) freeze-drying and further incubation in an aqeous solution of amikacin sulphate.

Some authors have proposed changing the nature of the acid present in the polymerization medium (HCl was employed in this study) when the drug dissolved in this medium has a basic character, as do aminoglucosides, expecting to produce a less soluble salt (Harmia et al., 1986). In this study, however, sulphuric, citric and hydrochloric acids in the medium led to little variation in the drug-polymer association efficiency (personal communication). Slow neutralization of the polymerization medium, during the particle formation process, was carried out to reduce the concentration of the ionised form of the drug. A significant effect was observed, which was attributed to the drug's high intrinsic solubility (Monteleone et al., 1983). Hence, a more fundamental modification was introduced in order to increase the intrinsic solubility (formulation A). A complex, which partially precipitated out as a fine suspension, was formed between the drug dissolved in the polymerization medium and the anionic surfactant SLS. The monomer, when added, polymerized onto the surface of the particles, increasing the stability of the initial suspension and enhancing the drug payload of the nanoparticles remarkably (Table 1). Changing the molar ratio of drug and surfactant modified the amount of amikacin that could associate with the nanoparticles (Fig. 1). A ratio of 1 : 2 (amikacin : SLS)

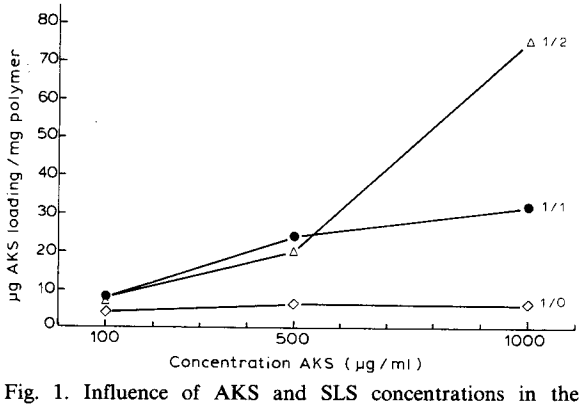


Fig. 1. Influence of AKS and SLS concentrations in the polymerization medium on drug loading.

TABLE 2

Minimum inhibitory concentrations of amikacin for an amikacin control solution and for amikacin liberated from the loaded nanoparticle formulations [(1) complex: Dextran-AKS; (2) non-loaded nanoparticles]

Control	Formulation				
	X	A	B	(1)	(2)
7.81	7.95	8	125	125	–

was found to give the highest association as well as the maximum yield of complex.

Since anionic surfactant agents are not desirable for injectable preparations another method was investigated based on the modification of the particle surface in order to link chemically the drug to surface groups, using the method proposed by Illum et al. (1984) for binding monoclonal antibodies to nanoparticles. Dextran aldehyde groups, which conveniently protrude from the nanoparticle surface, are oxidised and then react with the amine groups of amikacin. Covalent linkage between a drug and dextrans has also been employed by those proposing dextrans for drug carrying systems (Schacht et al., 1987). As expected, this formulation (B) gave a higher drug payload compared to the standard formulation (X) (Table 1), but the in vitro studies revealed a severely diminished antimicrobial activity (Table 2). No enzymes were present in the nanoparticle degradation medium implying that the drug must still have been linked to the dextran molecules after this process and, consequently, its activity is reduced.

Owing to the interest in aminoglucosides for the treatment of eye infections we developed some formulations for opthalmic use. Harmia et al. (1986) have indicated that, when opthalmic application is desired, the drug must preferably be adsorbed onto the surface of the colloidal carrier as opposed to being included within the carrier structure. To determine sorption isotherms, freeze-dried nanoparticles prepared in three different media (formulations C1–C3) were employed and the effect of the following variables on amikacin adsorption were studied: concentration

of amikacin in the polymerization medium; and nature of the stabilizing agent.

The stabilizer used for the first of these varibles was dextran 70. The drug was dissolved in the polymerization medium at several concentrations (formulation C1); once polymerization was over the suspension was neutralized and freeze-dried in order to adsorb the free drug not incorporated in the polymer matrix more efficiently. The large difference in drug-polymer association rate when the polymer was freeze-dried (cf. formulations X and C1, Table 1) shows that freeze-drying facilitates the drug-polymer interaction. However, an increase in particle size was noted as the drug concentration was increased (Table 3 and Fig. 2) and, moreover, at 20 mg/ml AKS concentration the final suspension obtained was not stable. This effect may be due to interference in the synthesis and deposition of the oligomeric chains caused by the presence of AKS. To elucidate the mechanism by which AKS and PBCA interact, the surface charge of the particles (Z potential) was determined. The results listed in Table 3 indicate that the negative surface charge of the particles is neutralized by adsorption of the drug; hence, assuming a mechanism of electrostatic attraction, if drug is present during polymerization it would probably impede formation of the oligomeric chains due to the large drug molecular volume.

This drug-polymer interaction mechanism is more clearly highlighted when AKS is not dissolved in the polymerization medium but in an incubation medium for the freeze-dried carrier (formulation C2). At the highest drug concentrations the surface charge (Z potential) was almost completely neutralized (Table 4). The reduction of the zeta potential is in keeping with the observed increase in the drug-polymer association rate; particle size, however, was not altered by adsorption of the drug.

In Fig. 3 the adsorption isotherms obtained when AKS was incorporated during polymerization or adsorbed after freeze-drying (formulations C1 and C2, respectively) are compared. For AKS concentrations below 5 mg/ml drug-polymer association was more efficient when AKS was included and adsorbed (formulation 1); above this concentration, however, more AKS associated to

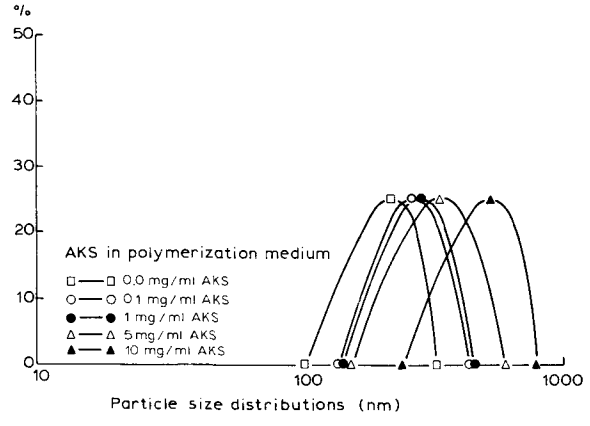


Fig. 2. Influence of concentration of AKS in polimerization medium on particle size distribution.

TABLE 3

Average particle size, Z potential and drug loading (adsorbed and included) of freeze-dried PBCA nanoparticles for different concentrations of AKS in the polymerization medium (formulation C_1)

AKS concentration (mg/ml)	Particle size (nm)	Z potential (μm/s.v)	Drug loading (μg/mg polymer)
0.1	237.8 (2.45) [a]	−7.41 (1.50) [a]	10.54 (0.67) [a]
1.0	264.7 (3.00)	−4.15 (0.76)	66.16 (0.37)
5.0	296.6 (6.68)	−1.76 (0.45)	136.6 (22.5)
10.0	485.3 (11.9)	−2.73 (0.92)	142.8 (24.2)

[a] (S.D.) 4 determinations.

TABLE 4

Average particle size, Z potential and drug loading (adsorbed) of freeze-dried PBCA nanoparticles after incubation in AKS solution (formulation C_2)

AKS concentration (mg/ml)	Particle size (nm)	Z potential (μm/s.v)	Drug loading (μg/mg polymer)
0.1	223.3 (13.6) [a]	−8.65 (2.19) [a]	8.200 (0.86) [a]
1.0	210.1 (2.93)	−4.67 (0.93)	38.56 (7.84)
5.0	204.5 (4.64)	−1.41 (0.08)	120.1 (15.5)
10.0	208.1 (5.96)	−0.68 (0.37)	318.3 (68.4)
20.0	213.1 (3.77)	−0.26 (0.08)	453.3 (94.5)

[a] (S.D.) 4 determinations.

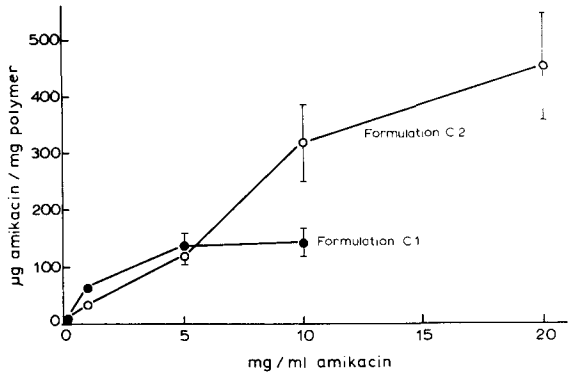


Fig. 3. Adsorption isotherm of AKS to freeze-dried Dextran 70-stabilized nanoparticles.

the nanoparticles when only adsorbed (formulation 2). This finding is in keeping with the observed increase in particle size (Fig. 2) when the drug is included in the polymer structure, leading to a reduction in surface adsorption.

To evaluate the influence of the surfactant Synperonic F 68 on the drug adsorption rate, freeze-dried nanoparticles were incubated in AKS solutions at several concentrations (0.1–20 mg/ml). The corresponding adsorption isotherm (Fig. 4) reveals a lower adsorption efficiency compared to the other adsorbate formulations (C2, C3). The surfactant appears to hinder the drug-polymer interaction due to modification of the particle surface and also probably due to the inclusion of drug into surfactant micelles. Similar results were obtained by Harmia et al. (1986) for pilocarpine adsorbates.

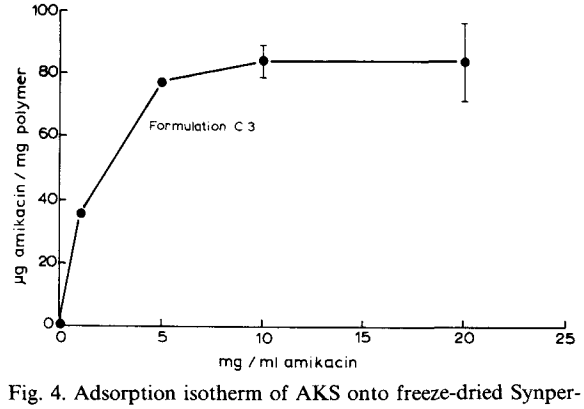


Fig. 4. Adsorption isotherm of AKS onto freeze-dried Synperonic F68-stabilized PBCA nanoparticles.

TABLE 5

Average molecular weights and particle sizes for loaded and non-loaded nanoparticle formulations

Polimerization medium	Particle size (nm)	Molecular weight
Dextran (1%)	210 (2.56) [a]	1480 (114) [a]
Dextran (1%), amikacin (0.1%)	260 (361)	1735 (148)
Dextran (1%), SLS (0.1%)	188 (2.14)	746 (15)
Dextran (1%), SLS (0.1%), AKS (0.1%)	235 (1.31)	1113 (53)
Symperonic F68 (2%)	80 (1.13)	2245 (17)

[a] (S.D.) 3 determinations.

The polymerization conditions used during the experiment seemed to affect the polymer molecular weight (Table 5). The increase due to the presence of AKS is explicable in terms of the supposed nanoparticle formation process (Douglas et al., 1985). According to this theory the polymerization reaction is initiated by hydroxyl ions and halted at the oligomer stage by protons. The oligomers then clumped together to form nuclei to which further oligomers could attach, undergoing a subsequent polymerization process that produces much longer chains. The presence of sulphate anions from AKS prolongs this secondary polymerization process leading to polymers of higher molecular weight. In the same way, SLS must have the opposite effect due to the greater accessibility of sodium cations to the particle nuclei, which terminate chain formation earlier, compared to the lauryl sulphate anions. On the other hand, the increase in molecular weight caused by Synperonic F 68 is also interpreted as a consequence of more intense secondary polymerization due to the formation of a greater number of particle nuclei, shown by the reduction in particle size.

Conclusions

The present results demonstrate that the manufacturing process of PBCA nanoparticles can

be controlled in order to obtain an adequate drug-polymer association rate, with particle sizes and polymer molecular weights falling within desired intervals of values. Moreover, the procedure for nanoparticle loading and, consequently, the drug-polymer association mechanism can be selected to suit the intended form of administration.

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