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Optimization of pilocarpine loading onto nanoparticles by sorption procedures

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Summary

The sorption behaviour of pilocarpine salts, such as nitrate and hydrochloride, onto poly(methylmethacrylate), (PMMA), poly(butylcyanoacrylate), (PBCA) and poly(hexylcyanoacrylate), (PHCA) nanoparticles was studied. Pilocarpine nitrate was more suitable for adsorption, because of its lower water solubility. The PBCA nanoparticles showed the best adsorption properties of the 3 above mentioned polymer materials. With electrolytes, i.e. sodium salts such as nitrate, chloride and sulfate, the amount of pilocarpine nitrate adsorbed onto the PBCA-nanoparticles could be increased significantly. Sodium sulfate had the highest sorption enhancing effect caused by its ionic strength and its system stabilizing effect. Surfactants used above their CMC appeared to have no adsorption promoting effect. However, non-ionic surfactants below the CMC, used after a pretreatment of either lyophilization alone or washing and additional lyophilization prior to adsorption of pilocarpine, improved the adsorption behaviour of this drug even more than electrolytes. The surfactants with the longest hydrocarbon chain length showed the best adsorption increasing effect. For the adsorption of pilocarpine nitrate onto washed, lyophilized PBCA nanoparticles both the Langmuir and Freundlich isotherms were observed. These isotherms are sigmoidal in shape indicating a building up of multilayers.

Introduction

Sustained release of pilocarpine for the treatment of glaucoma is of therapeutic interest because of the poor bioavailability of this drug in conventional ocular dose forms (Smolen, 1978; Juslin et al., 1981; Saettone et al., 1982; Harmia, 1984). One attempt that has been taken to improve ocular drug absorption is the decrease of the rate constant governing precorneal loss of drug. This strategy includes the use of viscous solutions, suspensions, inserts and, more recently, colloidal particles such as microspheres, liposomes and nanoparticles which are ultrafine solid particles (Kreuter, 1983a, Wood et al., 1985).

Rapidly biodegrading particles can be produced using alkylcyanoacrylates (Couvreur et al., 1977 and 1979a; Vezin and Florence, 1978; Lenaerts et al., 1984). The polymer carrier particles can be loaded by adsorption. The adsorption of fluorescein, daunorubicin and dactinomycin on polycyanoacrylate nanoparticles has been shown

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earlier (Couvreur et al., 1979b; Brasseur et al., 1980; Kante et al., 1980). However, very few workers have interpreted adsorption isotherms of drugs onto nanoparticles from a physico-chemical viewpoint.

Principally, adsorption isotherms obtained with drug-loaded nanoparticles can be linearized using the Freundlich equation (Brandl et al., 1964) or the Langmuir equation (Giles et al., 1960, Brandl et al., 1964; Blaug and Gross, 1965). In the case of multilayers, the BET isotherm (Zografi and Mattocks, 1963) becomes valid.

The aim of this work was to study the sorption pattern of pilocarpine onto polyacrylic nanoparticles and to evaluate some factors influencing its adsorption behaviour in order to select the conditions suitable to maximize the drug payload of such systems for ophthalamic purposes.

Materials and methods

Preparation of nanoparticles

The preparation of polymethylmethacrylate (PMMA) nanoparticles is described in detail by Kreuter (1974 and 1983a; Kreuter and Speiser, 1976). For the preparation of the polybutylcyanoacrylate (PBCA) nanoparticles 0.5 g butylcyanoacrylate (Sicomet, Sichel-Werke, Hannover, F.R.G.) was added drop by drop to a solution of 100 g Pluronic F68 (Wyandotte Chemicals Corp. Wyandotte, U.S.A.) in 50 ml 0.01 N HCl and stirred for 2 h at room temperature with a magnetic stirrer at 400 rpm. The resulting polymer suspension was neutralized with 0.1 N NaOH at pH 7 and stirring was continued for 6 h after neutralization. Polyhexylcyanoacrylate (PHCA) nanoparticles (hexylcyanoacrylate, Sicomet, Sichel-Werke, Hannover, F.R.G.) were prepared in a similar way, but instead of Pluronic F68, 250 mg of Tween 20 (Atlas Chemie, Essen, F.R.G.) was used as the surfactant. Analytical grade chemicals including pilocarpine hydrochloride and nitrate obtained from Merck AG (Darmstadt, F.R.G.) were used. The resultant PBCA nanoparticles had a diameter of 98 ± 14 nm, as measured by photon correlation spectroscopy (ALV-laser, BBC Goerz Laser doppler velocimeter, LSO 01 ISC-computer) compared to a value of 316 ± 45 nm for PHCA nanoparticles.

Shape of the particles

Both scanning and transmission micrographs were taken of the PBCA-nanoparticles (Figs. 3 and 4).

Lyophilization

The polymeric suspensions were centrifuged (Du Pont Instruments, Sorvall OTD 75) at 30000 r.p.m. at 10°C for 1 h. Sediment was then separated and dissolved in a mixture of water and alcohol (1 : 1). The procedure was repeated 3 times.

Quantitative determination of pilocarpine

The colorimetric method of Gibbs and Tuckermann (1970) was used for the quantitative determination of pilocarpine hydrochloride and nitrate.

Solubility of pilocarpine nitrate

The influence of buffer systems on the solubility of pilocarpine nitrate in aqueous media was determined by adjusting the pH with citrate or acetate buffer to pH 5.0 and with tris maleate buffer to pH 5.2. Also, the effect of electrolytes, such as NaCl, $NaNO_3$, and Na_2SO_4, on the solubility of pilocarpine nitrate was studied. The shaking time was 12 h at 18°C at a rate of 200 cycles/min. After dilution, the supernatant was assayed colorimetrically using the method of Gibbs and Tuckermann (1978).

Determination of the sorption isotherms

Samples (100 mg) of nanoparticles were transferred to a series of glass tubes (20 ml) fitted with tight screw caps. Ten ml of the appropriate concentration of the drug, surfactants and electrolytes in buffer solution were added. The tubes were shaken horizontally, wholly immersed in a thermostated water bath (18 ± 1°C) at 200 cycles/min. After 1 week, the samples were centrifuged and the supernatant was assayed quantitatively as described before. At the pH used (5.0 and 5.2) no degradation of particles or drug was observable.

The effect of the following variables on pilo-

carpine adsorption was studied: pilocarpine concentration; type of nanoparticles (PMMA, PBCA, PHCA); effect of electrolytes and surfactants on pilocarpine adsorption and solubility; and the influence of the following procedures:

1. no prior purification or lyophilization of the nanoparticles;
2. freeze-drying of non-purified particles;
3. removal of the surfactants by centrifugation and washing 3 times before lyophilization

Results and discussion

Influence of polymer on adsorption

The PBCA nanoparticles showed the best adsorption properties of the 3 polymer materials, polybutylcyanoacrylate (PBCA), polyhexylcyanoacrylate (PHCA) and polymethylmethacrylate (PMMA), (Figs. 1 and 2, Table 1) for pilocarpine nitrate. The PBCA-particles were approximately monodisperse with an average particle size of 100 nm (Fig. 3). This transmission electron microscopic picture was taken of the unpurified suspension. The scanning electron photomicrograph (Fig. 4) shows the product obtained after lyophilization. No surface structure could be observed in any case. Consequently, the high adsorption capacity of the PBCA nanoparticles was presumably due to their large specific surface area, about 100 m^2/g (Kreuter, 1983b) and to the favourable hydrophobicity of PBCA particles, in that this polymer

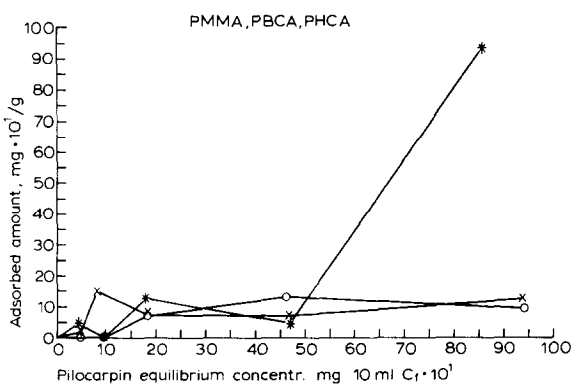

Fig. 1. Adsorption isotherms of pilocarpine hydrochloride onto (○) PMMA, (×) PBCA and (*) PHCA nanoparticles.

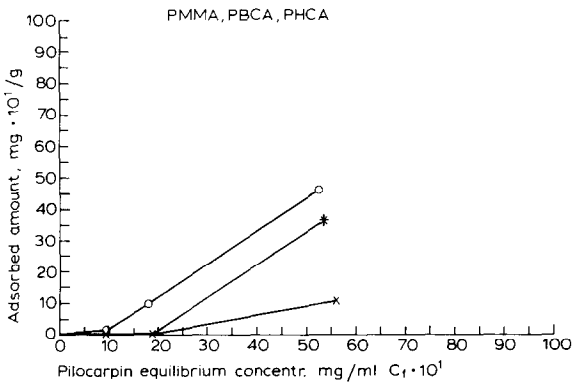

Fig. 2. Adsorption isotherms of pilocarpine nitrate onto (×) PMMA (○) PBCA and (*) PHCA nanoparticles.

is the least hydrophobic of the 3 above used polymers. Two other less hydrophobic cyanoacrylate derivates, ethyl- and methyl-, were not in-

TABLE 1

ADSORPTION OF PILOCARPINE NITRATE ONTO PMMA, PBCA AND PHCA NANOPARTICLES; INFLUENCE OF ELECTROLYTES (NaCl, $NaNO_3$, Na_2SO_4) ON THE ADSORPTION ONTO PBCA PARTICLES

Adsorbent	Adsorbed amount x/m (m = 1)	Initial pilocarpine concentration %			
		1	2	4	6
PMMA	mg	0.0	0.0	7.5	10.9
	%	0.0	0.0	2.0	1.9
	S	0.2	0.1	0.5	0.4
PHCA	mg	0.0	0.0	13.9	36.0
	%	0.0	0.0	3.7	6.3
	S	0.3	0.1	2.1	4.0
PBCA	mg	4.5	9.6	23.6	45.7
	%	4.5	5.1	5.9	8.0
	S	1.1	0.9	0.8	1.3
NaCl	mg	0.0	0.0	5.1	18.1
	%	0.0	0.0	1.3	3.2
	S	0.0	0.1	0.3	1.2
$NaNO_3$	mg	4.5	30.7	62.7	45.0
	%	4.5	15.3	15.7	7.5
	S	1.3	0.9	3.8	3.5
Na_2SO_4	mg	5.2	9.9	50.3	85.4
	%	5.2	5.2	12.6	15.0
	S	0.8	0.7	3.7	6.4

n = 3; S, standard deviation.

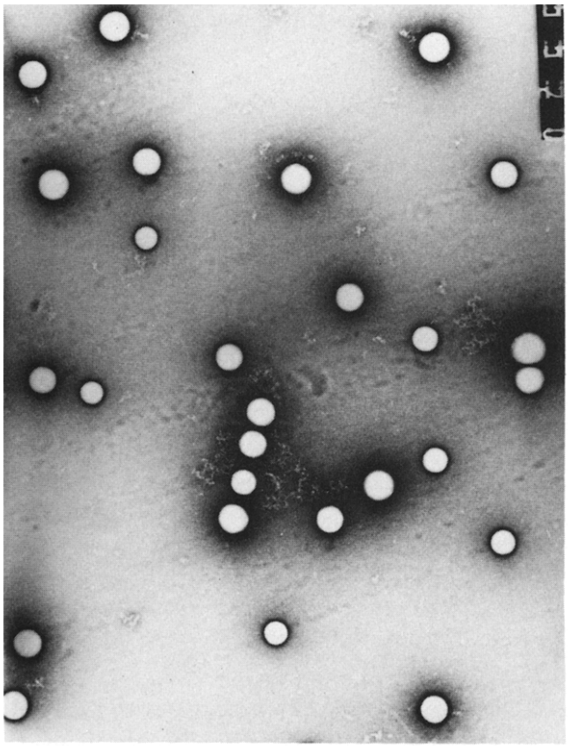

Fig. 3. TEM-photo of PBCA nanoparticle suspensions, ——— = 240 nm.

vestigated because of their rapid degradation which seems to lead to toxic effects (Woodword, 1968; Gibbs, unpublished results).

Solubility

Table 2 shows the solubilities of pilocarpine nitrate in water and in acetate, citrate and tris maleate buffer solutions adjusted to pH 5 and 5.2, respectively. Its solubility in distilled water is about 242 mg/ml which is in agreement with the literature (Dolder and Skinner, 1983). The citrate buffer reduced the solubility of pilocarpine nitrate to half of its water solubility.

Table 3 shows that the decrease in solubility is mainly caused by the citrate buffer: sodium chloride and nitrate even increased the solubility, while sodium sulfate had a small reducing effect.

These results are in agreement with the Hofmeister, or lyotropic series which ranks cations and anions in order of their hydratation

TABLE 2

SOLUBILITY OF PILOCARPINE NITRATE

Buffer solution	pH	Dissolved substance (mg/ml)	Solubility [parts/part of solvent]
Trismaleate	5.2	156.7	1:6.4
Acetate	5.0	156.3	1:6.4
Citrate	5.0	148.7	1:6.7
Water	6.9	243.9	1:4.1

power (Martin et al., 1969; Cutler and Davis, 1972): anions in decreasing order of hydratation are citrate, tartrate, sulfate, acetate, chloride, nitrate, bromide and iodide. Consequently, citrate buffer caused the greatest decrease in solubility.

Adsorption of pilocarpine salts without or with the aid of electrolytes

Pilocarpine nitrate was more suitable for adsorption than the hydrochloride salt, due to its

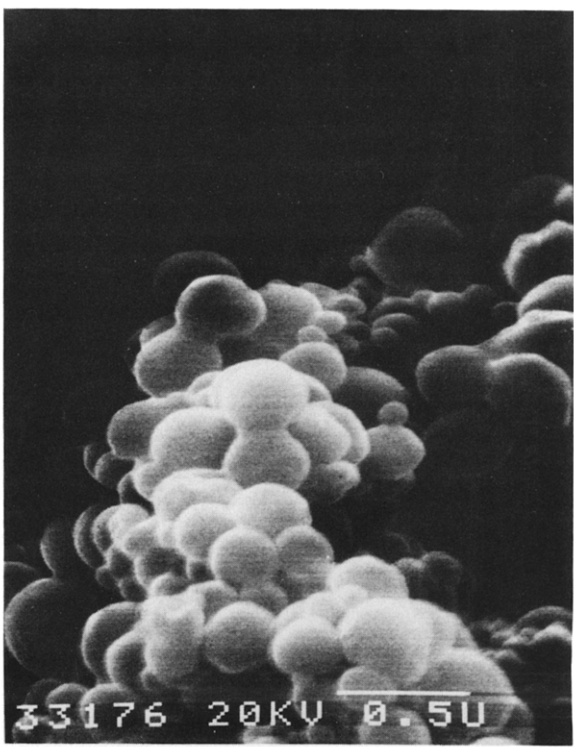

Fig. 4. SEM-photo of freeze-dried PBCA nanoparticles.

TABLE 3

THE INFLUENCE OF ELECTROLYTES ON THE SOLUBILITY OF PILOCARPINE NITRATE IN CITRATE BUFFER, pH 5

Molarity	Electrolyte					
	NaCl		$NaNO_3$		Na_2SO_4	
	mg/ml	L	mg/ml	L	mg/ml	L
0.025	178.6	1 : 5.6	156.3	1 : 6.4	146.4	1 : 6.8
0.05	185.5	1 : 5.4	162.4	1 : 6.2	143.3	1 : 7.0
0.10	182.9	1 : 5.5	154.4	1 : 6.5	146.7	1 : 6.8
0.15	181.0	1 : 5.5	148.3	1 : 6.7	146.4	1 : 6.8
0.20	x	x	161.0	1 : 6.2	141.4	1 : 7.1
0.25	x	x	156.3	1 : 6.4	141.4	1 : 7.1

x, no experiments; L, solubility [parts/parts of solvent].

lower water solubility (1 part in 4 parts, in comparison to 1 part in 0.3 parts for the hydrochloride, Figs. 1 and 2).

As expected, the adsorption of drug onto nanoparticles was influenced by the presence of electrolytes (Table 1) which increased the ionic strength of the medium. Sodium sulfate showed the strongest adsorption enhancing effect.

Surfactant-electrolyte combinations

Fig. 5 shows the adsorption enhancing effect of sodium salts in combination with Pluronic F68 on the sorption of pilocarpine nitrate onto PBCA nanoparticles. A significant increase in adsorption of the drug resulted due to the presence of electrolytes. Again sodium sulfate had the most significant influence, especially at concentrations around 0.05 M. Besides the higher ionic strength of sulfate in comparison to nitrate and chloride ions, the sulfate ions are possibly capable of becoming an integral part of the suspended nanoparticles (Blaug and Gross, 1965; Cutler and Davis, 1972). This may increase the charge of the particles and stabilize the system. The resulting negative charge in turn may attract the pilocarpine cations.

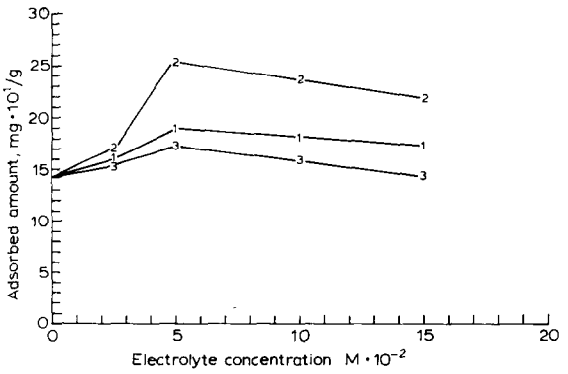

Fig. 5. Adsorption of pilocarpine nitrate onto PBCA nanoparticles in the presence of a surfactant, Pluronic F68 (0.05%) and of electrolytes: 1, NaCl; 2 Na_2SO_4; and 3, $NaNO_3$.

Influence of freeze-drying and purification prior to adsorption on pilocarpine uptake

The uptake of pilocarpine using electrolytes and detergents in concentrations above the critical micelle concentration (CMC) was too low for sustained drug release purposes. If the nanoparticles were used without purification or lyophilization, the surfactants present during the manufacturing remained associated with the particles and only small amounts of either pilocarpine hydrochloride or nitrate were adsorbed. The sorption isotherm of such systems is shown in Fig. 6. It follows the Freundlich equation,

$$C_g = kC_f^m, \quad (1)$$

whereby C_g is the amount of bound drug and C_f is free, non-bound drug (equilibrium concentration). Since m, the Freundlich exponential, is equal to 1, the isotherm can be linearized using the Langmuir equation:

$$\frac{C}{y} = \frac{1}{by_m} + \frac{C}{y_m} \quad (2)$$

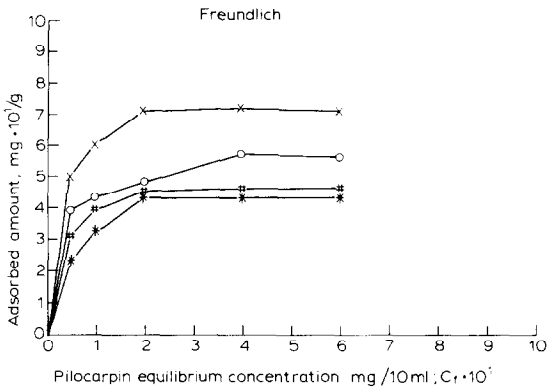

Fig. 6. Adsorption isotherms of (×) pilocarpine nitrate, (○) pilocarpine nitrate + 0.05 M Na_2SO_4, (*) pilocarpine hydrochloride and (#) pilocarpine hydrochloride + 0.05 M Na_2SO_4 onto non-purified, unlyophilized PBCA nanoparticles.

where y (= x/m) is the ratio of adsorbed drug (x) to adsorbate (m), C is the equilibrium concentration of drug. b and y_m are constants related to the surface area of adsorbent and to the enthalpy of adsorption, respectively.

No significant difference in the adsorption behaviour between pilocarpine hydrochloride and nitrate was observed. With both, a saturation concentration was obtained at the equilibrium concentration of about 20 mg pilocarpine/ml solution yielding an adsorbed monolayer on the surface of the particles.

The Langmuir isotherm indicated that pilocarpine does not penetrate into the polymeric matrix but rather is adsorbed onto the surface without forming multilayers. The amount of unoccupied adsorption sites is larger than the amount of occupied sites; both the Freundlich and Langmuir equations are valid in this case. The surfactants do prevent higher adsorption ratios.

Sorption onto freeze-dried particles

A. Without addition of electrolytes. Another type of adsorption isotherm was observed after adsorption of pilocarpine nitrate onto freeze-dried but non-purified PBCA nanoparticles (Fig. 8). At low concentrations about 20% of the drug was adsorbed independently of the initial pilocarpine concentration, indicating a constant equilibrium between free and bound drug. Above 6.0% initial

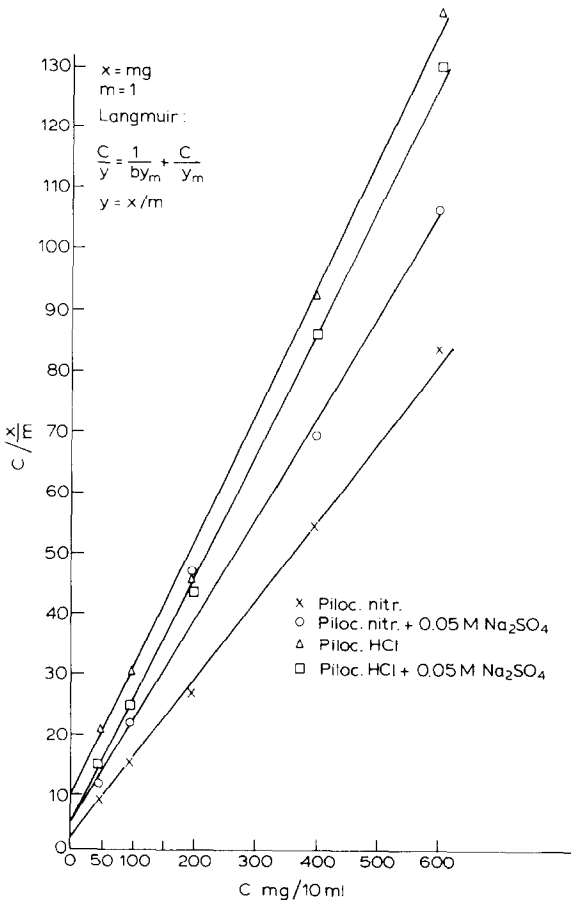

Fig. 7. Adsorption isotherms according to Langmuir. Adsorption of pilocarpine hydrochloride and nitrate on non-purified, unlyophilized PBCA nanoparticles. C, pilocarpine equilibrium concentration.

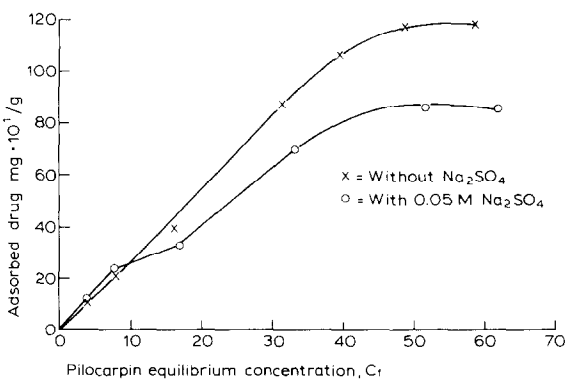

Fig. 8. Adsorption isotherms of pilocarpine nitrate onto non-purified, lyophilized PBCA nanoparticles. C_f, pilocarpine equilibrium concentration, C_g, adsorbed amount of pilocarpine (x/m = mg × 10/g).

pilocarpine concentrations saturation of the particle surface was achieved. The amount of pilocarpine adsorbed was more than two times higher than without lyophilization.

The freeze-drying seems to activate the surface of the particles leading to a higher sorption capacity. The cause of this activation process is so-far not totally clear. Nevertheless, it has been our experience, that the nature of the drying process as well as the surfactant used has a great influence on the interaction of the surfaces with the wetting agent and its contents during the rehydration process (Johnson et al., 1986). In addition, we found that the surface of a surfactant-containing sample appears to be different during scanning electron microscopical investigation depending on the method of drying: air-drying leads to a very smooth surface while lyophilization leads to a more rough surface (Kreuter, 1974, 1983b; Kopf et al., 1976). These changes of the surface properties of the particles seem to be responsible for the "activation" process of the nanoparticles and for the increased adsorption capacity in comparison to non-lyophilized particles that was observed in this study. It has to be mentioned though, that the presence of surfactants in freeze-dried nanoparticle samples may lead to unwanted effects: lyophilized nanoparticle samples with surfactants present originating from the manufacturing process are often more difficult to rehydrate than those in which these surfactants were removed. This latter observation, however, is not a general trend, and depends on the polymer, the surfactants involved and other additives (unpublished observations).

B. Addition of sodium sulfate. Electrolytes only yielded a higher pilocarpine adsorption at the lowest pilocarpine concentrations. In the sodium sulfate adsorption curve a small shoulder can be observed, which may be due to competitive adsorption of pilocarpine and surfactants or due to interaction between the electrolyte and drug.

Sorption onto washed, lyophilized particles

The lyophilized nanoparticles that were additionally purified by washing from the surfactants resulting from the manufacturing process were then resuspended in pilocarpine nitrate solutions containing different amounts of other surfactants. This led to the occurrence of an interesting phenomenon: surfactants at concentrations above the CMC did not have any significant influence on the uptake of pilocarpine. However, concentrations below the CMC considerably improved adsorption (Table 4). This effect was clearly dependent on the hydrocarbon chain length of the surfactant. In the Tween group, increased amounts of pilocarpine were adsorbed with increasing hydrocarbon chain length (Tween 80 with 18, Tween 40 with 16 and Tween 20 with 12 C-atoms). Only

TABLE 4

THE INFLUENCE OF SURFACTANTS IN CONCENTRATIONS BELOW THE CMC ON THE ADSORPTION OF PILOCARPINE NITRATE ONTO PBCA-NANOPARTICLES; INITIAL PILOCARPINE CONCENTRATION = 1% SOLUTION

Surfactant	Concentration %	HLB	Adsorbed drug/nanoparticles (mg × 10/g)			
			With Na_2SO_4		Without Na_2SO_4	
			mg	S.D.	mg	S.D.
Plu. F68	5.3×10^{-3}	29	36.7	2.3	44.6	3.1
Tween 80	1.1×10^{-3}	15.0	36.0	1.5	27.8	2.1
Tween 40	2.7×10^{-3}	15.6	30.8	2.0	36.0	2.3
Tween 20	5.8×10^{-3}	16.7	28.4	1.7	31.9	2.2
Brij 35	5.8×10^{-3}	16.9	30.4	1.2	34.3	1.9
Myrj 49	4.7×10^{-3}	15	41.5	1.6	37.8	1.6
Myrj 51	7.8×10^{-3}	16	42.9	1.7	37.9	1.5

Pluronic F68 = Polyoxyethylene-polyoxypropylene; Tween 80 = PEG(20-sorbitan-monoleate; Tween 40 = PEG(20)-sorbitan-monopalmitate; Tween 20 = PEG(20)-sorbitan-monolaurate; Brij 35 = PEG(23)-lauryl ether; Myrj 49 = PEG(20)-stearic acid ester; Myrj 51 = PEG(30)-stearic acid ester. HLB = hydrophile–lipophile classification; PEG = polyethyleneglycol.

small differences in the adsorption behaviour between Tween 20 and Brij 35 were observed. Like Tween 20, Brij 35 has 12 C-atoms in its hydrophobic chain and only 3 PEG-groups more than the latter. The highest adsorption yields were achieved with the Myrj 49 and 51 both containing 18 C-atoms in their hydrocarbon chain. Pluronic F68 also was a comparable suitable adsorption enhancing agent.

Compared to the previous experiments (maximal uptake at 1% initial pilocarpine concentration was only about 20 mg), much higher amounts (uptake > 40 μg) were adsorbed using the purified lyophilized nanoparticles in combination with surfactants below their CMC. With the latter particles, sodium sulfate only had a minor adsorption enhancing effect, in some cases (Table 4) it even decreased adsorption.

Fig. 9 shows the amount of drug adsorbed at different pilocarpine nitrate concentrations onto PBCA nanoparticles, using Pluronic F68 and Myrj 51 as surfactants. Higher amounts were adsorbed, but the increase in adsorption was not uniform. There is even more evidence from the isotherms plotted according to Langmuir (Fig. 10). At low pilocarpine concentrations, sodium sulfate increased the uptake in combination with Pluronic F68 but not with Myrj 51. However, the reverse was the case at high pilocarpine concentrations.

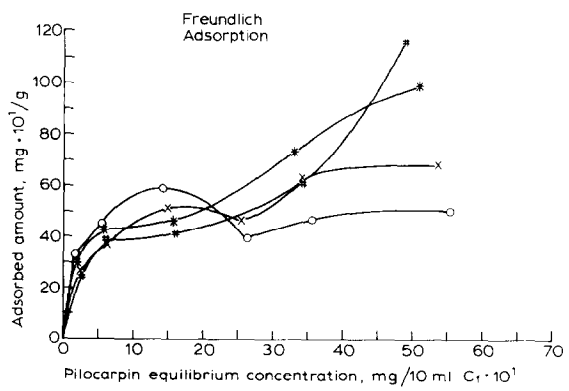

Fig. 9. Adsorption isotherms of pilocarpine nitrate onto purified, freeze-dried PBCA nanoparticles. Surfactants used were (×) 5.3×10^{-3}% Pluronic F68, (○) 5.3×10^{-3}% Pluronic F68 + 0.05 M Na_2SO_4, (*) 4.7×10^{-3}% Myrj 51 and (#) 4.7×10^{-3}% Myrj 51 + 0.05 M Na_2SO_4.

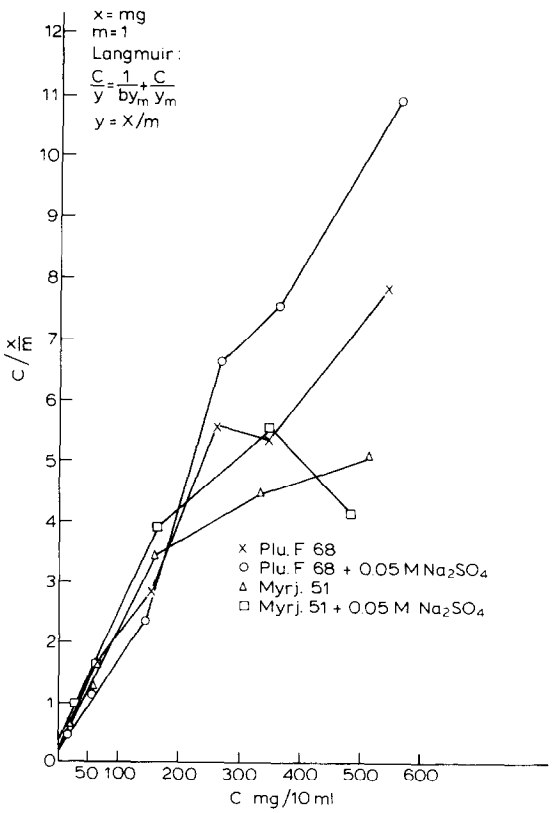

Fig. 10. Adsorption isotherms according to Langmuir. Adsorption of pilocarpine nitrate onto freeze-dried, purified PBCA nanoparticles. C, pilocarpine equilibrium concentration.

Conclusions

It can be concluded, that pilocarpine uptake onto polybutylcyanoacrylate nanoparticles is very complex: electrolytes may increase or decrease the uptake depending on the presence and the amount of surfactants. Below the CMC of the surfactants, the influence of the electrolytes was diminished. These results are in accordance with findings that the hydratation and the electrostatic repulsion of surfactants are decreased due to electrolytes (Tabor, 1977; Tadros and Vincent, 1979; Wilson and Kennedy, 1979, Kiefer and Wilson, 1980; Piirma and Chen, 1980).

Surfactants above their CMC, which may still be present originating from the manufacturing process or which may be added after removal of the original surfactants, have no adsorption-pro-

moting influence. As mentioned above, they may even render the wetting more difficult after the nanoparticles have been dried. The interaction of certain polymers and surfactants with similar semihydrophobic surfaces has already been studied by Johnson et al. (1986), but no clear picture about the events occurring on a molecular scale could be elucidated. Nevertheless, the freeze-drying process seems to expose structures on the particle surface that facilitate pilocarpine sorption even at surfactant concentrations of above the CMC.

Below their CMC, our results demonstrate an increase in pilocarpine sorption by surfactants, whereby an increase in the hydrophobic chain length of the surfactant enhances the extent of the sorption process. The nature of this interaction between surface, adsorbed species and surfactant is not totally understood. The understanding of this process will require additional extensive studies and is not necessarily within the scope of this study. One possibility is that cooperative adsorption is taking place similar to what has been observed during adsorption of proteins on polymer surfaces (Söderqvist and Walton, 1980). A first rudimental model of this cooperative process is shown in Fig. 11. This model can also take into account the possibility of a build-up of a double layer as suggested by the results presented in Fig. 9.

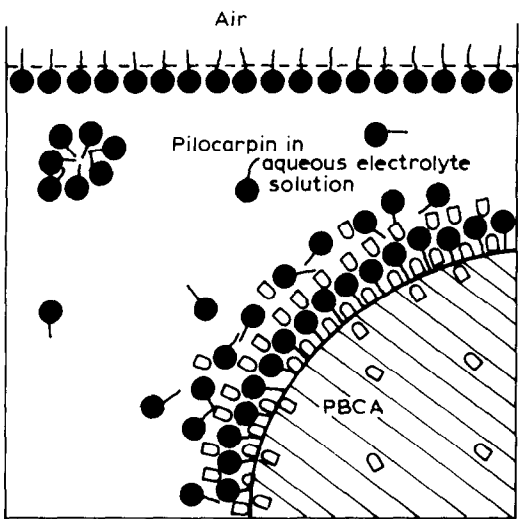

Fig. 11. Illustrative presentation of the adsorption and build-up of multilayers onto PBCA nanoparticles. ⌂, pilocarpine salt; ●, non-ionic surfactant.

The optimal preparations developed in this study are presently tested in rabbits. Preliminary results showed a 30% increase in bioavailability and miotic response over a comparable pilocarpine solution (submitted to *Int. J. Pharm.*).

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