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Methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles for drug delivery Part II: arecaidine propargyl ester and pilocarpine loading and in vitro release

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Abstract

Methylmethacrylate (MMA) sulfopropylmethacrylate (SPM) copolymer nanoparticles were prepared by free radical polymerization and the loading characteristics of the muscarinic agonists arecaidine propargyl ester (APE) and pilocarpine were investigated. The loading efficiency was mainly influenced by the concentration of the copolymer carrier system and followed Langmuir's adsorption equation. The in vitro drug release was mainly influenced by the composition of the acceptor phase used and the nanoparticle content of the donor phase. The bound drug was released from the carrier by competitive replacement by other ions from the binding sites of the particles. By this mechanism the nanoparticle system achieved a prolonged drug release, which was not the result of the increased viscosity at higher nanoparticle concentrations. © 1997 Elsevier Science B.V.

Keywords: Nanoparticles; Copolymer; Pilocarpine; Arecaidine propargyl ester; In vitro release; Drug loading

1. Introduction

One of the major problems of the most frequently used polymers for preparation of nanoparticles, namely the polyalkylcyanoacrylates and the polyalkylmethacrylates, is their low loading capacity for hydrophilic drugs. In order to increase the hydrophilicity of the particle surface, in the first part of this study nanoparticles were prepared by copolymerization of sulfopropylmethacrylate and methylmethacrylate (Langer et al., 1996). The polymerization resulted in stable

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dispersions with a strong negative surface charge. The surface charge of the nanoparticles was constant over a pH range between 3.0 and 9.0 due to the high acidity of the sulfonic acid group containing comonomer sulfopropylmethacrylate-potassium (SPM).

The objective of the present study was the evaluation and characterization of the loading capacity and characteristics of the proposed carrier system for the muscarinic drugs arecaidine propargyl ester (APE) and pilocarpine. Both drugs are compounds of high hydrophilicity in their protonated forms existing under acid conditions. The proposed copolymer nanoparticles were further characterized with regard to their in vitro APE and pilocarpine release using a dialysis technique.

2. Materials and methods

2.1. Reagents and chemicals

Methylmethacrylate (MMA) (Merck-Schuchardt, Hohenbrunn, Germany), SPM, ammonium persulfate (Hüls, Marl, Germany), arecoline hydrobromide (Fluka, Buchs, Switzerland), sodium hydrogen carbonate (Roth, Karlsruhe, Germany), dichloromethane, mannitol, pilocarpine hydrochloride, sodium chloride, sodium dihydrogenphosphate-monohydrate and disodium hydrogenphosphate-dodecahydrate (Merck, Darmstadt, Germany) were used. Water was purified by ion exchange followed by boiling under a stream of nitrogen to remove oxygen.

The arecaidine propargyl ester hydrobromide (APE · HBr) was synthesized by EMKA-Chemie (Markgröningen, Germany) according to a method previously described (Moser et al., 1989).

2.2. Preparation of nanoparticles

Copolymer nanoparticles were prepared by a process of free radical polymerization in water in closed beakers of 100 ml (Langer et al., 1996). The total monomer content and the ammonium persulfate concentration were fixed to 5.0 and 0.03%, respectively. The resulting suspensions were concentrated to obtain polymer contents of about 25% (w/v) using an Amicon Stirring Unit Series 8400 equipped with an Amicon YC05-filtration membrane (Amicon, Witten, Germany). The polymer contents of the resulting suspensions were determined by gravimetry. The particle size of the resulting nanoparticles was determined by photon correlation spectroscopy (PCS).

2.3. Determination of APE

The gaschromatographic determination of APE was conducted by an analytical method reported earlier (Langer et al., 1997).

2.4. Determination of pilocarpine

An aliquot of a solution of pilocarpine hydrochloride in water, containing between 0.13 and 0.36 mg of the drug was diluted with phosphate buffer pH 5.6 to 10.0 ml. The absorbance of the solution was assayed spectrophotometrically (Hitachi U-3000, Hitachi, Berkshire, UK) at 215 nm against the corresponding control samples. The phosphate buffer consisted of 0.13 M sodium dihydrogenphophate-monohydrate and 0.005 M disodium hydrogenphosphate-dodecahydrate.

2.5. Drug loading

Stock solutions of each drug (APE · HBr or pilocarpine hydrochloride) were prepared at a concentration of 10% (w/v) in water. An aliquot of each polymer suspension was placed in a volumetric flask and an aliquot of the drug solution was added. If necessary the pH of the mixture was adjusted to 4.0. The suspensions were diluted with water to 5.0 ml and stirred over a period of 3 h using a magnetic stirrer. If isotonicity was required, sodium chloride or mannitol were added before diluting the samples.

After equilibration 450 μl of each suspension were transferred to Microcon 10 microconcentrators (Amicon, Witten, Germany) followed by centrifugation at 10 000 $\times$ g over a period of 1 h in an Eppendorf centrifuge 5417 (Gerätebau Eppendorf, Engelsdorf, Germany). The filtrates were

assayed for the drug content as described before. The percentage of drug that was bound to the particles was calculated relative to a drug reference that was treated in the same manner as the particulate systems (relative loading). The absolute loading (x/m) was calculated as the amount of drug x bound to 1 mg of the carrier system.

2.6. Pilocarpine transport through dialysis membranes

In order to determine the pilocarpine release from the nanoparticles the drug-diffusion profiles of the loaded copolymer nanoparticles were studied using dialysis tubings size 9 (Medicell, UK). The following acceptor media were employed: (1) a 5.0% (w/v) solution of mannitol in water; and (2) a 0.9% (w/v) solution of sodium chloride in water. Samples were taken after 0, 30, 60, 90, 120, 180 and 240 min. The diffused drug amounts were assayed as described above. For statistical analysis the unpaired Students t-test was performed.

3. Results and discussion

In the present study the loading behavior of copolymer nanoparticles (Langer et al., 1996) for the muscarinic agonists pilocarpine and APE as well as the in vitro release of the drugs were investigated. APE, a highly potent muscarinic agonist, may offer new perspectives in the therapy with cholinergic drugs (Wolf-Pflugmann et al., 1989). For example, APE was found to be 600-fold more potent than pilocarpine when exposed to the rat iris (Hagan et al., 1988).

In this part of the study methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles were prepared at a total monomer concentration of 5%. The concentration of the charged comonomer SPM was fixed to 0, 5 and 10% of the total monomer content, respectively. The effective diameters of the resulting particles were calculated to be 283.8 ± 24.0, 151.6 ± 18.3 and 122.7 ± 25.9 nm (mean $\pm$ S.D.; $n = 4$), respectively.

3.1. Drug loading study

The drug loading capacity of the new copolymer nanoparticles was determined relative to the nanoparticle content, the drug content and the composition of the copolymer. During the loading experiments the pH of the suspensions was fixed at 4.0. At this pH both drugs, pilocarpine and APE, were present in their cationic form due to protonation of their amino groups.

3.2. Drug loading—influence of copolymer composition

In order to evaluate the influence of the copolymer composition onto the loading behavior of the nanoparticle system, copolymer nanoparticles were prepared at concentrations of the comonomer SPM of 0, 5 and 10% of the total monomer. The loading experiments were performed at a total polymer content of 4% and an APE concentration of 1%. A linear correlation between the comonomer amount in the carrier system and the loading efficiency was observed. APE showed no affinity towards PMMA nanoparticles without comonomer. This finding agrees with similar results of Harmia et al. (1986) with pilocarpine. These authors studied the sorption behavior of the latter drug onto different acrylic acid homopolymer nanoparticles. Consequently, it can be concluded that the negatively charged functional groups of SPM served as the binding sites for cationic drugs like APE and pilocarpine.

3.3. Drug loading—influence of nanoparticle concentration

In order to determine the influence of different particle concentrations on the loading characteristics of the proposed carrier system, a copolymer 10/90-carrier (copolymer composition: 10% SPM, 90% MMA) was used at a fixed APE concentration of 1% (Fig. 1). A free availability of the functional SPM-groups at the surface of the copolymer nanoparticles was observed for polymer concentrations below 5% (w/v). Above 5% polymer contents, the accessability of the binding sites

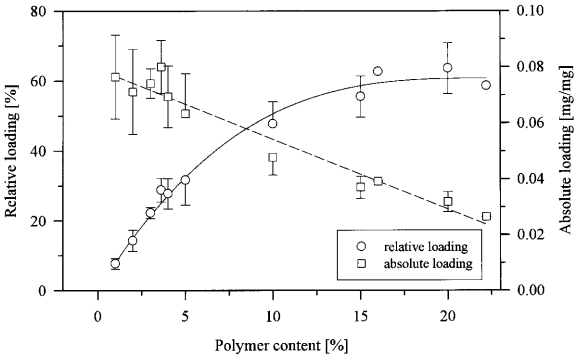

Fig. 1. Influence of the nanoparticle content on the APE binding to copolymer 10/90-nanoparticles (mean ± S.D.; $n > 3$). The binding capacity was calculated as relative (– ○ –) and absolute loading (– □ –). A linear correlation between the relative drug loading efficiency and polymer content was observed for carrier concentrations below 5% (w/v).

was gradually reduced and the relative loading attained a plateau at polymer contents higher than 15% (w/v). This reduction in accessability of the particle surface is due to interaction of the particles with each other at high particle concentrations.

A corresponding loading behavior was observed for pilocarpine at a concentration of 1% (Fig. 2). Absolute and relative loading was slightly lower because of the lower molecular weight of the drug in comparison to APE. A calculation of the loading efficiency in mole drug per mass polymer led to similar results for both drugs. This

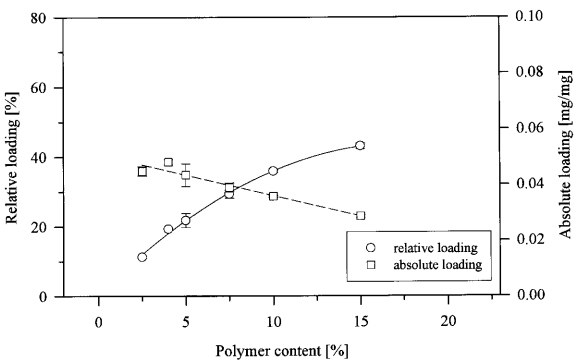

Fig. 2. Influence of the nanoparticle content on the pilocarpine binding to copolymer 10/90-nanoparticles (mean ± S.D.; $n = 2$). The binding capacity was calculated as relative (– ○ –) and absolute loading (– □ –).

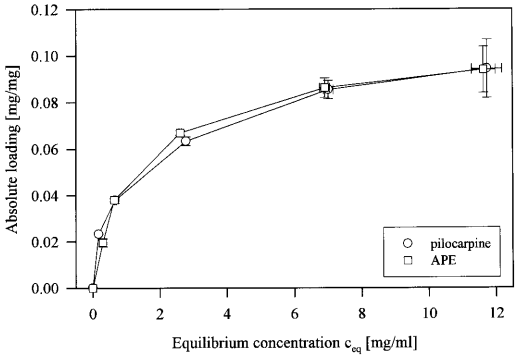

Fig. 3. Freundlich plot for the APE (– □ –) and pilocarpine (– ○ –) binding to copolymer 10/90-nanoparticles (mean ± S.D.; $n = 3$).

indicates a comparable availability of the binding sites for monovalent cationic drugs.

3.4. Drug loading—influence of drug concentration

For the evaluation of the influence of the drug concentration on the loading of the copolymer carrier system, adsorption isotherms were established by adding various amounts of the drug to a copolymer 10/90-carrier system at a concentration of 4% (w/v). As mentioned above, at this concentration a free availability of the sulfonate groups of APE for drug binding was anticipated. The results were plotted according to the theory of Freundlich (Fig. 3) and Langmuir (Fig. 4). A

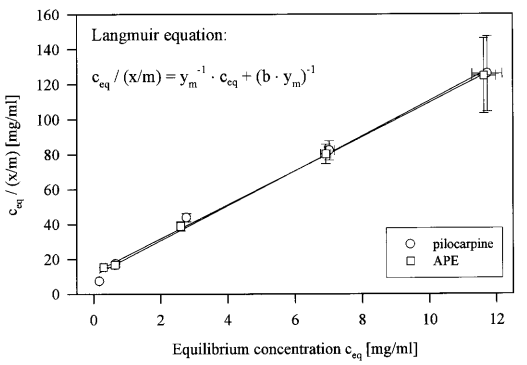

Fig. 4. Langmuir plot for the APE (– □ –) and pilocarpine (– ○ –) binding to copolymer 10/90-nanoparticles (mean ± S.D.; $n = 3$).

similar loading behavior for both drugs was observed. At plateau drug concentrations an amount of drug bound to nanoparticles between 80 and 100 $mg \cdot g^{-1}$ was estimated. Compared to earlier studies of Harmia et al. (1986) that employed PMMA, PBCA and PHCA nanoparticles for the adsorption of pilocarpine, the copolymer nanoparticles used in the present study exhibited by far the best binding properties for this drug.

The Langmuir isotherm (Fig. 4) indicated that no significant penetration of the drugs into the particle matrix occurred and that the drugs were rather bound onto the surface without the formation of multilayers. The Langmuir equation offers the possibility to calculate the maximum drug binding y_m to the carrier system as the reciprocal value of the slope of the curve. The linearization led to y_m values of 102.8 $mg \cdot g^{-1}$ ($n = 3$) and 100.04 $mg \cdot g^{-1}$ ($n = 3$) for APE·HBr and pilocarpine hydrochloride, respectively. Calculations based on the total amounts of monomers employed for the preparation of the copolymer nanoparticles yielded y_m values of 105.61 $mg \cdot g^{-1}$ (APE·HBr) and 99.35 $mg \cdot g^{-1}$ (pilocarpine hydrochloride), assuming that all of the charged comonomer SPM was placed on the surface of the carrier system. Since these calculated theoretical values are very close to the values obtained by the Langmuir calculation based on the experimental measurements, an almost quantitative placement of SPM on the particle surface and an easy accessability for drug binding can be deduced from these experiments at carrier concentrations below 4% (w/v).

3.5. Drug loading—influence of medium composition

The copolymer carrier systems exhibits ion-exchange characteristics. As a result, other ionic compounds besides the drugs can influence the loading results (Fig. 5). Sodium chloride reduced pilocarpine binding, demonstrating the anticipated competition for the binding sites on the copolymer carrier. Addition of phosphate buffers to the systems yielded comparable results (data not shown). Mannitol led to an opposite effect on drug binding. Mannitol seems to modify pilocarpine binding either by alteration of the structural arrangement of the polymer chains on the particle surface or by cooperative binding with the drug.

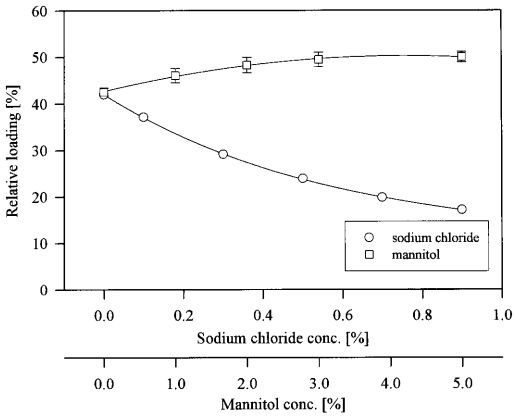

Fig. 5. Influence of sodium chloride and mannitol on the pilocarpine binding to copoylmer 10/90-nanoparticles; polymer content 10% (mean ± S.D.; $n = 2$).

3.6. Pilocarpine transport through dialysis membranes

In the present study dialysis was chosen for obtaining data about the drug delivery by the nanoparticles. All of the experiments were performed with 1% pilocarpine as the model compound loaded onto the copolymer 10/90-carrier system. In vitro drug diffusion experiments were performed using 0.7% saline or 4.0% mannitol as the auxiliary isotonic compounds in the nanoparticle donor phase and a 5% mannitol solution (isotonic without drug) as the acceptor phase. Mannitol in the donor compartment revealed only little influence on the diffusion profile (Fig. 6). Saline showed a slightly enhanced total amount diffused across the membrane, probably because of the above mentioned displacement of adsorbed pilocarpine by the sodium ions.

Diffusion of pilocarpine from the copolymer nanoparticles at a concentration of 4% also was influenced by the composition of the acceptor medium (Fig. 7). Mannitol was used for the isotonicity of the nanoparticle donor compartment. Up to 120 min no significant difference between

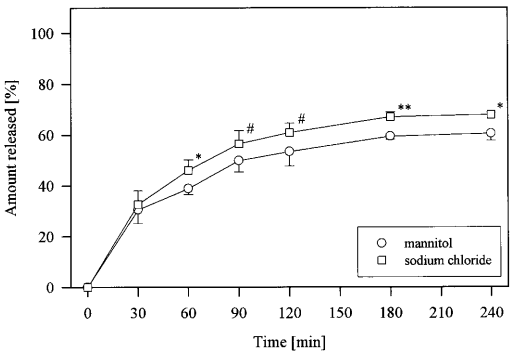

Fig. 6. Influence of the compound used to achieve isotonicity in the donor phase (mannitol – ○ –; saline – □ –) on the pilocarpine release from copolymer 10/90-nanoparticles at a polymer concentration of 4% (mean ± S.D.; $n \geq 3$). A 5% mannitol solution served as the acceptor phase. $^{\#}p = 0.10$; $^{*}p = 0.05$; $^{**}p = 0.01$ (Students t-test).

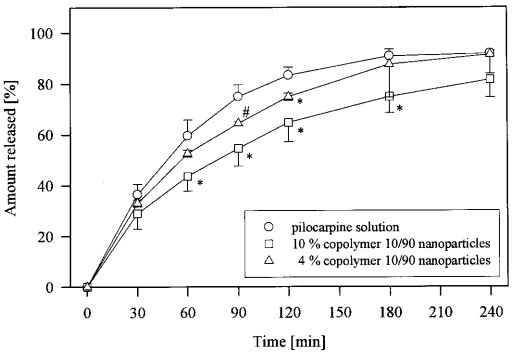

Fig. 8. Influence of the polymer concentration in the donor phase on the pilocarpine release of copolymer 10/90-nanoparticles (mean ± S.D.; $n = 3$). $^{\#}p = 0.10$; $^{*}p = 0.05$ (Students t-test).

saline or mannitol in the acceptor phase existed. After 2 h with mannitol only the unbound drug was transported. This amount corresponded to the free amount of pilocarpine obtained after ultrafiltration of the nanoparticle suspension during the determination of drug loading. In the case of saline as the acceptor phase, however, additional nanoparticle-bound drug was released and diffused through the membrane as a result of the reverse diffusion of the sodium ions from the acceptor compartment and the subsequent ion-exchange on the particle surface.

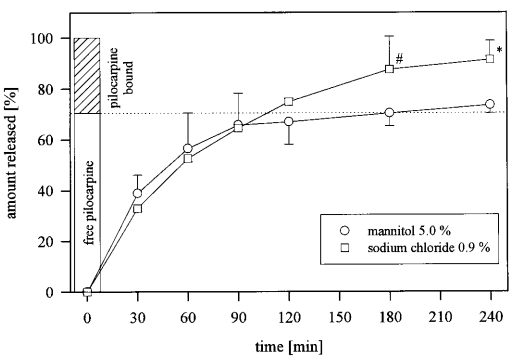

Fig. 7. Influence of the composition of the acceptor phase (mannitol – ○ –; saline – □ –) on the pilocarpine release from the nanoparticles. Copolymer 10/90-nanoparticles at a polymer content of 4% served as the donor phase (mean ± S.D.; $n = 3$). Mannitol was used for the isotonicity of the nanoparticle donor compartment. $^{\#}p = 0.10$; $^{*}p = 0.05$ (Students t-test).

As mentioned above, the loading behavior of the proposed carrier system was mainly influenced by the nanoparticle content in the suspensions. Consequently, a significant influence of the polymer content on the release profile from the carrier was observed (Fig. 8). The diffusion became slower with increasing polymer content due to a higher amount of bound drug.

Increasing amounts of nanoparticles led to an enhanced viscosity of the resulting donor compartment. For example, a copolymer 10/90-carrier system at a polymer concentration of 11% exhibited a viscosity of 8.1 mPas. To evaluate whether the delay in the diffusion of the drug was due to the viscosity of the nanoparticle systems or the nanoparticles itself, the release profiles obtained for the nanoparticle systems were compared with the transport profiles of pilocarpine solutions across the dialysis membranes adjusted to the same viscosity by addition of methylhydroxyethylcellulose (Tylose MH). Tylose/pilocarpine solutions with viscosities up to 9.0 mPas showed no delayed diffusion as observed for the particulate systems (Fig. 9). Therefore, it can be concluded that the delayed release of the particulate systems was caused by the ionic interactions between the carrier and the drug and not by the enhanced viscosity.

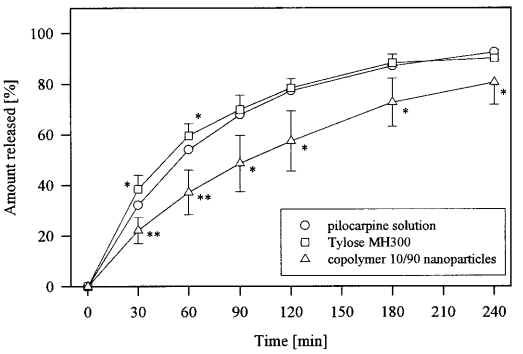

Fig. 9. Influence of the viscosity on the pilocarpine diffusion in vitro (mean ± S.D.; $n = 3$). *$p = 0.05$; **$p = 0.01$ (Students t-test).

4. Conclusions

The results of this study demonstrate the feasibility of the loading of hydrophilic drugs such as the muscarinic agonists APE and pilocarpine with a high efficacy onto copolymer nanoparticles by ionic interactions. The loading efficacy was mainly influenced by the nanoparticle content and the composition of the copolymer. The ionic composition of the loading media was shown to modify drug binding. The release behavior of the carrier system with the model compound pilocarpine determined by dialysis showed a prolonged release by the particulate system compared to the transport of the simple aqueous drug solutions across the dialysis membranes. Ionic compounds induced the release of bound drug from the carrier by competitive interaction with the drug for the binding sites of the particles.

The proposed carrier system enables rational surface modifications. This may improve the development of nanoparticulate carrier systems. Methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles seem to represent promising carriers for hydrophilic cationic drugs with low affinity to previously frequently employed polymers for the preparation of nanoparticles such as polyalkylcyanoacrylates and methylmethacrylates.

Acknowledgements

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