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European Journal of Pharmaceutics and Biopharmaceutics 47 (1999) 203–213

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

Evaluation of aminoalkylmethacrylate nanoparticles as colloidal drug carrier systems. Part I: synthesis of monomers, dependence of the physical properties on the polymerization methods

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Received 4 June 1998; accepted 30 November 1998

Abstract

Conventional nanoparticles based on acrylic compounds are lipophilic and possess a negative surface charge. This is due to their manufacturing process and to the chemical structure of the polymer. Hence, these particles are not suitable for the adsorption of hydrophilic anionic drugs. In the present investigation, positively charged copolymer nanoparticles prepared from aminoalkyl- and methylmethacrylates were evaluated, with regard to their physical properties. This report provides a detailed description of the synthesis of the non-commercially available monomers and their polymerization procedure. Various parameters were investigated, such as comonomer content, total amount of monomer, concentration of the radical initiator, and the composition of the polymerization medium. The resulting particle diameter and the surface charge were found to be strongly dependent on the polymerization conditions and on the pH. Optimization of the polymerization procedure yielded nanoparticles of about 200 nm exhibiting a positive surface charge. The charges of the different copolymer particles were then compared at different pH values. N-trimethylaminoethylmethacrylate (TMAEMC) nanoparticles with quaternary ammonium groups located at their surfaces, possessed a nearly constant positive zeta potential at various pH values and, consequently, pH-independent particle diameters. The physical characteristics of the other aminoalkyl copolymers correlated with the basicity of the monomers employed and were found to be strongly dependent on the pH of the dispersion medium. Aminoethylmethacrylate (AEMC), methylaminoethylmethacrylate (MMAEMC), and aminohexylmethacrylate (AHMC) as well as aminoethylmethacrylamide (AHMAC) copolymer nanoparticles exhibited a strong positively charged surface even at physiological pH and, therefore, are useful candidates for the adsorption of anionic drugs. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Nanoparticles; Copolymer; Aminoalkylmethacrylate; Methylmethacrylate; Surface charge; Physicochemical characterization

1. Introduction

For the last 20 years, nanoparticles were employed as colloidal drug delivery systems to improve the performance of various drugs. The main goal of this dosage form is to achieve a prolonged, controlled, or targeted action of the incorporated or encapsulated drugs [1]. Nanoparticles were most frequently manufactured from acrylic acid derivatives such as poly(alkylcyanoacrylates) and poly(alkylmethacrylates) [1–4]. However, these compounds are not suitable for the binding of hydrophilic ionic drugs due to their hydrophobicity. Several attempts were undertaken to overcome this limitation. Alkylcyanoacrylates were polymerized in the presence of anionic and cationic dextrane derivatives [5–7]

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PII: S0939-6411(98)00100-3

to obtain nanoparticles with hydrophilic surfaces exhibiting different surface charges. Among the methacrylates, the hydrophilicity of their particle surface was increased by copolymerization of alkylmethacrylates with 4-vinylpyridine and various acrylic acid derivatives [4,8,9]. The copolymerization of methylmethacrylate with the strong acid sulfopropylmethacrylate (SPM) led to the formation of nanoparticles possessing a strong negative surface charge at all relevant pH-values, i.e. 2–13. These particles were suitable for the adsorption of the cationic drugs pilocarpine and arecaidine propargyl ester [10,11]. Although a large number of drugs such as oligonucleotides, nucleoside analoga (5-fluorouracil), foscarnet, and others, are anionic at physiological pH-values, so far very little systematic work has been done to develop cationic particulate carrier systems. In earlier studies, the commercially available cationic compound dimethylaminoethylmethacrylate (DMAEMC) was employed for the synthesis of homopolymers [12] and copolymers, respectively [13]. This compound was also used in a recent study of our workgroup [14], where the three cationic methacrylate copolymers N-dimethylaminoethylmethacrylate (DMAEMC), N-trimethylammoniumpropyl-methacrylamide (MAPTAC), and trimethylammoniumethyl-methacrylate (TMAEMC) were investigated to determine their physical properties and cytotoxicity.

Another approach for the binding of plasmid to poly((2-dimethylamino)ethyl methacrylate) was reported by Cheng et al. [12]. In this case, the formation of the nanoparticles was the result of a precipitation due to ionic interactions of the soluble polymer with the plasmid.

The main goal of the present study was to synthesize new acrylic monomers with partial positively charged structures and to characterize nanoparticles, prepared from these commercially non-available compounds. In the first part of the present paper, the synthesis of aminoethylmethacrylate **4a** (AEMC), methylaminoethylmethacrylate **4b** (MMAEMC), trifluoracetylaminohexylmethacrylate **3d** (AHMC), and aminohexylmethacrylamide **4c** (AHMAC) is described. The second part deals with the influence of various polymerization parameters on the physical properties of the formed particles. Different comonomer compositions, initiator concentrations, total monomer concentrations, as well as the composition of the polymerization medium were varied to obtain optimal polymerizing conditions and to establish a reproducible preparation procedure for each monomer. In the third part of the present study, the nanoparticles that differed from each other by the substitution pattern of their aminoalkyl groups, were compared with regard to their particle size and surface charge.

2. Materials and methods

2.1. Materials

Commercially available compounds for the synthesis of the monomers were purchased from Fluka AG (Buchs, Switzerland) and Merck (Darmstadt, Germany) and were used without further purification. CH_2Cl_2 was distilled over P_20_5. Pyridine was distilled over KOH. Solvents for chromatography and petrolether also were distilled prior to use. The comonomer N-trimethylaminoethylmethacrylate chloride (TMAEMC) and ammonium persulfate (APS) were provided by Hüls (Marl, Germany), the comonomer N-dimethylaminoethylmethacrylate (DMAEMC) was purchased from Fluka AG (Buchs, Switzerland). Methylmethacrylate (MMA) and all other chemical compounds used for the preparation of nanoparticles were provided by Merck (Darmstadt, Germany).

2.2. Synthesis of the monomers

2-Methacrylic acid-2-aminoethyl ester hydrochloride **4a**, 2-methacrylic acid-*N*-methyl-2-aminoethyl ester hydrochloride **4b**, 6-aminohexyl methacrylic acid amide hydrochloride and 2-Methacrylic acid-6-aminohexyl ester hydrochloride **4c** had to be synthesized, because they were not commercially available (Fig. 1). To avoid *N*-acylation, the amino group of the amino alcohols was first selectively protected by a *tert*-butyloxycarbonyl (BOC) group or by a trifluoroacetate group. Both protective groups are cleaveable and can be removed both at the monomeric and polymeric stage of the methacrylates. The synthesis of the desired methacrylates was achieved by reaction of methacryloyl chloride with the *N*-protected aminoalcohols **2a-2c** (Fig. 1) followed by *N*-deprotection and were both practicable in large scale synthesis. The *N*-trifluoroacetamide aminohexyl methacrylate **3d** (Fig. 1) was directly used for the preparation of nanoparticles without deprotection.

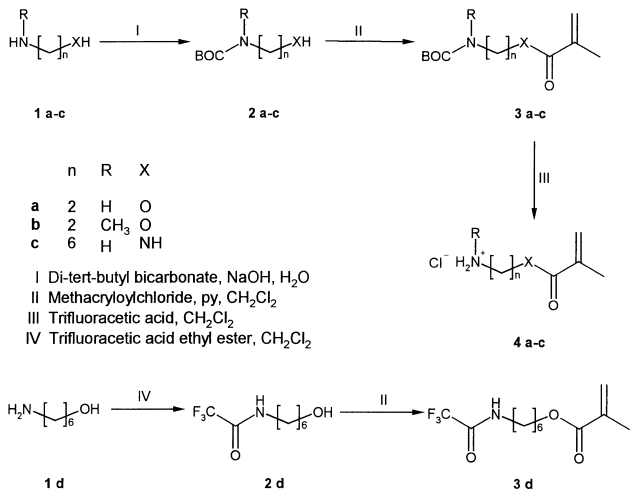

Fig. 1. Synthesis conditions for different aminoalkylmethacrylate comonomers.

Table 1

Results of products **2a-2b**

Product	Yield (%)	R_f	Molecular formula	^{1}H-NMR ($CDCl_3$/300 MHz) δ
2a	84.2	0.36	$C_7H_{13}NO_3$ (175.23)	5.02 (bs, 1H; NH), 3.66 (t, 2H; OCH_2), 3.25 (q, 2H; NCH_2), 2.75 (bs, 1H; OH), 1.42 (m, 9H; $C_3(CH)_3$)
2b	89.4	0.41	$C_8H_{17}NO_3$ (161.20)	4.08 (q, 2H; OCH_2), 3.71 (t, 2H; NCH_2), 2.87 (s, 3H; NCH_3), 1.41 (m, 9H: $C(CH_3)_3$)

*2.2.1. General procedure for the synthesis of N-BOC-aminoalkyl alcohol **2a-2b** (Fig. 1)*

The synthesis was performed according to Mattingly [15]. One equivalent (eq.) of di-tert-butyl dicarbonate was added dropwise to a stirred solution of 1 eq. aminoalkyl alcohol **1a-1c** (Fig. 1) and 1.8 eq. NaOH in water at 0°C. The mixture was allowed to warm up to room temperature (r.t.) and was stirred overnight. The reaction mixture was extracted several times with ethyl acetate and the pooled organic phases were washed with water. The organic phase was dried over Na_2SO_4, and the solvent was evaporated to end up with the N-BOC-aminoalkyl alcohol **2a-2b** as a pale yellow oil, which was pure as determined by thin-layer chromatography (TLC) (Table 1).

*2.2.2. General procedure for the synthesis of N-BOC-aminoalkyl methacrylates **3a-3b** (Fig. 1)*

One eq. pyridine was added to the stirred solution of 1 eq. *N-BOC*-aminoalkyl alcohol in CH2Cl2 (150 ml). The mixture was stirred at r.t. for 1 h, methacryloylchloride was added drop by drop and stirring was continued for another 3 h. The reaction mixture was then extracted with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4), and the solvent was evaporated to yield the N-BOC-aminoalkyl alcohol **3a-3b** (Fig. 1) which was pure, as determined by TLC (Table 2).

*2.2.3. Synthesis of N-BOC-1,6-diaminohexane **2c** (Fig. 1)*

The synthesis of N-BOC-1,6-diaminohexane was performed according to Callahan et al. [16]. and Stahl et al. [17]. 1,6-Diaminohexane **3** (Fig. 1) (100 g, 0.86 mol) was dissolved in CH_2Cl_2 (300 ml) and cooled in an ice bath to 0–3°C. To the stirred solution 0.3 eq. di-tert-butyl bicarbonate (62.6 g, 0.29 mol) was added slowly over a period of 1 h. The reaction was allowed to warm up to r.t., and proceeded overnight. The reaction mixture was extracted with saturated aqueous $NaHCO_3$ (50 ml, three times). The organic phases were pooled, dried, and evaporated under reduced pressure. The resulting oil was dissolved in 200 ml, 1 N HCl and extracted with ether. The aqueous phase was washed with ether, made basic to a pH of 10 with aqueous 2 N NaO_3, and extracted with ethyl acetate. The organic phases were pooled, dried, and evaporated under reduced pressure. The resulting oil was dissolved in 200 ml, 1 N HCL and extracted with ether. The aqueous phase was washed with ether, made basic to a pH of 10 with aqueous 2 N NaOH, and extracted with ethyl acetate. The organic extracts were pooled, dried over Na_2SO_4 and evaporated to give 10.5 g of homogeneous *N-BOC*-1, 6-diaminohexane **2c** (Fig. 1) as a yellow oil which was used without further purification. TLC R_f 0.51; 1H NMR ($CDCL_3$) δ 4.54 (bs, 1H; NH), 3.05 1 2.62 (m, 4H; NCH_2), 1.41 (m, 9H; CH_3), 1.29 (m, 8H; CH_2).

*2.2.4. Synthesis of N-BOC-N′-(2-methacryl)-1,6-diaminohexane **3c** (Fig. 1)*

N-BOC-1,6-diaminohexane **2c** (Fig. 1) (31 g, 0.15 mol) was dissolved in CH_2Cl_2 (200 ml), One eq. Pyridine was added and stirred for 1 h at r.t. To the stirring solution, 1 eq. 2-methacryloylchloride (15.67 g, 0.15 mol) was added dropwise. After 3 h the reaction mixture was washed with saturated aqueous $NaHCO_3$ (50 ml, three times) and water. The organic phase dried over Na_2SO_4 and evaporated under reduced pressure to give 35.9 g (84%) *N-BOC-N′*-(2-methacryl)-1,6-diaminohexane **3c** (Fig. 1). m.p. = 47°C, TLC R_f = 0.76; ^{1}H NMR ($CDCl_3$) δ 5.95/4.55 (bs, 1H; NH), 5.65/5.29 (m, 2H; CH_2), 3.26/3.07 (m, 4H, NCH_2), 1.94 (s, 3H; CH_3), 1.41 (m, 8H; CH_2) 1.32 (m, 8H; CH_2).

*2.2.5. Synthesis of (6-hydroxyhexyl)trifluoroacetamide **2d** (Fig. 1)*

Ethyl trifluoroacetate was slowly added to a solution of 6-amino-1-hexanol (100 g, 0.853 mol) in CH_2Cl_2 (200 ml). The reaction was stirred for 5 h, concentrated in vacuo, and then petrolether was added and the mixture stored at r.t. for 12 h, resulting in the formation of a white precipitate. The precipitate was collected by filtration, washed with petrolether and dried in vacuo to yield 174.6 g (96%) of pure product of 6-hydroxyhexyl)trifluoroacetamide **2d** (Fig. 1). m.p. = 49–50°C; R_f = 0.42 (CH_2Cl_2:EtOH 9:1); ^{1}H NMR ($CDCl_3$) 7.16 (bs, NH), 3.63 (t, J = 7.1 Hz, CH_2OH), 3.34 (q, J = 7.1 Hz, CH_2NH), 1.6-1.4 (m, 8H, CH_2). ^{13}C NMR ($CDCl_3$) 158.14–156.68 (q, CO), 121.58–110.15 (q, CF_3), 62.43 (t, CH_2OH), 39.75 (t, CH_2NH), 32.48/32.25/26.24/25.13 (t, CH_2).

Table 2

Results of products **3a-3b**

Product	Yield (%)	R_f	Molecular formula	^{1}H-NMR ($CDC1_3$/300 MHz) δ
3a	69.5	0.65	$C_{10}H_{19}NO_4$ (229.28)	6.1/5.56 (2H; $=CH_2$), 4.79 (bs, 1H; NH), 4.18 (m, 2H: N-CH_2), 3.41 (m, 2H; O-CH_2), 1.92 (m, 3H; CH_3), 1.42 (m, 9H; $C(CH_3)_3$)
3b	75	0.57	$C_{12}H_{21}NO_4$ (243.30)	6.06/5.52 (m, 2H; $=CH_2$), 4.19 (m, 2H; N-CH_2), 3.44 (m, 2H; O-CH_2), 2.86 (m, 3H; N-CH_3), 1.88 (m, 3H; CH_3), 1.39 (m. 9H: $C(CH_3)_3$)

*2.2.6. Synthesis of 6-(trifluoroacetylamino)hexyl methacrylate **3d** (Fig. 1)*

One hundred grams (0.47 mol) of compound **2d** was dissolved in a mixture of CH_2Cl_2 (200 ml) and pyridine (80 ml). Methacryloylchloride, 68.2 ml (0.7 mol) was added dropwise at r.t. to the mixture and refluxed for 2 h. The cooled reaction mixture was diluted with ether and extracted with $CuSO_4$ solution and saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to give 87.9 g (0.31 mol) 6-(trifluoroacetylamino)hexyl methacrylate **3d** (Fig. 1) as a viscous oil. $R_f = 0.36$ (CH_2Cl_2); ^1H NMR ($CDCl_3$) 6.65 (bs, NH), 6.1/5.6 (s, $=CH_2$), 4.15 (t, 2H; O-CH_2), 3.35 (q, 2H, NCH_2), 1.93 (s, 3H, CH_3), 1.8–1.3 (m, 8H;CH_2). ^{13}C NMR ($CDCl_3$) 167.41/158.14 (C = O), 136.25 (C), 125.18 (CH_2), 121.58–110.2 (CF_3), 64.22 (CH_2O), 39.62 (CH_2NH), 28.66/28.29/26.63/25.31 (CH_2), 18.11 (CH_3). $ESI^+ = 282.1$.

2.2.7. General procedure for the cleavage of the N-BOC-group

N-BOC-aminoalkyl methacrylates **3a-3c** (Fig. 1) were dissolved in CH_2Cl_2 (200 ml) and were ice-cooled to 0–3°C. Ice-cooled 10 eq. trifluoroacetic acid was added dropwise to the stirring solution of the protected acrylates. The mixture was allowed to warm up to r.t. and was stirred for 3 h. The residue was obtained after evaporation and dissolved in water. The aqueous solution was given to a pretreated ion exchange resin (ion exchange resins (IER), see Section 2.3) and was slowly stirred overnight. After filtration of the IER, the aqueous solution was lyophilized to give a white solid substance **4a-4c** (Fig. 1, Table 3).

2.2.8. Pre-treatment of ion exchange resins (IER)

Basic IER (Merck, Darmstadt, Germany) was activated with 2.5 eq. aqueous 6% NaCl. The IER was then washed neutral with deionized water and kept under deionized water until use.

2.3. Analysis of the monomers

NMR spectra were recorded on a Bruker ARX-300 NMR spectrometer; shifts reported are relative to the appropriate internal standard; coupling constants (J) are reported in Hertz and refer to apparent peak multiplicities and not true coupling constants. Abbreviations used are as follows: bs, broad singlet; s, singlet; t, triplet; q, quartet; m, multiplet. The melting points were taken on a Kofler melting apparatus and are uncorrected. TLC was performed using silica gel 60 F_{254} precoated plates (Merck, Darmstadt, Germany); plates were visualized under UV light or by treatment with ninhydrin reagent (0.1% ninhydrin in ethanol). The chromatograms were developed in the top-phase of ^{n}BuOH:H_2O:HOAc 5:4:1 or CH_2Cl_2:EtOH 9:1.

2.4. Preparation of nanoparticles

2.4.1. General preparation procedure

The different methacrylate nanoparticles were produced by free radical polymerization according to Stieneker and Kreuter [18]. The amounts of monomers were calculated as free bases and dissolved in preheated water or in water acetone mixtures at 78°C. At this temperature, a stock solution of ammonium persulfate was added as initiator. The reaction was carried out under stirring on a thermostatic well plate in closed beakers of 100 ml at 400 rpm. After 24 h, when the polymerization was completed, the acetone of the polymerization medium was evaporated for 1 h. The resulting unpurified dispersions (raw dispersions) were used to investigate particle size and surface charge in dependence on the polymerization conditions. For further physical characterization, copolymer nanoparticles were produced from each basic monomer under optimized polymerization conditions, to yield particles with diameters of about 200 nm. The resulting suspensions were concentrated to a polymer content of between 5 and 20% (w/v) with an ultrafiltration unit (model 402, Amicon, Witten, Germany), equipped with a Diaflo YC05 filtration membrane (Amicon) in order to obtain stock suspensions. These stock solutions were purified by dialysis through a semipermeable membrane with an exclusion size of 12 000–14 000 dalton (Dialysis Tubing-Visking, Medicell, London, UK) prior to characterization. For the monomer trimethylammoniumethylmethacrylate (TMAEMC), the resulting nanoparticles were lyophilized after purification (Lyovac GT2, Leybold Heraeus, Hürth, Germany). Resuspending of the lyophilized substance was performed by using an ultrasonication dispenser (Transsonic Digital, Elma, Singen, Germany; 5 min 240 W).

Table 3

Results of products **4a-4c**

Product	Yield (%)	R_f	Molecular formula	^1H-NMR (DMSO/300 MHz) δ	m.p. (°C)	ESI-MS (M+)
4a	100	0.57	$C_6H_{12}NO_2CI$ (165.62)	8.31 (m, 3H; NH_3), 6.2/5.70 (m, 2H; $=CH_2$), 4.23 (m, 2H; NCH_2), 3.43 (m, 3H; OCH_2), 1.87 (s, 3H; CH_3)	84	129.9
4b	100	0.43	$C_7H_{14}NO_2CI$ (179.79)	9.16 (m, 3H; NH_3), 6.2/5.71 (m, 2H; $=CH_2$), 4.27 (m, 2H; NCH_2), 3.19 (m. 2H; CH_2OH), 2.53 (m, 3H; NCH_3), 1.88 (s, 3H; CH_3)	67	144.0
4c	75.6	0.49	$C_{10}H_{20}N_2OCI$ (219.67)	7.97 (m, 3H; NH_3), 5.6/5.26 (m, 2H; $=CH_2$), 3.03 (2m, 4H; NCH_2), 1.8 (s, 3H; CH_3), 1.5-1.1 (m, 8H; CH_2)	70	185.1

2.4.2. Preparation of PMMA homopolymer nanoparticles

The preparation of PMMA nanoparticles was carried out according to following standard protocol: 1% (w/w) of the monomer was dissolved in water at 78°C. A 5% (w/w) stock solution of APS in water was added to give a final concentration of 0.03% (w/w). The resulting nanoparticle suspension was concentrated to a content of 5% (w/w) and dialyzed prior to use.

2.4.3. Preparation of AEMC copolymer nanoparticles

The monomer aminoethylmethacrylate hydrochloride was dissolved in water-acetone mixtures at 78°C as a 33.3% (w/w) stock solution. Methylmethacrylate and a 5% (w/w) APS solution were immediately added. Different batches were prepared by variations within the limits of one of the following reaction conditions.

- The acetone content of the dispersion medium was varied over the range of 0–30% (w/w) while the total monomer content (1% w/w) and the portion of the comonomer (30% w/w) were kept constant.
- Nanoparticles with a variable total monomer content were produced at a constant portion of the basic comonomer (30% w/w) in 10% (w/w) acetone solutions.

Particle characterization of AEMC nanoparticles was carried out with particles produced using the following reaction components: 1.25% (w/w) total monomer, 30% (w/w) comonomer, 0.03% (w/w) APS, and 10% (w/w) acetone.

2.4.4. Preparation of MMAEMC copolymer nanoparticles

The monomer N-monomethylaminoethylmethacrylate hydrochloride **4b** was dissolved as a 33.3% (w/w) stock solution in water-acetone mixtures (10% w/w acetone) at 78°C. Methylmethacrylate and APS were immediately added. Different batches were prepared by variation of the following reaction conditions.

- Nanoparticles with a variable total monomer content were produced at a constant portion of the basic comonomer (30% w/w).
- The portion of the basic comonomer was varied between 0 and 50% (w/w).
- The concentration of the initiator APS was varied between 0.01 and 1% (w/w).

MMAEMC nanoparticles used for physical characterization were produced using the following reaction components: 1% (w/w) total monomer, 30% (w/w) comonomer, 0.03% (w/w) APS, and 10% (w/w) acetone.

2.4.5. Preparation of DMAEMC nanoparticles

In contrast to the standard procedure, polymerization of dimethylaminoethylmethacrylate was carried out at basic pH using deionized water adjusted to pH 11–12. At a temperature of 78°C, methylmethacrylate and the basic monomer DMAEMC were added to obtain a final total monomer content of 1% (w/w) consisting of 30% (w/w) of the basic monomer. The APS initiator concentration was adjusted to 0.03% (w/w). The resulting suspension was immediately dialyzed and concentrated to a polymer content of 12.7% (w/w).

2.4.6. Preparation of TMAEMC nanoparticles

The monomer trimethylaminoethylmethacrylate chloride (50% w/w aqueous stock solution) was dissolved in demineralized water at 78°C. Methylmethacrylate and a 5% (w/w) APS solution were immediately added. The total monomer concentration was 3% (w/w), the concentration of the basic comonomer was 30% (w/w), and the initiator used was adjusted to 0.03% (w/w). The suspensions were purified by dialysis and subsequently freeze-dried.

2.4.7. Preparation of AHMAC nanoparticles

Aminohexylmethacrylamide hydrochloride **4c**, methylmethacrylate, and APS were dissolved in a 13% (w/w) acetone solution to obtain a final total monomer content of 1.18% (w/w) and an APS concentration of 0.03% (w/w). The portion of the basic monomer was varied between 29 and 49% (w/w). This portion was fixed at 32% (w/w) for pH-profile measurements of the particles.

2.4.8. Preparation of AHMC nanoparticles

AHMC nanoparticles were prepared by polymerization in a 37% (w/w) aqueous acetone solution, followed by organic solvent evaporation. The basic modified comonomer exhibiting a protected amino group (trifluoroacetylaminoethylmethacrylate **3d**) and methylmethacrylate were copolymerized using 0.03% (w/w) APS as initiator. After the acetone was evaporated, the particles were purified by dialysis for the subsequent particle size and zeta potential determinations. Afterwards, the amino groups were deprotected in a 12.5% ammonium hydroxide solution by autoclaving at 2 bar and 121°C for 1 h. Finally, the deprotected AHMC nanoparticles were purified by dialysis.

2.5. Size determination of nanoparticles

The particle diameters were measured by photon correlation spectroscopy (PCS). The PCS instrument consisted of a BI-200 SM Goniometer Ver. 2.0 (Brookhaven Instruments, Holtsville, NY), equipped with a 30 mW He-Ne-Laser (Melles Griot, Cincinnati, CA), and was connected to a BI-2030 AT Digital Correlator (Brookhaven). The measurements were carried out at a scattering angle of 90° and a temperature of 25°C. The count rate was adjusted to 20–30 kHz by diluting each sample with deionized, filtered water (0.22 μm cellulose nitrate filter, Schleicher&Schuell, Dassel, Germany) and sodium chloride solutions of pH values between 3 and 12. The samples were prepared by adjusting the pH using 0.01 or 0.1 M hydrochloric acid as well as sodium hydroxide

2.6. Surface charge

The surface charge (zeta potential) was determined by measuring the elecrophoretic mobility of the nanoparticles using a Lazer Zee Meter™ Model 501 (PenKem, Bedford Hills, NY). The polymer content of the nanoparticle suspensions was calculated by the determination of the drying weight loss. Samples were afterwards adjusted to a final concentration of 100 μg/ml by diluting the suspensions with deionized water and the sodium chloride solutions of different pH values, respectively. The measured zeta potential was calculated and corrected for a standard reference temperature of 20°C.

3. Results and discussion

The preparation of the methacrylate copolymer nanoparticles was performed by a free radical polymerization process in the presence of APS as an initiating reagent using the monomers shown in Fig. 2. The polymerization process followed an earlier described method [1,14,19]. A mixture consisting of water and acetone was preferentially employed as polymerization medium, because this composition led to decreased particle diameters of the most copolymer derivatives. The portion of the basic comonomer, the total amount of monomer, the concentration of the redox initiator APS, and the acetone content of the polymerization medium were varied, to characterize the influence of the different reaction conditions.

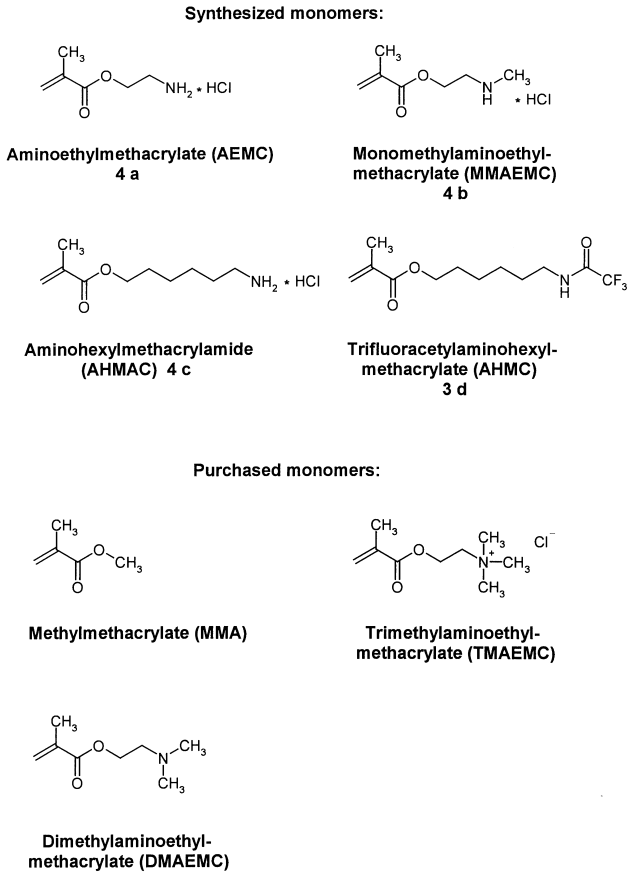

Fig. 2. Chemical structure of the monomers used for the preparation of the aminoalkylmethacrylat copolymer nanoparticles.

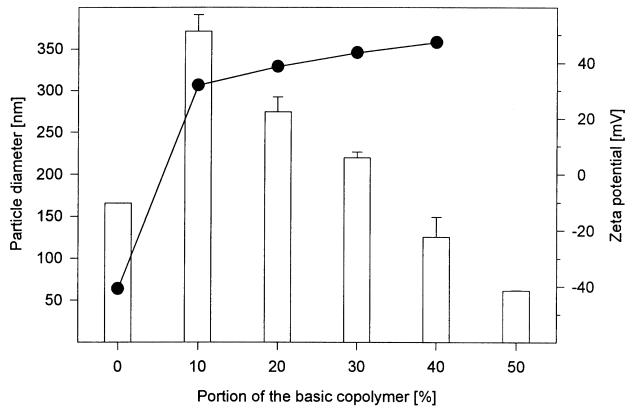

Fig. 3. Particle diameter and zeta potential of aminoalkylmethacrylate copolymer nanoparticles versus the content of the basic copolymer. White bars: diameter of MMAEMC nanoparticles (mean ± SD, $n = 4$). ●: Zeta potential of MMAEMC nanoparticles.

3.1. Influence of basic monomer concentration

Size and surface charge of the resulting nanoparticles were found to be dependent on the portion of the basic monomer (Fig. 3). Polymerization of pure methylmethacrylate resulted in stable latices with an average particle size of about 160 nm. The homopolymer nanoparticles are prevented from aggregation by repulsion forces generated by charged initiator molecules which are incorporated into the polymeric matrix during the polymerization process [20]. Consequently, the homopolymer PMMA nanoparticles showed a strong negative zeta potential of about −45 mV. The addition of 10% basic monomer inverted the surface charge and nanoparticles with positive zeta potential were obtained. While increasing the portions of the basic monomer up to 50% (w/w) the zeta potential was raised to $>$ +45 mV and, consequently, the particle diameter was reduced to $<$70 nm. In an earlier study, corresponding results were obtained by addition of the negative derivatives methacrylic acid [21] and sulfopropylmethacrylate [10] also resulting in stronger repulsion forces and, therefore, smaller particle diameters. The charged substituents of the copolymer preferentially concentrate on the particle surface, because of their relative hydrophilicity compared with the more hydrophobic nature of the acrylic polymer portions. This increase in charged surface groups with increasing amounts of the basic monomer leads to smaller particle sizes due to two factors: increasing surface hydrophilicity and increasing surface charge repulsion.

Aminohexylmethacrylate copolymer nanoparticles were produced according to a different procedure. Due to their instability and their tendency to spontaneous polymeriza-

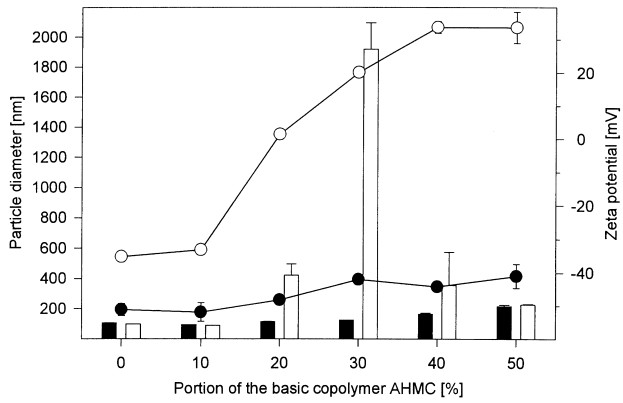

Fig. 4. Influence of the comonomer concentration on the particle diameter and zeta potential of aminohexylmethacrylate copolymer nanoparticles (AHMC). Black bars: diameter of the N-protected particles; white bars: diameter of the deprotected particles. ●: Zeta potential of the N-protected nanoparticles. ○: Zeta potential of the deprotected nanoparticles (mean ± SD, $n = 4$).

tion, the synthesis of the monomer had to be carried out by reaction of the N-trifluoroacetyl protected amino alcohol and methacryloyl chloride. As a consequence, the resulting trifluoroacetyl aminohexylmethacrylate copolymer nanoparticles had a much more hydrophobic character and the compound was more uniformly distributed in the nanoparticle matrix. Hence, an influence of the copolymer content on the surface charge or on the particle diameters was not detectable (Fig. 4). The synthesis of the nanoparticles was followed by the deprotection of the aminohexyl groups using a basic environment. After the purification by dialysis, the deprotected free amino groups in the final copolymer nanoparticles were protonated at neutral pH, which led to more positive surface charges that correlated to the portion of the basic copolymer. Contents of 20–30% (w/w) copolymer led to neutral surface charges. As a consequence, these particles tend to aggregate resulting in larger particle diameters (>1800 nm). At a copolymer portion above 40% (w/w) particles were charged positively. This charge prevented the growing particles from aggregation during the polymerization process by strong repulsion forces. Thus, stable suspension with diameters below 300 nm were obtained.

The monomers AEMC **4a**, MMAEMC **4b** and AHMAC **4c** were employed for polymerization in the form of hydrochlorides. Their dissociation led to a pH-decrease of the polymerization medium. Non-dialyzed dispersions varied between pH 2 and 3. The protonization of the amino groups led to strong positive surface charges (40% MMAEMC: +45 mV). At lower MMAEMC monomer portions the repulsion forces between the polymers decreased resulting in larger final nanoparticle diameters. At higher contents of the cationic derivative, the growing polymer chains were protected from aggregation by strong positive electrostatic forces and a larger amount of particles with small diameters was obtained. This was indicated by a decrease in turbidity of the resulting suspensions and the occurrence of a bluish shine. The further addition of the hydrophilic monomer above concentrations of 50% (w/w) led to water soluble non-particulate polymer molecules. For AEMC and MMAEMC nanoparticles pH-values also were determined after the purification by dialysis. Residual monomers, acetone and hydrochloric acid were removed by this procedure. The dialyzed particles possessed an increased pH that correlated with their copolymer content.

3.2. Influence of the total monomer concentration

Increasing monomer concentration resulted in larger particle diameters (Fig. 5). The results of the present study are in concordance with the outcome of earlier studies [1,8,10, 19,22,23]. According to Fitch [20], the number of the generated radicals remains constant at a definite temperature and concentration of the initiator APS. Additional monomer does not influence the number of radicals but leads to the increase of the molecular weight of the formed particles. In one of the previous studies, a strong correlation between particle diameters and molecular weights was observed for sulfopropyl-methacrylate copolymer nanoparticles [10]. Hence, it is very likely, that also in our study the increased particle diameters at higher monomer concentrations can be attributed to increasing molecular weights of the copolymer nanoparticles.

Additionally, higher zeta potentials of the suspensions were observed. With raising monomer concentration, the hydrophilic aminoalkyl groups accumulated at the interface between the growing particles and the dispersion medium, while the apolar methoxygroups of the MMA monomer were intruded into the polymeric matrix.

3.3. Influence of the polymerization medium

The dependence of the physical properties of aminoalkylmethacrylate copolymer nanoparticles on the

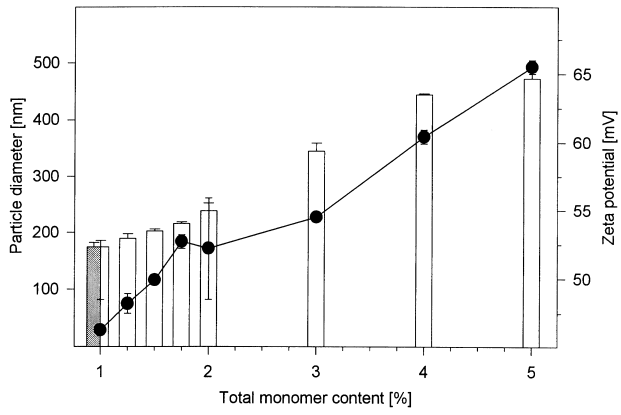

Fig. 5. Effect of the total monomer content on the diameter and the zeta potential of the resulting aminoethylmethacrylate copolymer nanoparticles (AEMC). White bars: diameter of the AEMC copolymer nanoparticles. Shaded bar: diameter of PMMA homopolymer nanoparticles. ●: Zeta potential of AEMC copolymer particles (mean ± SD, $n = 4$).

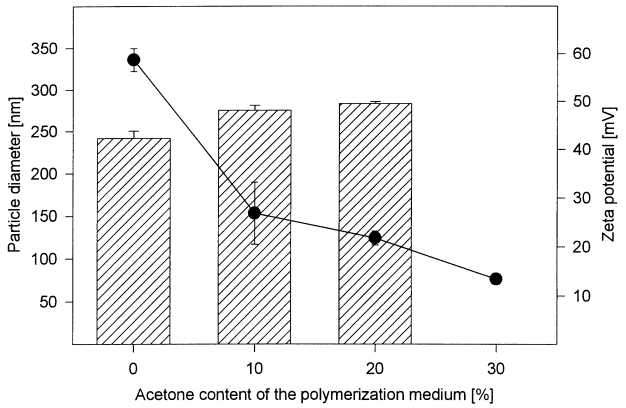

Fig. 6. Influence of the polymerization medium composition on the size and zeta potential of the resulting aminoethylmethacrylate (AEMC) copolymer nanoparticles. ●: Particle diameters, bars represent the zeta potential of the nanoparticle suspensions (mean ± SD, $n = 4$).

employed polymerization medium is shown in Fig. 6. Increasing acetone contents led to decreasing particle sizes. Due to their charge-to-mass ratio, the smaller nanoparticles possessed higher surface potentials. The zeta potential of nanoparticles synthesized with 30% (w/w) cosolvent could not be determined because of their small diameters. The observation can be explained by the theory proposed by Kreuter [1] for the termination of the polymer chain growth reaction. Colloidal macroradicals coagulate prior to termination. According to that theory, the chain termination by the capture of a macroradical in the coagulated monomer is more likely to occur than the termination by a water-soluble solvatized smaller radical. In the present study, the solubility of the monomer was raised by an increasing acetone content. Consequently, the resulting growing polymer chains remained in solution for a longer period of time, before the coagulation to nanoparticles occurred. At higher acetone concentrations (30% w/w), particles were formed after the evaporation of acetone. In this case, the termination of the polymer chain growth took place before phase separation and formation of solid nanoparticles.

3.4. Influence of the polymerization initiator

The concentration of APS used as radical initiator during the emulsion polymerization is another important parameter which influences the particle diameter of the resulting suspension. Larger particle diameters were determined for MMAEMC copolymer nanoparticles by raising the APS concentration from 0.01 towards 0.03% (w/w) (Table 4). At a constant content of co- and total monomer the further increase of the redox initiator concentration led to an uncontrollable polymerization process, resulting in polymer flocules in addition to nanoparticles. The polydispersity indices reported in Table 4 indicated the reduced dispersity of these systems.

Table 4

Influence of the APS concentration on the physical properties of MMAEMC copolymer nanoparticles (mean ± SD, $n = 4$).

APS concentration (% w/w)	Diameter (nm)	Polydispersity index	Observation
0.01	137.0 ± 3.5	0.0408 ± 0.00289	Homogeneous suspension
0.03	244.1 ± 7.2	0.0934 ± 0.0173	Homogeneous suspension
0.05	633.8 ± 231.4	0.4645 ± 0.0764	Agglomeration
0.10	1314.7 ± 191.0	0.7072 ± 0.1133	Agglomeration

3.5. Monitoring of particle size

In a further experiment, the compensation of the opposing effects of increasing acetone and total monomer content was investigated, with the objective to yield small particle diameters and high surface charges. The portion of the basic monomer was kept constant at 30% (w/w) for all preparations. The mean diameters of AEMC nanoparticles increased from 175 towards 475nm by raising the total monomer content from 1 to 5% (w/w) during polymerization in a 10% (w/w) acetone solution (Table 5). Simultaneously, the zeta potential was increased from +46 towards +66 mV. Raising the acetone content of the polymerization medium up to 30% (w/w) at a definite total monomer concentration (1% w/w) led to a decrease in particle diameters from 175 to 76 nm. Summarizing these effects, small diameters (131 nm) and high positive surface charges (+59 mV) were obtained by the combination of higher acetone and higher total monomer content.

3.6. Optimization of particle preparation and storage conditions

In order to compare the different aminoalkylmethacrylate nanoparticles with regard to their size and surface charge at different pH-values, each polymer was produced under optimized polymerization conditions to obtain particles of about 200 nm. The content of the basic monomers was kept constant at 30% (w/w). The yield of the polymerization process was determined by calculating the mass relation between the monomers used in the reaction and the dried polymer after purification by dialysis. For each particle species, the

Table 5

Variation of different polymerization conditions for the preparation of AEMC copolymer nanoparticles (mean ± SD, $n = 4$)

Total monomer content (%)	Acetone content (%)	Diameter (nm)	Zeta potential (mV)
1	10	174.6 ± 11.2	46.3 ± 2.2
5	10	473.3 ± 24.6	65.5 ± 0.5
1	30	76.2 ± 1.5	Not available
5	30	130.7 ± 0.8	59.6 ± 1.1

yield of the reaction was found to be more than 85%. All undialyzed suspensions were stable for several months, no sedimentation or aggregation occurred. Due to their strong permanent positive surface charge, TMAEMC nanoparticles could be lyophilized after purification and were easily redispersed in purified water by sonication. AEMC-, MMAEMC-, TMAEMC-, and AHMAC-nanoparticles should be stored as concentrated raw dispersions without further purification because of the higher zeta potential of the particles in acidic media. The storage of AHMC- and DMAEMC-nanoparticles as unpurified suspensions was not suitable due to their polymerization in a basic environment or the elimination of the N-protecting group under basic conditions. These particles were immediately dialyzed after preparation, to avoid a basic ester hydrolysis and were stored as purified suspensions.

3.7. pH-profile of the copolymer nanoparticles

Physical characterization of the different particle species was performed at different pH-values to explore the influence of the functional groups located on their surfaces. Hydrochloric acid and sodium hydroxide solution were added to a 0.01 molar sodium chloride solution in order to reduce electrolyte effects. The pH-profiles of the different particles are shown in Fig. 7. Pure PMMA homopolymer nanoparticles possessed no chargeable functional groups on their surface. As a consequence, negative zeta potentials

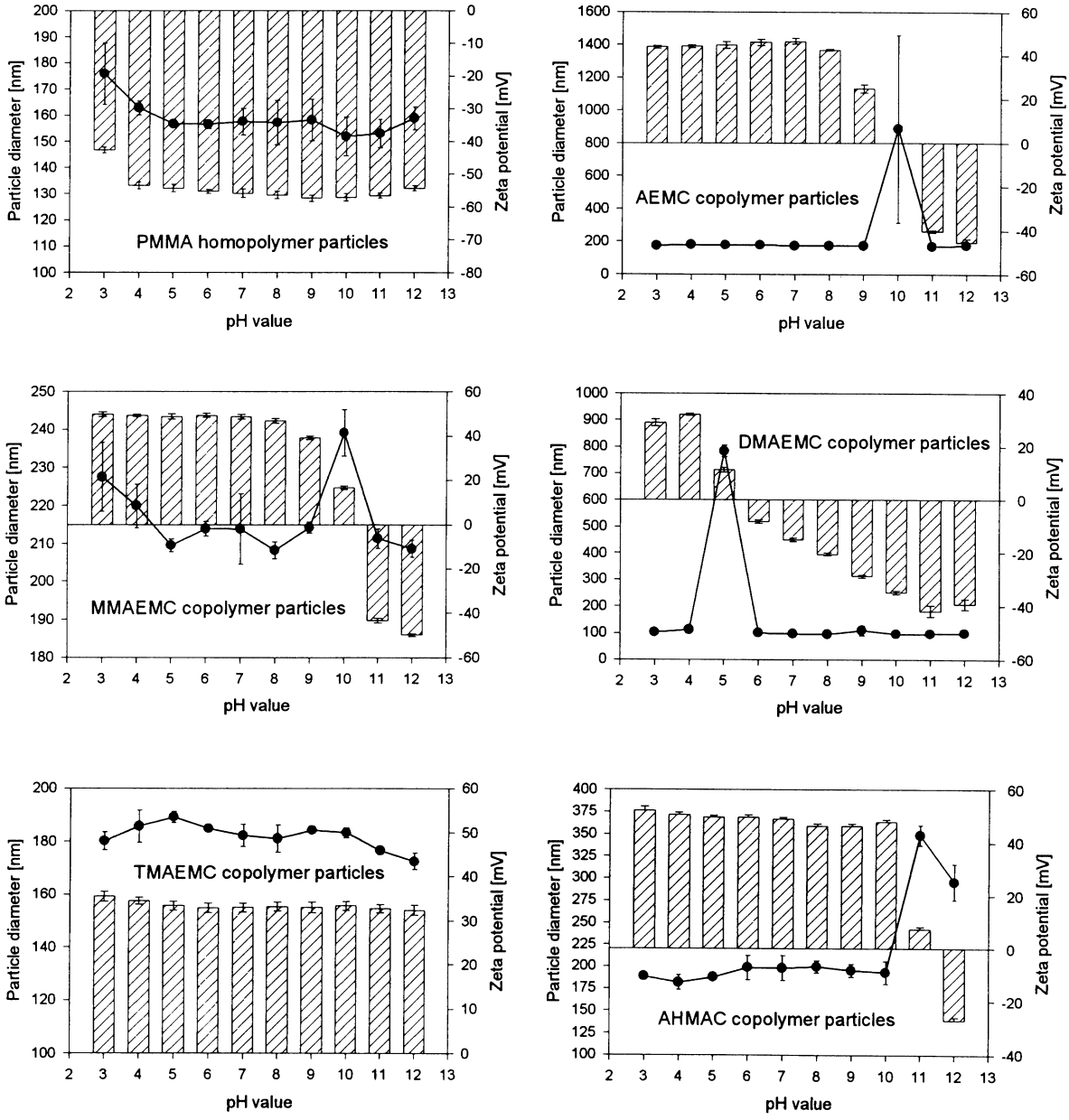

Fig. 7. pH profiles of different aminoalkylmethacrylate nanoparticles. Effect of pH on the diameter and the zeta potential of the copolymer nanoparticles. ●: Particle diameters, bars represent the zeta potential of the nanoparticle suspensions (mean ± SD, $n = 3$).

that are typical for net neutral polymer particles were measured over the whole pH-range. TMAEMC nanoparticles showed a positive surface charge at every pH-value. However, because of their nearly constant surface charge, no dependence of the resulting particle size on the final pH of the dispersions was observed. The other aminoalkylmethacrylates exhibited a characteristic inversion point at which the positive surface charge turned to negative. At this pH-value, the suspensions possessed minimal positive surface charges and minimized electrostatic repulsion forces leading to nanoparticle aggregation. The aggregates were responsible for large mean particle diameters measured by PCS. The inversion points correlated to the basicity of the different particles, which were dependent on the substitution of the surface located amino groups. Due to the steric hindrance, the alkyl groups prevented the protonization of the amino groups. Protonization of the N-dimethylaminoethyl group in DMAEMC nanoparticles only was possible at pH-values below 5, leading to strong repulsions forces by positive zeta potentials and, consequently, to stable dispersions. DMAEMC nanoparticles showed strong negative surface charges above pH 6 resulting in decreased particle diameters. The negative repulsion forces originated from entrapped charged redox initiator molecules. Hence, these pH-sensitive particles might not be suitable for an adsorption of anionic drugs under physiological conditions. MMAEMC and AHMAC nanoparticles exhibited a higher basicity than unsubstituted aminoethylmethacrylate (AEMC) particles, due to the positive inductive effect of additional alkyl groups and extended alkyl chains. AEMC, MMAEMC, and AHMAC nanoparticles possessed a positive surface charge even at neutral pH and should enable the adsorption of anionic drugs under physiological conditions.

4. Conclusions

In conclusion, the copolymerization of different aminoalkylmethacrylates with methylmethacrylate resulted in stable nanoparticle suspensions. The particle diameters and the surface charges of the suspensions could be influenced by different polymerization conditions. Each nanoparticle species was reproducibly obtained by specific polymerization conditions. Due to their positive surface charge, the copolymer nanoparticles described, may be useful as colloidal carriers for hydrophilic anionic drugs, as well as oligonucleotides and DNA. Drug binding onto the cationic nanoparticles can be achieved by the formation of ion-pairs, and the subsequent release by ion-exchange. Studies concerning the loading behavior of drugs and the cytotoxicity of these differently substituted carriers will be described in a second publication in this journal.

A remaining problem is that of the biodegradability of the polymer. A similar nanoparticles polymer, polymethylmethacrylate, was shown to be very slowly biodegradable [24,25]. It is possible that the more hydrophilic particles used here, may be more rapidly biodegradable. Nevertheless, these particles anyway, may be useful for the delivery of essential drugs and of genetic material that are required only in small amounts.

Acknowledgements

We are grateful to Anja Hildebrand and Christina Spanheimer for their expert technical assistance. Also, we gratefully acknowledge Hüls, Marl (Germany) for the donation of the monomer trimethylaminoethylmethacrylate chloride.

References

[1] J. Kreuter, Evaluation of nanoparticles as drug-delivery systems. I. Preparation methods, Pharm Acta Helvetiae 58 (1983) 196–209.
[2] J. Kreuter, P.P. Speiser, New adjuvants on a polymethylmethacrylate base, Infect. Immun. 13 (1976) 204–210.
[3] P. Couvreur, M. Roland, P. Speiser, Biodegradable submicroscopic particles containing a biologically active substance and compositions containing them, U.S. Patent 4 (1982) 329–332.
[4] J. Kreuter, E. Liehl, U. Berg, M. Soliva, P.P. Speiser, Influence of hydrophobicity on the adjuvant effect of particulate polymeric adjuvants, Vaccine 6 (1988) 253–256.
[5] S.J. Douglas, L. Illum, S.S. Davis, Poly(butyl 2-cyanoacrylate) Nanoparticles with differing surface charges, J. Control. Rel. 3 (1986) 15–23.
[6] W.M. Bertling, M. Gareis, V. Paspaleeva, A. Zimmer, J. Kreuter, E. Nürnberg, P. Harrer, Use of liposomes, viral capsids, and nanoparticles as DNA carriers, J. Biotech. Appl. Biochem. 13 (1991) 390–405.
[7] H.-P. Zobel, J. Kreuter, D. Werner, C.R. Noe, G. Kümel, A. Zimmer, Cationic polyhexylcyanoacrylate nanoparticles as carriers for antisense oligonucleotides, Antisense Nucl. Acid Drug Dev. 7 (1997) 483–493.
[8] A. Rembaum, S.P.S. Yen, Synthesis and reactions of hydrophilic functional microspheres for immunological studies, J. Macromol. Sci. Chem. A 13 (1979) 603–632.
[9] G. Lukowski, R.H. Müller, B.W. Müller, M. Dittgen, Acrylic acid copolymer nanoparticles for drug delivery: I. Characterization of the surface properties relevant for in vivo organ distribution, Int. J. Pharm. 84 (1992) 23–31.
[10] K. Langer, C. Marburger, A. Berthold, J. Kreuter, F. Stieneker, Methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles for drug delivery. Part I: preparation and physicochemical characterization, Int. J. Pharm. 137 (1996) 67–74.
[11] K. Langer, F. Stieneker, G. Lambrecht, E. Mutschler, J. Kreuter, Methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles for drug delivery. Part II: arecaidine propargyl ester and pilocarpine loading and in vitro release, Int. J. Pharm. (1997), in press.
[12] J.Y. Cherng, P. van de Wetering, H. Talsma, D.J. Crommelin, W.E. Hennink, Effect of size and serum proteins on transfection efficiency of poly ((2-dimethylamino)ethyl methacrylate)-plasmid nanoparticles, Pharm. Res. 13 (1996) 1038–1042.
[13] A. Denizli, M. Kiremitci, E. Piskin, Nonspecific adsorption and covalent coupling of heparin on polyacrylate based microbeads, Biomater. Artif. Cells Immobil. Biotech. 21 (1993) 183–198.
[14] F. Hoffmann, J. Cinatl Jr., , H. Kabicova, J. Cinatl, J. Kreuter, F. Stieneker, Preparation, characterization and cytotoxicity of methylmethacrylate copolymer nanoparticles with a permanent positive surface charge, Int. J. Pharm. 157 (1997) 189–198.
[15] P.G. Mattingly, Mono-protected diamines. N^{α}(-tert-butoxycarbonyl

α, ω-alkanediamine hydrochlorides from amino alcohols, Synthesis (1990) 366–368.
[16] J.F. Callahan, D. Ashton-Shue, H.G. Bryan, W.M. Bryan, G.D. Heckmann, L.B. Kinter, J.E. McDonald, M.L. Moore, D.B. Schmidt, J.S. Silvestri, F.L. Stassen, S. Lynn, N.C.F. Yim, W.F. Huffmann, Structure-activity relationships of novel vasopressin antagonists containing C-terminal diaminoalkanes and (aminoalkyl)guanidines, J. Med. Chem. 32 (1989) 391–396.
[17] G.L. Stahl, R. Walter, C.W. Smith, General procedure for the synthesis of mono-N-acylated 1,6- diaminohexane, J. Org. Chem. 43 (1978) 2285–2286.
[18] F. Stieneker, J. Kreuter, Nanoparticles as a potential antigen delivery system, in: J.L. Cleland, R. Langer (Eds.), Formulation and Delivery of Proteins and Peptides. ACS Symposium Series 567, American Chemical Society, Washington, 1994, pp. 306–321.
[19] U.E. Berg, J. Kreuter, P.P. Speiser, M. Soliva, Herstellung und in-vitro-prüfung von polymeren adjuvantien für impfstoffe, Pharm. Ind. 48 (1986) 75–79.
[20] R.M. Fitch, M.B. Prenosil, K.J. Sprick, The mechanism of particle formation in polymer hydrosols. I. Kinetics of aqueous polymerization of methyl methacrylate, J. Poly. Sci. 27 (1969) 95–118.
[21] A. Rembaum, S.P. Yen, E. Cheong, S. Wallace, R.S. Molday, I.L. Gordon, W.J. Dreyer, Functional polymeric microspheres based on 2-hydroxyethyl methacrylate for immunochemical studies, Macromolecules 9 (1976) 328–336.
[22] V. Bentele, U.E. Berg, J. Kreuter, Molecular weights of poly(methylmethacrylate) nanoparticles, Int. J. Pharm. 13 (1983) 109–113.
[23] R.S. Molday, W.J. Dreyer, A. Rembaum, S.P. Yen, New immunolatex spheres: visual markers of antigens on lymphocytes for scanning electron microscopy, J. Cell Biol. 64 (1975) 75–88.
[24] J. Kreuter, M. Nefzger, E. Liehl, R. Czok, R. Vogues, Distribution and elimination of poly(methyl methacrylate) nanoparticles after subcutaneous administration to rats, J. Pharm. Sci. 72 (1983) 1146–1149.
[25] M. Nefzger, J. Kreuter, R. Vogues, R. E Liehl, Czok, Distribution and elimination of polymethyl methacrylate nanoparticles after peroral administration to rats, J. Pharm. Sci. 73 (1984) 1309–1311.