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Preparation, characterization and cytotoxicity of methylmethacrylate copolymer nanoparticles with a permanent positive surface charge

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Abstract

Methylmethacrylate copolymer nanoparticles containing different cationic comonomers such as *N*-trimethylammoniumethylmethacrylate (TMAEMC), *N*-dimethylammoniumethylmethacrylate (DMAEMC), *N*-trimethylammoniumpropylmethylacrylamide (MAPTAC) or the anionic comonomer sulfopropylmethacrylate (SPM), respectively, were prepared by free radical polymerization. Particle size was determined by photon correlation spectroscopy (PCS), transmission and scanning electron microscopy (TEM, SEM), and surface charge by microelectrophoresis. Pure poly(methylmethacrylate) nanoparticles served as control. Depending on the method, mean diameters of permanently positively-charged nanoparticles MMA-TMAEMC and MMA-MAPTAC were 243 or 207 nm (PCS), 161 or 201 nm (TEM), and 158 or 197 nm (SEM), respectively. Zeta potential examined in demineralized water or NaCl solution was $+63.4$ or $+32.1$ mV for MMA-TMAEMC nanoparticles and $+49.2$ or $+32.0$ mV for MMA-MAPTAC nanoparticles, respectively. Cytotoxicity of nanoparticles was determined by MTT assay in three different cell cultures including human foreskin fibroblasts (HFF) and two monkey kidney cell lines MA-104 and Vero. Cell viability profiles of TMAEMC and MAPTAC containing nanoparticles were different, showing IC_{50} values for MMA-TMAEMC nanoparticles of 189.6 ± 11.4 μg/ml (MA-104), 110.9 ± 3.1 μg/ml (Vero) and 27.2 ± 4.0 μg/ml (HFF). Cell viability at maximum concentration of 500 μg/ml MMA-MAPTAC nanoparticles was 98.3% (Vero), 85.7% (MA-104), or 94.0% (HFF), respectively. © 1997 Elsevier Science B.V.

Abbreviations: DMAEMC, *N*-dimethylammoniumethylmethacrylate; MAPTAC, *N*-trimethylammoniumpropylmethylacrylamide; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; SPM, Sulfopropylmethacrylate; TMAEMC, *N*-trimethylammoniumethylmethacrylate.

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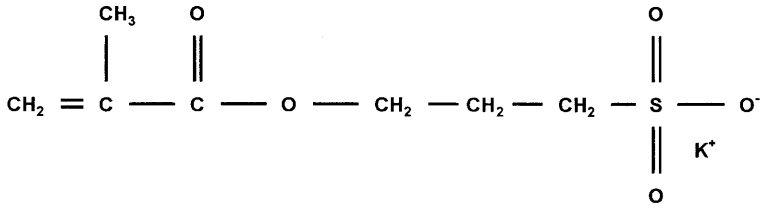
1. Introduction

Acrylic acid derivatives such as poly(alkylcyanoacrylates) and poly(methylmethacrylates) are the most frequently employed polymers for the preparation of nanoparticles (Kreuter and Speiser, 1976; Couvreur et al., 1982; Kreuter, 1983, 1991). Because of their hydrophobicity such nanoparticles showed only poor adsorption properties for hydrophilic, ionic drugs (Harmia et al., 1986). This problem was overcome by the employment of copolymers made of different acrylic acid derivatives (Rolland et al., 1986; Kreuter et al., 1988). Similar copolymer nanoparticles made of acrylic amide, acrylic acid, acrylic acid butylester and styrene showed a strong dependency between pH value and surface charge (Dittgen et al., 1988). These results induced the development of copolymer nanoparticles consisting of methylmethacrylate (MMA) and the strong acid sulfopropylmethacrylate (SPM) (Langer et al., 1996). MMA-SPM copolymer nanoparticles possessed a non-pH dependent negative surface charge between pH 3–9. Adsorption studies with the cationic, hydrophilic model drug pilocarpine (Langer, 1996) revealed a higher loading capacity compared to simple poly(methylmethacrylate) (PMMA) nanoparticles. Although considerable work was performed for the development of a number of new methacrylic carrier systems with enhanced binding properties for hydrophilic drugs, very little information is available about cationic methacrylic carrier systems.

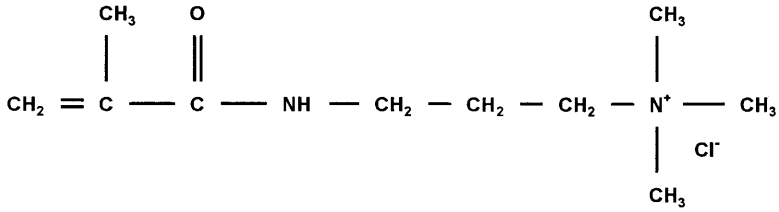
In the present study, the preparation of three copolymer nanoparticle specimen consisting of the monomer methylmethacrylate (MMA) and the comonomer *N*-trimethylammoniumethylmethacrylate (TMAEMC), *N*-trimethylammoniumpropylmethylacrylamide (MAPTAC) or *N*-dimethylammoniumethylmethacrylate (DMAEMC) is described. Since TMAEMC and MAPTAC comonomers include a trimethylammonium group (Fig. 1) the resulting particles possess a positive surface charge which is not pH-dependent over a large range. In contrast to these particles the surface charge of DMAEMC copolymer nanoparticles depends on the pH value of the surrounding medium. The nanoparticles were characterized by the measurement of particle size and surface charge and were compared to MMA-SPM copolymer and PMMA nanoparticles.

The evaluation of drug carriers also has to focus on the cytotoxicity of such systems. Other cationic carriers such as the polycations protamine, poly-L-lysine, and histone showed toxic effects in various cell culture systems, whereas cationized bovine serum albumin and diethylaminoethyl-dextran, exhibited only little cytotoxicity (Choksakulnimitr et al., 1995). Various studies described the preparation of positively-charged liposomes (Campbell, 1983; Felgner et al., 1987; Ballas et al., 1988; Pinnaduwage et al., 1989). The positive charge was achieved by introduction of a quaternary ammonium group. Felgner et al. (1987) reported that vesicles containing cationic lipids such as *N*-(1-(2,3-dioleyloxy)propyl)-*N*,*N*,*N*-trimethylammonium chloride could induce cytotoxic effects. Further studies showed that these effects were attributable to the presence of the cationic detergent, that caused a disruption-solubilization of cell membranes (Lappalainen et al., 1994). These findings were supported by a study examining the mechanisms of toxicity of the cationic detergent cetyltrimethylammonium bromide (Bragadin and Dellantone, 1996). In contrast, PMMA nanoparticles showed no cytotoxicity in rat hepatocytes as shown by electron microscopy and lactate dehydrogenase assay (Rolland et al., 1989). Furthermore no toxic effects were observed in vivo after i.v. and i.m. administration in rats and mice (Kreuter et al., 1979). Eye drop application of MMA-SPM copolymer nanoparticles did not show any macroscopic irritations or inflammations of the rabbit eye (Langer, 1996).

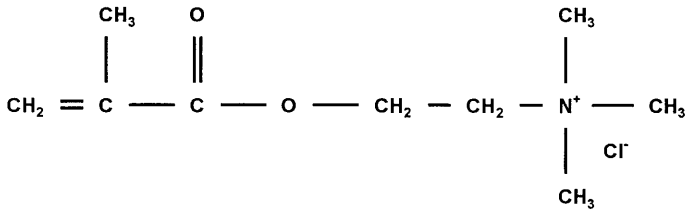
Sulfopropylmethacrylate (SPM), potassium salt



N-trimethylammoniumpropyl-methylacrylamide (MAPTAC), chloride



N-trimethylammoniumethyl-methacrylate (TMAEMC), chloride



N-dimethylammoniumethyl-methacrylate (DMAEMC), hydrochloride

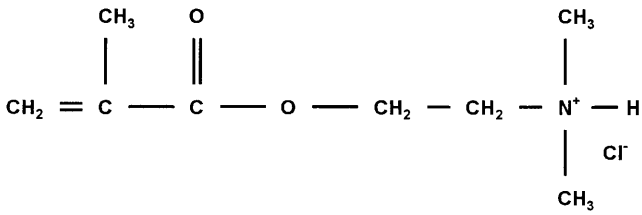


Fig. 1. Chemical structure of different comonomers used for preparation of MMA copolymer nanoparticles.

In the present study, MMA-TMAEMC, MMA-MAPTAC, MMA-DMAEMC, MMA-SPM copolymer and PMMA nanoparticles were examined in three different cell cultures in order to determine their cytotoxicity.

The aim of this work was the development and evaluation of copolymer nanoparticles exhibiting the properties of anion-exchange resins. Nanoparticles with a permanently positive surface charge might represent a suitable carrier system for hydrophilic, anionic salts of drugs at physiological pH values.

2. Materials and methods

2.1. Reagents and chemicals

Methylmethacrylate (MMA) (Merck-Schuchardt, Hohenbrunn, Germany), *N*-trimethylammoniumpropylmethylacrylamide chloride (MAPTAC), *N*-trimethyl-ammoniumethylmethacrylate chloride (TMAEMC), *N*-dimethylammoniumethylmethacrylate hydrochloride (DMAEMC), and sulfopropylmethacrylate potassium salt (SPM) (Hüls, Marl, Germany) were used as monomers.

Ammonium persulfate (APS) was purchased from Hüls (Marl, Germany). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was obtained from Serva (Heidelberg, Germany), dimethylformamide (DMF) from Merck (Darmstadt, Germany) and sodium dodecyl sulfate (SDS) from ICN (Meckenheim, Germany). Demineralized water was prepared by passage over an ionic exchange resin column. Formvar was purchased from Agar (Essex, UK) and copper grids from TESLA Brno (Prague, Czech Republic).

2.2. Cell cultures

Cultures of human foreskin fibroblasts (HFF) were established in our laboratory. The embryonic african green monkey kidney cell line MA-104 was purchased from Serva (Heidelberg, Germany) and the monkey kidney cell line Vero was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in Eagle's minimal essential medium (MEM, Seromed, Berlin) supplemented with 10% fetal bovine serum (FBS, Seromed) and subcultured at 5-day intervals at a seeding density of $2 \times 10^4/cm^2$. All cells were routinely tested for mycoplasmae by the Hoechst 33 258 DNA staining method and found to be free of contamination.

2.3. Nanoparticle preparation

2.3.1. Preparation of PMMA nanoparticles

PMMA nanoparticles were prepared by free radical polymerization as described earlier (Stieneker and Kreuter, 1994). Briefly, the monomer was dissolved in a concentration of 1% (v/v) in water at 78°C. APS was added to obtain a final concentration of 0.03% (w/v). The mixture was stirred at 400 rev./min on a hot well plate in closed beakers of 100 ml. The polymerization was carried out for 24 h. In order to obtain stock suspensions nanoparticles were concentrated to a polymer content of 5% with an ultrafiltration stirring unit (model 402, Amicon, Witten, Germany) equipped with a Diaflo YC05 filtration membrane (Amicon). For the cytotoxicity studies the nanoparticles were sterilized by autoclaving at 2 bar and 121°C for 20 min.

2.3.2. Preparation of MMA-SPM copolymer nanoparticles

For the preparation of MMA-SPM nanoparticles we used the same preparation method as described for PMMA nanoparticles using different monomer concentrations. The total monomer content of the latter preparations was 5% (w/v), the SPM comonomer content amounted to 10% (w/v) of the total monomer concentration. For the cytotoxicity studies the nanoparticles were sterilized as described under Section 2.3.1.

2.3.3. Preparation of MMA-TMAEMC, MMA-MAPTAC and MMA-DMAEMC copolymer nanoparticles

To prepare nanoparticles consisting of MMA and either of the comonomers TMAEMC, MAPTAC, or DMAEMC, a mixture of 67.5 g water and 7.5 g acetone as polymerization medium was used. This mixture was heated to 78°C under stirring in closed beakers of 100 ml. One of the comonomers dissolved in 500 μl water was added together with MMA to the polymerization medium. The total monomer concentration was 1% for DMAEMC and MAPTAC or 3% for TMAEMC respectively. The comonomer concentration amounted to 30% (w/v) of the total monomer in all cases. The polymerization process was started by addition of 0.03% (w/v) of APS and was terminated after 24 h. Afterwards, the particles were concentrated to a final concentration of 5% and autoclaved as described under Section 2.3.1.

2.4. Particle size measurement

2.4.1. Photon correlation spectroscopy (PCS)

For the PCS study, a BI-200SM Goniometer Vers. 2.0 (Brookhaven Instruments, Holtsville, NY) equipped with a 30 mW He-Ne laser and connected to a BI-2030AT Digital Correlator (Brookhaven) was used; 50 μl of nanoparticle suspension were diluted to 100 ml and measured at 25°C. Particle size was expressed by mean effective diameter and size distribution was characterized by the polydispersity index. Demineralized water or 0.1 N NaCl solution used for dilution were filtered through a 0.8-μm Minisart NML filter unit (Sartorius, Göttingen, Germany).

2.4.2. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) of nanoparticles was performed by negative staining. Briefly, Formvar/carbon-coated grids were floated on a droplet of nanoparticle suspension on Parafilm to permit adsorption of the specimen. After blotting with a filter paper the grid was transferred onto a drop of negative stain, afterwards blotted with a filter paper and air-dried. Staining reagents uranylacetate (UA) or phosphothungstic acid (PTA) were obtained from BDH (Great Britain) or Polysciences (Eppelheim, Germany), respectively. Samples were examined in JEM 200 CX (Jeol, Tokyo, Japan) at 100 kV.

2.4.3. Scanning electron microscopy (SEM)

Nanoparticles for scanning electron microscopy (SEM) were prepared similarly to the preparations for TEM. After adsorption of the specimen on Formvar/carbon-coated grids, the samples were coated with a thin layer of gold or platinum by sputter coating. Samples were examined in JSM 6400 (Jeol, Tokyo, Japan) at 25 kV.

2.5. Surface charge

The surface charge of the nanoparticles was determined by microelectrophoresis (Fricke and Hüttenrauch, 1991) with a Lazer Zee Meter Model 501 (Penkam, Bedford Hills, NY) equipped with a CCD video camera head LDH 0460/xx (Philips, Eindhoven, Netherlands) and a video monitor LDH 2132/10 (Philips). An electric field of 150 V was applied to observe the electrophoretic mobility of the particles. The potential of the particles was measured directly after polymerization; 50 μl of each suspension was diluted to 100 ml either with demineralized water or 0.1 N NaCl solution in order to examine the influence of the surrounding medium on the surface charge. For the determination of the pH profiles the suspensions were diluted with 0.1 N NaCl solution followed by adjusting the pH to values between 2.0 and 12.0 with HCl and NaOH. The added NaCl solution served as a compensation for the conductivity effect resulting from the addition of HCl or NaOH. Measured values were transformed to standard values at the reference temperature of 20°C.

2.6. Cytotoxicity studies

The evaluation of the in vitro toxicity of the nanoparticles was based on the cell viability as determined by the MTT assay (Mosmann, 1983). Briefly, 5% stock suspensions of different nanoparticle preparations were diluted with MEM supplemented with 10% FBS starting with a 1:100 dilution and proceeding with 1:2 dilution steps. These dilutions were added to confluent cell layers in 96-well plates 4–5 days after seeding. Cells were incubated for 4 days at 37°C and 5% CO_2-atmosphere. After addition of 25 μl MTT solution/well (2 mg/ml MTT in phosphate buffered saline) and incubating for another 2 h, the crystals formed were dissolved by addition of 100 μl SDS solution (20% SDS in an 1:1 DMF/H_2O solution). Plates were read on a multiwell scanning spectrophotometer at a wavelength of 620 nm and a reference wavelength of 690 nm. The extent of cytotoxicity is defined as the relative reduction of the optical density (OD) which correlates with the amount of viable cells in relation to cell control (= 100%).

3. Results and discussion

3.1. Nanoparticle preparation

The polymerization conditions and the use of closed beakers resulted in a yield of about 98% of polymerized material as determined by gravimetric measurement. In all cases, milky white homogenous suspensions were obtained, that were stable for several months. The autoclaving procedure did not cause any changes in particle size or surface charge.

3.2. Physicochemical characterization

3.2.1. Particle size measurement

Mean diameters of nanoparticles determined by PCS, TEM or SEM are shown in Table 1. The particle sizes of all nanoparticles except MMA-MAPTAC measured by PCS were larger than those obtained by the quantitative analysis of the transmission or scanning electron micrographs.

Table 1
Particle size and surface charge of different nanoparticles

Copolymer composition	Mean diameter (nm)			Zeta potential (mV)	
	PCS[a]	TEM[b]	SEM[b]	Demin. water	0.1 N NaCl
MMA/TMAEMC, 70:30	243 ± 8 (0.09)	161 ± 8 $n = 216$	158 ± 10 $n = 110$	$+63.4 \pm 1.0$	$+32.1 \pm 0.7$
MMA/DMAEMC, 70:30	111 ± 3 (0.12)	71 ± 12 $n = 443$	76 ± 12 $n = 150$	-35.0 ± 1.1	-14.4 ± 1.4
MMA/MAPTAC, 70:30	207 ± 3 (0.04)	201 ± 24 $n = 231$	197 ± 32 $n = 148$	$+49.2 \pm 1.3$	$+32.0 \pm 1.3$
MMA/SPM, 90:10	150 ± 2 (0.07)	138 ± 8 $n = 210$	139 ± 9 $n = 220$	-77.3 ± 2.4	-46.4 ± 1.5
MMA	169 ± 1 (0.10)	114 ± 9 $n = 249$	141 ± 11 $n = 280$	-43.0 ± 0.8	-25.9 ± 0.1

Number of experiments for PCS and zeta potential, $n = 6$.
[a]Values in parentheses represent polydispersity index.
[b]Values for n represent number of measured nanoparticles on electron micrographs.

The explanation of this difference was given by Finsy et al. (1992) for PMMA nanoparticles and can equally be employed for the charged copolymer nanoparticles: The contrast of the EM pictures allows only the visualization of the nanoparticle core, whereas the hydrodynamic radius of the particles is measured by PCS. These differences were moderate in the case of MMA-SPM, MMA-DMAEMC copolymer, and PMMA nanoparticles, but conspicuous in the case of MMA-TMAEMC nanoparticles. We assume that TMAEMC containing particles were surrounded by a large hydrodynamic layer because the trimethylammoniumethyl chain covers the particle surface in a thread-like manner. Under EM vacuum the protruding chains collapsed resulting in a significantly reduced diameter. MAPTAC-containing particles should behave similarly, but the averaged particle size was almost the same after investigation by all three methods. Thus it can be assumed that the hydrodynamic layer is narrower. This might be the result of an intramolecular stabilization of the trimethylammoniumpropyl chain in the form of a pentagonal alignment. A lower energetic level could result from an interaction of the positively-charged ammonium group with the free electron pair of the amide nitrogen.

A selection of transmission and scanning electron micrographs is shown in Figs. 2–5. Nanoparticles were observed to be spherical (MMA-TMAEMC, MMA-SPM, MMA-DMAEMC) or pebble-like (MMA-MAPTAC) and were generally separated from each other.

3.2.2. Surface charge

Zeta potentials measured in demineralized water and in 0.1 N NaCl are shown in Table 1. MMA-TMAEMC and MMA-MAPTAC copolymer nanoparticles are characterized by remarkable positive potentials, MMA-SPM,

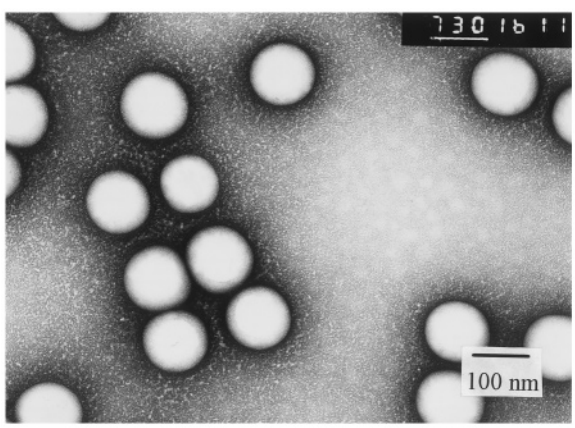


Fig. 2. Transmission electron micrographs of MMA-SPM copolymer nanoparticles (× 73 000) stained by uranylacetate. Bar represents 100 nm.

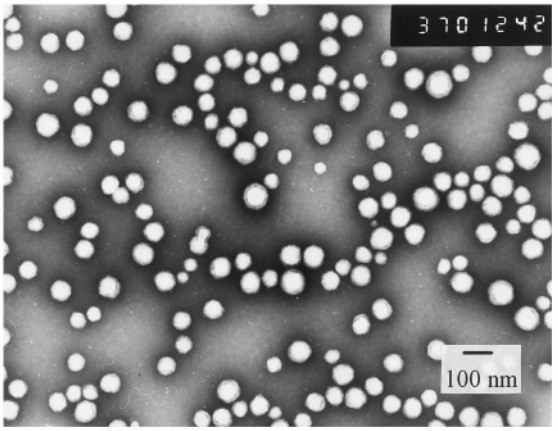


Fig. 3. Transmission electron micrographs of MMA-DMAEMC copolymer nanoparticles (× 37 000) stained by phosphothungstic acid. Bar represents 100 nm.

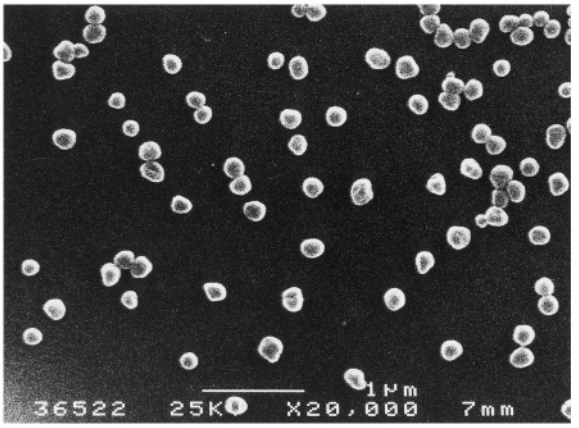


Fig. 5. Morphological appearance of MMA-MAPTAC copolymer nanoparticles analyzed by scanning electron microscopy seen at 20 000 magnification. Bar represents 1 μm.

MMA-DMAEMC and PMMA nanoparticles by clearly negative potentials. The differences between the species are more distinct in demineralized water than in NaCl solution. This is due to the fact that high salt concentrations in the surrounding medium reduce and equalize the zeta potentials due to the compression of the diffuse layer (Müller, 1996). Nevertheless, positively and negatively charged nanoparticles can be clearly distinguished even in high salt medium. The electrophoretic behavior of MMA-DMAEMC copolymer nanoparticles was similar to that of PMMA particles, which leads to the conclusion that the dimethylammonium group of DMAEMC is not dominating the surface charge. At pH 5.5, which was the result of the 0.1 N NaCl solution, DMAEMC is obviously not in a protonated state. Surface charge may be derived from negatively-charged sulfate groups of the starter APS. Comparing TMAEMC- and MAPTAC-containing particles a higher potential was measured in water for MMA-TMAEMC than for MMA-MAPTAC nanoparticles. Since both possess the same number of permanently positively-charged trimethylammonium groups, this difference in surface charge supports the previously discussed assumption of an intramolecular stabilization of the MAPTAC molecules.

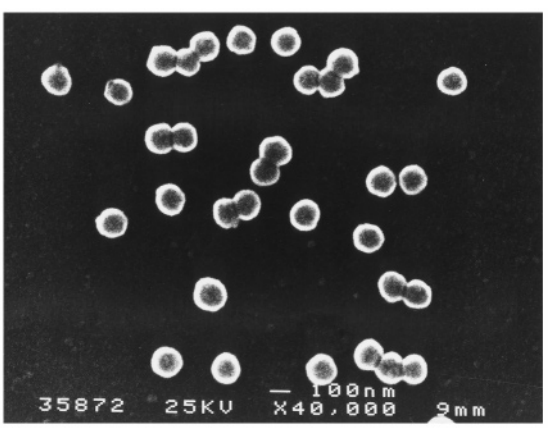


Fig. 4. Morphological appearance of MMA-TMAEMC copolymer nanoparticles analyzed by scanning electron microscopy seen at 40 000 magnification. Bar represents 100 nm.

3.2.3. pH dependency of particle size and surface charge

In order to clarify the dependence of particle size and surface charge on pH, MMA-TMAEMC and MMA-MAPTAC nanoparticle suspensions were measured at pH values 2–12 in 0.1 N NaCl solution. The effective diameter and the surface charge of MMA-TMAEMC nanoparticles (Fig. 6), ranging in size between 182 and 199 nm or between + 26.6 and + 32.4 mV in zeta potential, were not correlated with changes in pH values. MMA-MAPTAC nanoparticles were pH-independent in particle size and zeta potential at pH

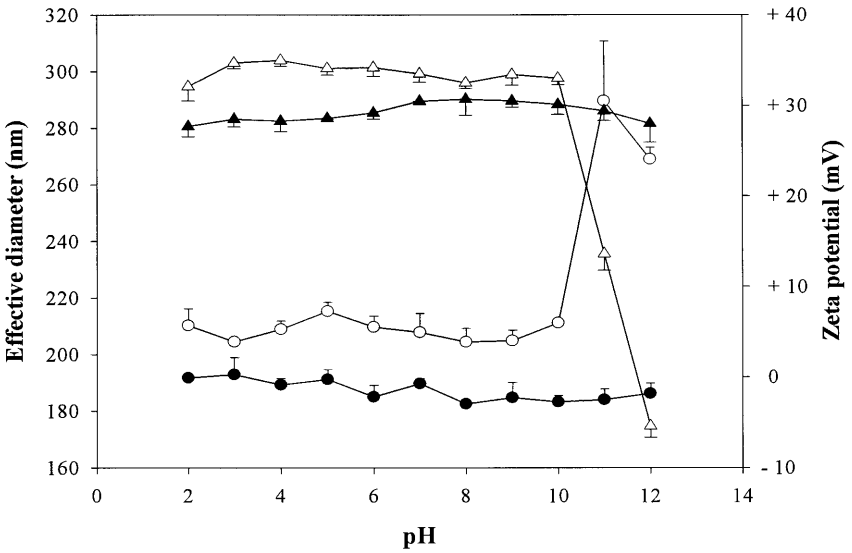


Fig. 6. pH-dependency of particle size and surface charge. (●) effective diameter of MMA-TMAEMC or (○) MMA-MAPTAC copolymer nanoparticles measured by PCS; (▲) zeta potential of MMA-TMAEMC or (△) MMA-MAPTAC copolymer nanoparticles. Number of experiments $n = 3$ for particle size and $n = 6$ for surface charge.

values 2–10 ranging between 200 and 212 nm or + 32.1 and + 35.7 mV, respectively. At pH 11 the mean effective diameter increased to 390 ± 21 nm and zeta potential was reduced to $+13.6 \pm 1.8$ mV. A pH of 12 reduced the mean effective diameter to 269 ± 4 nm and the zeta potential further to -5.4 ± 1.2 mV. These observations lead to the assumption that the methylacrylamide function becomes hydrolyzed at pH values higher than 10 exhibiting free methacrylic acid groups with a negative surface charge. Between pH 10 and 12, zeta potential is reduced to zero, leading to the agglomeration of uncharged particles, which then redisperse at higher pH values.

3.3. Cytotoxicity studies

Copolymer and PMMA nanoparticles were tested in three different cell cultures including human foreskin fibroblasts and two monkey kidney cell lines, Vero and MA-104. Fig. 7 shows representative data from three different experiments per cell line. Results show that the cell viability is not influenced significantly by MMA-MAPTAC, MMA-DMAEMC, MMA-SPM, and PMMA nanoparticles, but dramatically reduced by MMA-TMAEMC copolymer nanoparticles.

As stated previously in Section 1, MMA-SPM copolymer and PMMA nanoparticles did not show toxic effects in previous in vivo studies. These findings were now confirmed by the present in vitro results. The cell viability of MMA-SPM nanoparticles ranged between 92.7 and 135.7% of control in all experiments. At a maximum nanoparticle concentration of 500 μg/ml, the mean cell viability of two different batches in three different cell lines was $112.8 \pm 11.8\%$ of control. Cell viability of PMMA nanoparticles ranged between 82.8 and 119.3% of control. The mean cell viability at a maximum concentration of 500 μg/ml in three different cell lines was $90.8 \pm 4.5\%$ of control.

MMA-DMAEMC copolymer nanoparticles showed a cell viability profile similar to PMMA nanoparticles. Since the nanoparticle suspension was diluted 100-fold with medium of pH 7.4, the dimethylammonium-group of the DMAEMC molecule presumably existed in a deprotonated state. In contrast, the MMA-TMAEMC copolymer nanoparticles, which possessed positively charged *N*,*N*,*N*-trimethylammonium groups at pH 7.4, clearly exerted toxic effects in all experiments. The mean concentrations that led to a 50%

reduction of viable cells (IC_{50}) were 189.6 ± 11.4 μg/ml (MA-104 cells), 110.9 ± 3.1 μg/ml (Vero cells), and 27.2 ± 4.0 μg/ml (HFF). Since the DMAEMC monomer and the TMAEMC monomer are structurally-related substances and the comonomer contents in both copolymer particles were identical, the number of methyl substitutes of the ammonium group seems to be the decisive factor for in vitro toxicity. The cytolytic activity of cationic emulsifiers such as benzalconium bromide, cetylpyridinium chloride, or cetrimide is well known and is used for the conservation of pharmaceuticals. As stated previously in Section 1, substances containing quaternary ammonium functions interact with the negatively-charged cell-membrane and subsequently lead to its destruction. This phenomenon may explain the different results of MMA-TMAEMC and MMA-DMAEMC copolymer nanoparticles in the present study, since the former contain quaternary ammonium groups whereas the latter do not. On the other hand, this fact cannot explain the differences in cytotoxicity between MMA-TMAEMC and MMA-MAPTAC-nanoparticles, since both comonomers possess a quaternary *N*,*N*,*N*-trimethylammonium-group. In contrast to the described cytotoxic effect of MMA-TMAEMC copolymer nanoparticles, cell viability of MMA-MAPTAC at a maximum concentration of 500 μg/ml was 98.3% (Vero), 85.7% (MA-104) and 94.0% (HFF) of control. Therefore the attention here should be focused on the substitution of the methacrylic function (Fig. 1). While the trimethylammonium-group of the TMAEMC monomer is connected to the methacrylate by a C-2 bridge and an ester function, in the case of MAPTAC, the bonding consists of a C-3 bridge and a carbamide function. As previously discussed, these structural features of the MAPTAC molecule might result in an intramolecular pentagonal stabilization, reducing the surface charge of the copolymer particles and leading to a reduced cytotoxic potential of the trimethylammonium group.

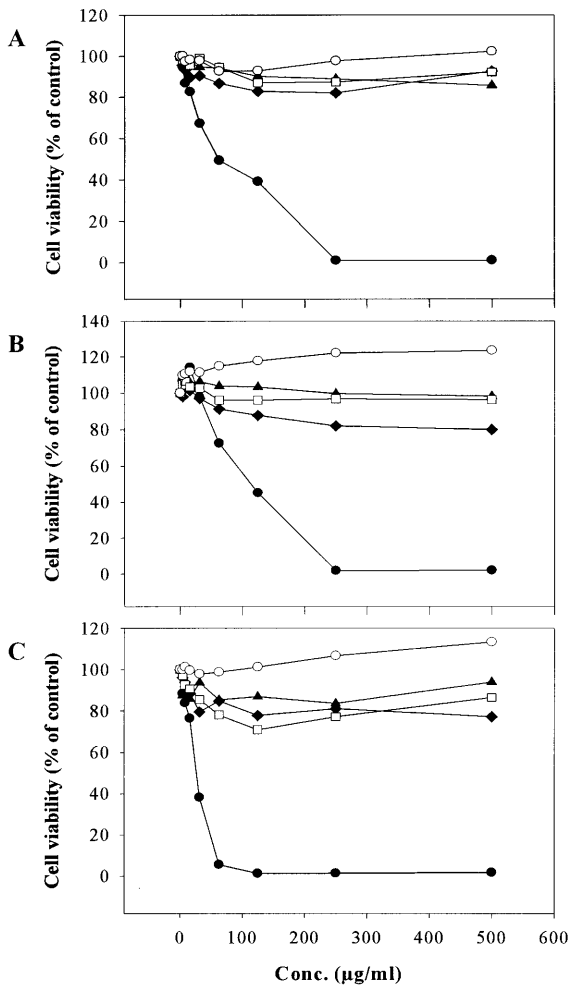


Fig. 7. In vitro cytotoxic effect of different nanoparticles on (A) MA-104-cells, (B) Vero-cells or (C) HFF measured by MTT assay. Nanoparticles consisted of (●) MMA-TMAEMC copolymer, (▲) MMA-MAPTAC copolymer, (◆) MMA-DMAEMC copolymer, (○) MMA-SPM copolymer or (□) PMMA. Reported results are representative data of three independent experiments. Intra assay S.D. $\leq 15\%$; inter assay S.D. $\leq 30\%$.

4. Concluding remarks

The results demonstrate that permanently positively-charged methylmethacrylate copolymer nanoparticles can be prepared in a reproducible manner. Since their particle size is in the nanometer range and since their surface charge is pH-independent, the copolymer nanoparticles described may be useful as colloidal drug carriers. Release of hydrophilic, anionic drugs would occur by ion

exchange and high loading capacities can be obtained. Problems regarding the cytotoxicity of quaternary ammonium functions seem to be related to structural differences between the different polymers. Therefore, further studies are required to investigate and draw final conclusions on the correlation between molecular structure and biological effects of cationic nanoparticles. In addition, further studies are necessary to examine the biodegradability of the described copolymer nanoparticles in order to evaluate the use of these particles as drug delivery systems.

Acknowledgements

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