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Methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles for drug delivery. Part I: preparation and physicochemical characterization

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Abstract

Methylmethacrylate (MMA) sulfopropylmethacrylate (SPM) copolymer nanoparticles were prepared by free radical polymerization. The conditions of preparation were varied with regard to the concentration of initiator and monomer, and copolymer composition. Nanoparticles with a yield greater than 80% were produced. The particles were characterized in terms of particle size, size distribution, particle charge (zetapotential) and molecular weight. The data were compared to pure polymethylmethacrylate (PMMA) nanoparticles. The copolymer composition was shown to influence particle size and particle charge. The influence of the total monomer amount in the polymerization medium on the particle size was characteristic up to a concentration of 2% depending on the solubility of MMA in water at the temperature of reaction. An increasing amount of total monomer led to particle sizes of 60–130 nm for low monomer concentrations (0.5%), depending on the proportion of SPM (0–10%), to 120–280 nm for higher total monomer concentrations (greater than 2%). Surface charge as well as particle size were influenced mainly by the proportion of the comonomer SPM in the copolymer. The negative surface charge increased from − 52 mV for pure PMMA nanoparticles to − 80 mV for the copolymer particles with an SPM content of 10%. In the same range of 0–10% SPM of the total monomer, the particle sizes decreased from 187 to 100 nm. The concentration of the initiator up to a concentration of 0.3% showed no effect on the particle size of the resulting nanoparticle suspension. Higher concentrations led to intolerably large variability in the polymerization process.

Keywords: Nanoparticles; Copolymer; Methylmethacrylate (MMA); Sulfopropylmethacrylate (SPM); Physicochemical characterization

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1. Introduction

The most frequently used polymers for the manufacture of nanoparticles are acrylic acid derivatives, mainly polyalkylcyanoacrylates and polyalkylmethacrylates (Kreuter and Speiser, 1976; Couvreur et al., 1982; Kreuter, 1983a, 1991). However, most homopolymer nanoparticles have low loading capacities, especially for hydrophilic drugs (Harmia et al., 1986). In order

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to increase the hydrophilicity of the particle surface, nanoparticles were manufactured by copolymerization of alkylmethacrylate with various acrylic acid derivatives (Rolland et al., 1986; Kreuter et al., 1988) including acrylamide, acrylic acid, acrylic acid butyl ester, and with styrene. Nanoparticles prepared with the weak acid acrylic acid as comonomer showed a pH-dependent surface charge (Dittgen et al., 1988). Zosel et al., 1988, studied the influence of chemical nature of anions and concentration of electrolytes on the zetapotential of acrylic acid/methylmethacrylate copolymers (Zosel et al., 1988; Dittgen and Zosel, 1991). Lukowski et al. showed that by replacing the comonomer styrene against methylmethacrylate led to nanoparticles with a surface hydrophobicity mainly influenced by the proportion of acrylic acid in the comonomer (Lukowski et al., 1992). Adsorption experiments revealed that the coating layer thickness of nonionic surfactants like poloxamer and poloxamine correlated to the hydrophobicity of the carrier system whereas the adsorption of the ionic surfactant Gafac RE 960 showed no correlation to the surface hydrophobicity (Lukowski et al., 1993). These copolymer nanoparticles were structurally characterized as being compact, without channels and holes and as existing in different size subpopulations possessing different hydrophobic areas on their surface (Müller et al., 1994).

Although considerable work has been performed in the field of methacrylic nanoparticle carrier systems, very little information about the use of monomers carrying a specific charge as comonomers for the preparation of nanoparticles is available.

The objective of the present study was the development of nanoparticles exhibiting the properties of strong-acid cation-exchange resins. Keeping in mind that a lot of pharmacologically active compounds like cholinomimetics for the therapy of ocular diseases are hydrophilic drugs with a low affinity to most common polymers used for nanoparticle preparation, SPM nanoparticles may be useful carriers for the binding of such drugs. Due to the fact that many drugs are charged at physiological pH values, good binding to the carrier systems under physiological conditions was anticipated. Copolymer nanoparticles consisting of methylmethacrylate (MMA) and the strong acid sulfopropylmethacrylate (SPM) as the second component were prepared. SPM served as a model compound for comonomers carrying a permanent negative charge.

Copolymer carrier systems with a variety of comonomer compositions, initiator concentrations and total monomer concentrations were prepared. Different kinds of reaction conditions were also tested with the aim of establishing a reproducible preparation procedure in combination with a high yield of reaction.

2. Materials and methods

2.1. Reagents and chemicals

Methylmethacrylate (MMA) (Merck-Schuchardt, Hohenbrunn, Germany) and sulfopropylmethacrylate-potassium (SPM) (Hüls, Marl, Germany) were used as monomers. Ammonium persulfate (APS) was purchased from Hüls (Marl, Germany). Tetrahydrofuran was obtained from Merck (Darmstadt, Germany). Toluene was purchased from Fluka (Buchs, Switzerland). Water used for all steps of preparation was first purified by ion exchange, then boiled under a nitrogen stream to completely remove oxygen.

2.2. Preparation of nanoparticles

Copolymer nanoparticles were prepared by a process of free radical polymerization in water in closed 100 ml beaker. SPM was dissolved in water at 78°C and MMA and ammonium persulfate were added under stirring on a hotplate at 400 rpm. The polymerization was completed over a period of 24 h. Different batches were prepared according to the following reaction conditions:

(1) The content of SPM was varied over the range 0–50% w/w total monomer.
(2) The total monomer concentration was varied between 0.5% and 5.0% w/v.
(3) The concentration of the initiator APS was varied between 0.01% and 1.0% w/v.

Three batches of nanoparticles were produced under each set of reaction conditions. The resulting suspensions were lyophilized in a Lyovac GT2 freeze-dryer (Leybold-Heraeus, Hürth, Germany) for 24 h under vacuum (2 • 10^{-3} bar) or used as prepared.

2.3. Particle size by PCS

The particle size of the resulting nanoparticles was determined by photon correlation spectroscopy (PCS) (De Jaeger et al., 1991; Finsy and De Jaeger, 1991; Finsy et al., 1992; Finsy et al., 1993). A BI-200SM Goniometer Vers. 2.0 (Brookhaven Instruments Corp., Holtsville, NY, USA) equipped with a 30 mW He-Ne laser and connected to a BI-2030AT Digital Correlator was used. The count rate was adjusted to a value of 20 kHz by diluting the samples with water, the measuring angle was fixed at 90°, and the pinhole selection of the photomultiplier was set at 100 μm. The water used for dilution was first filtered through a 0.22 μm filter unit (Millex-GS, Millipore, Molsheim, France).

The particle size was expressed by the effective diameter (De Jaeger et al., 1991), and the width of the size distribution was characterized by the polydispersity index (Koppel, 1972). The software data analysis for calculating the size distribution of the nanoparticle samples was based on the fitting method of non-negative constrained least-squares (NNLS) (Finsy et al., 1992).

2.4. Surface charge

The surface charge of the nanoparticles was determined by microelectrophoresis (Fricke and Hüttenrauch, 1991) with a Lazer Zee Meter Model 501 (Penkam Inc., Bedford Hills, NY, USA) equipped with a CCD camera. An electric field of 150 V was applied to observe the electrophoretic mobility of the particles. The potential of the particles was measured immediately after polymerization. For these measurements, 1.0 ml of each suspension was diluted with water to 100.0 ml. For the determination of the pH-profiles dependency of the surface charge, suspensions were diluted with 0.1 M NaCl solution followed by adjustment of the pH to values between 3.0 and 9.0 with HCl and/or NaOH. All measurement were performed in 0.1 M NaCl to compensate for the conductivity effect of the addition of HCl and NaOH when adjusting the pH. All values were transformed to standard values at the reference temperature of 20°C.

2.5. Molecular weight of copolymer nanoparticles

The molecular weight of the resulting copolymer was determined by viscometry. Polystyrol standards with molecular weights ranging from 10200 to 194000 (Macherey-Nagel, Düren, Germany) were dissolved in toluene at concentrations of 0.5%, 1.0%, 2.0%, 3.0% w/v and used for calibration. The density of the solutions was measured in a DMA 48 density meter (AP PAAR, Graz, Austria). The flow time of each solution was determined in a Cannon-Fenske viscometer (k = 0.002897, Schott, Hofheim, Germany). The reduced specific viscosity was calculated and plotted against the concentration of the solution followed by the extrapolation to zero concentration. The resulting intercept represents the intrinsic viscosity. To calculate the calibration curve, the logarithm of the intrinsic viscosity was plotted against the logarithm of the molecular weight of the polystyrol standards.

The freeze dried nanoparticles were treated in the same way. The concentration of the dissolved copolymer ranged from 0.25% to 1.5%. Using the calibration curve, the molecular weights of the copolymers were calculated. All measurements were taken at a temperature of 20°C.

2.6. Molecular weight of PMMA nanoparticles

The molecular weights of PMMA nanoparticles were determined using the method of gelpermeation chromatography (GPC). The PMMA polymers were prepared under the same conditions as the copolymer nanoparticles used for the determination of the molecular weights. Founded upon the data of the GPC analysis, the number average molecular weight M_n and the weight average molecular weight M_w were determined. The system consisted of a HPLC-chromatography pump

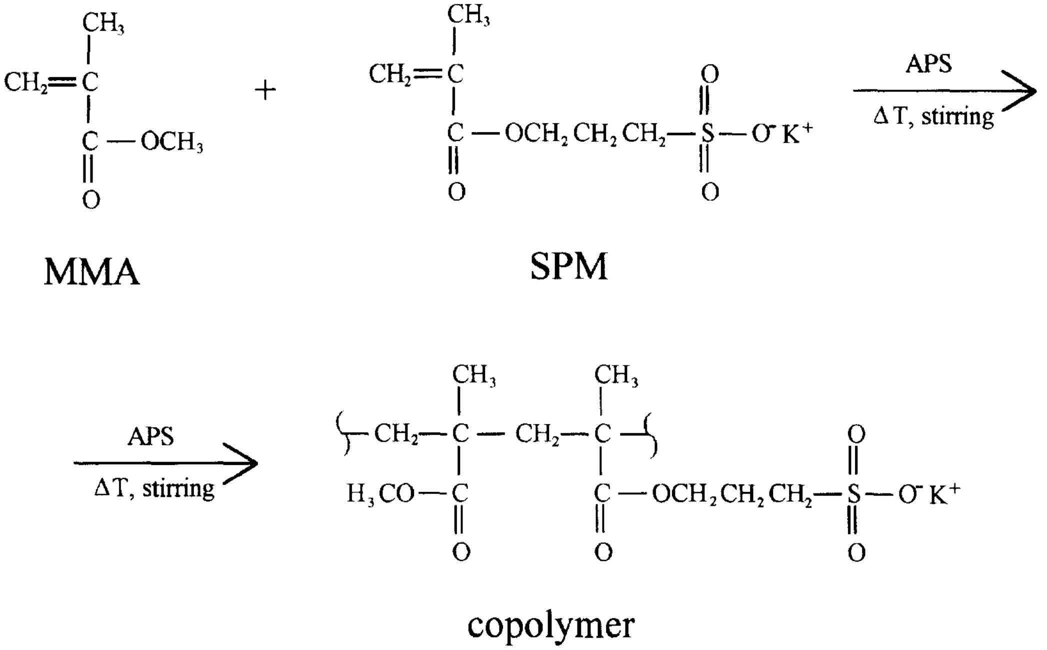

Fig. 1. Scheme of copolymerization of MMA and SPM.

(HPLC pump 64, Knauer, Berlin, Germany) fitted with a refractive index detector (Waters 410 differential refractometer, Millipore, Eschborn, Germany); 5μ Styragel 10^3 and 5μ Styragel 10^5 (Polymer Standard Service, Mainz, Germany) served as columns. Tetrahydrofuran at a flow rate of 1 ml/min was used as the mobile phase. Ten micrograms of freeze dried PMMA nanoparticles were dissolved in 5 ml of tetrahydrofuran followed by filtration through a 0.45 μm filter of regenerated cellulose (Schleicher and Schüll, Dassel, Germany). Toluene at a concentration of 0.05% v/v was used as an internal standard. The chromatograms were registered on a PC integration pack (Kontron Instruments, Neufahrn, Germany). PMMA standards with molecular weights between 2030 and 10^6 (Polymer Standard Service, Mainz, Germany) were used for column calibration.

3. Results and discussion

For the preparation of copolymer nanoparticles, methylmethacrylate (MMA) and sulfopropylmethacrylate (SPM) (Fig. 1) were copolymerized by the method of free radical polymerization using ammonium persulfate as an initiator (Berg, 1979; Kreuter, 1983b). The presence of the comonomer SPM with its permanent negative charge resulted in stable suspensions. Even after storage of the suspensions for several months, no sedimentation or agglomeration of the nanoparticles occurred. The yield of the polymerization process was determined by calculating the mass balance between the monomers used in the reaction and the freeze-dried polymer after completion of the polymerization followed by dialysis against water over a period of 24 h in order to separate from monomers. In each case, the yield of the reaction was determined to be greater than 80%.

To characterize the factors influencing the formation of the nanoparticles, the particles were prepared at fixed concentrations of 2.0% w/v total monomer and 0.03% w/v APS. The concentration of the charged comonomer SPM was varied between 0% and 50% w/w of the total monomer.

The size of the resulting nanoparticles was dependent on the concentration of the comonomer (Fig. 2). With increasing SPM content, the turbidity of the resulting suspension decreased and a bluish shine occurred indicating a reduction of the particle size compared to pure PMMA nanoparticles. In the absence of SPM, PMMA nanoparticles had an effective diameter of 187 nm. The particle size was reduced to 100 nm at a concentration of 10% SPM with no further changes in particle size at higher SPM concentrations. SPM concentrations up to 25% showed polydispersity indexes smaller than 0.1 indicating a very narrow size distribution. For higher concentrations, the polydispersity indexes were in the range of 0.1–0.5 indicating considerably broader size distributions. Data analysis by the method of NNLS indicated that size distributions were bimodal consisting of a small fraction of the particles falling in the size range 10–20 nm with the majority of the particles having a size of about 100 nm. Bimodal size distributions can be attributed to the formation of different copolymer compositions in the same reaction sample, i.e. copolymer particles with a high content of MMA having larger sizes than copolymer particles with a high content of SPM. In order to evaluate this hypothesis, a GPC method had to be developed. However, as discussed below, the solubility of the copolymer in common solvents for GPC/RI-detection limited these measurements. Characterization by hydrophobic interaction chromatography (HIC) might be a suitable method to get further information regarding the formation of different particle subpopulations (Carstensen et al., 1991).

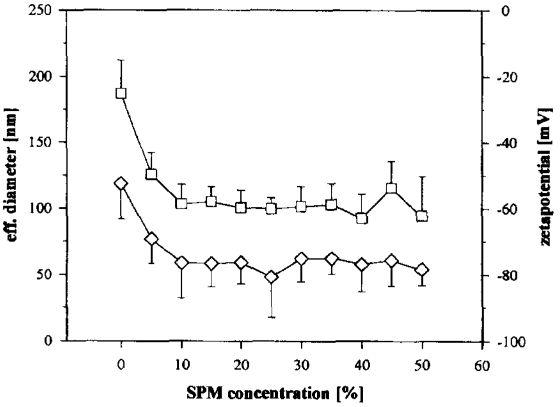

Fig. 2. Influence of the SPM content on the effective diameter (-□-) and zetapotential (-◇-) of the resulting nanoparticles. Standard deviations are indicated by vertical bars (n = 4).

The zetapotential showed the same dependency on the comonomer concentration as the particle size. The zetapotential of pure PMMA nanoparticles was determined to be −52.5 mV, whereas with increasing SPM concentration the zetapotential increases to values ranging from −75 mV to −80 mV. The results indicated that the comonomer increased the negative surface charge of the colloidal system. It has been hypothesized that a more negative surface charge on the copolymer nanoparticles leads to a electrostatic repulsion between the polymerizing particles, resulting in decreased particle growth and a lower particle size (Lukowski et al., 1992).

Using a fixed comonomer concentration of 5.0%, the zetapotential was determined at different pH values. Shifting the pH from 3.0 to 9.0 showed no significant effect on the zetapotential of the nanoparticles. This lack of effect was attributed to the strong dissociation of the SPM functional groups on the particle surface. In earlier studies in which the weak acid acrylic acid was used as the comonomer, a pH-dependent zetapotential of the colloidal system has been observed (Lukowski et al., 1992). This was due to pH dependent dissociation of the carboxylic acid groups of this weak acid.

The concentration of the total monomer was then varied over the range of 0.5% to 5.0% at fixed SPM concentrations of 0%, 5% or 10% w/w (Fig. 3). Ammonium persulfate was used in a concentration of 0.03%. In the case of the pure PMMA nanoparticles, the concentration of total monomer influenced the size of the resulting particles. An increasing concentration of MMA led to a larger effective diameter of the particles attaining a plateau for concentrations above 2.5%. This result is in agreement with earlier results of Berg et al. who used a slightly different method of preparation of PMMA nanoparticles (Berg et al., 1986). For systems containing the como-

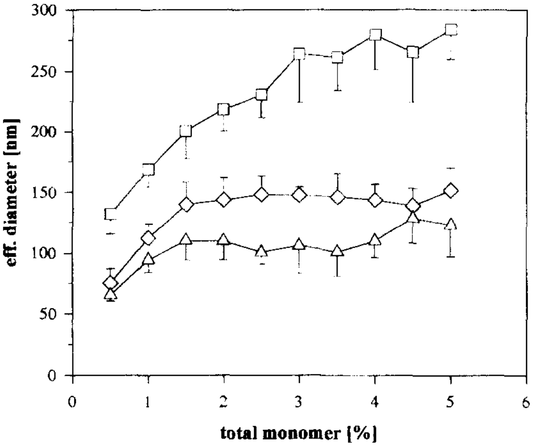

Fig. 3. Effect of the total monomer content on the effective diameter of the resulting nanoparticles. Copolymer composition: (–□–) 0% SPM, (–◇–) 5% SPM, (–△–) 10% SPM. Standard deviations are indicated by vertical bars (n = 4).

nomer SPM, comparable results were observed except that the plateau was observed at lower concentrations. The solubility of MMA in water at the temperature of reaction appeared to influence the polymerization. At 78°C, the saturation concentration of MMA is approximately 2%. Taking into account that the polymerization takes place in the aqueous phase at higher concentrations, the equilibrium between dissolved and dispersed MMA serves as a reservoir, ensuring a constant concentration of monomer available for polymerization and thus resulting in a narrow particle size distribution.

In each case, nanoparticles containing the comonomer SPM were smaller than pure PMMA nanoparticles. These results also support the hypothesis that higher electrostatic repulsion between the polymerizing comonomers leads to smaller nanoparticles.

Increasing the amount of ammonium persulfate up to concentrations of 0.3% m/v at a fixed concentration of 2% w/v total monomer showed no significant effects on the effective diameter of the resulting nanoparticles (Fig. 4) in agreement with previous observations of pure PMMA nanoparticles (Berg et al., 1986). Higher concentrations of the starter led to uncontrollable polymerization processes resulting in polymer flocules in addition to nanoparticles. Lack of interstate reproducibility is indicated by the high standard deviations at APS concentrations of 0.5% w/v. At low APS concentrations (0.01%), only a small number of radicals was generated, ensuring in a particulate system comprised of polymers of a high molecular weight. Higher APS concentrations (0.02%–0.3%) increased the number of radicals resulting in a reduction in the molecular weight of the copolymer. However, the particle size was not changed. It is assumed that in this situation more polymer molecules were required to form a single particle with the result of an even particle size. It was observed that with APS concentrations up to 0.3%, the reaction mixture containing 10% SPM led to smaller particles than the mixture containing 5% of the charged comonomer at the same total amount of the monomer mixture. Above 0.3%, the higher amount of generated radicals led to aggregation of preformed nanoparticles and to the formation of flocules.

For PMMA nanoparticles prepared over the range of 0.5–5.0% of methylmethacrylate, the effective diameter of the resulting particles was in good correlation to the molecular weight of the polymer, calculated as M_{GPC}, representing the mode of the molecular weight taken from the chromatogram (Fig. 5).

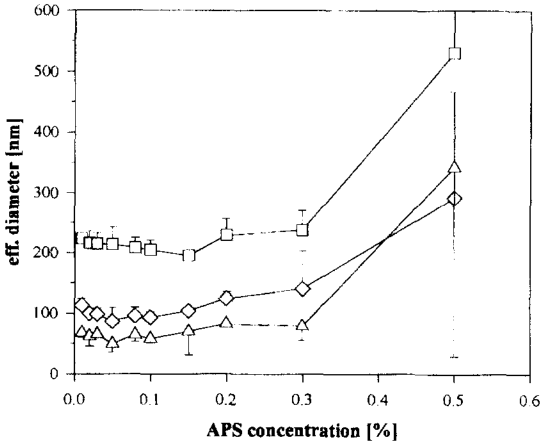

Fig. 4. Effect of the concentration of the initiator APS on the effective diameter of the resulting nanoparticles. Copolymer composition: (–□–) 0% SPM, (–◇–) 5% SPM, (–△–) 10% SPM. Standard deviations are indicated by vertical bars (n = 3).

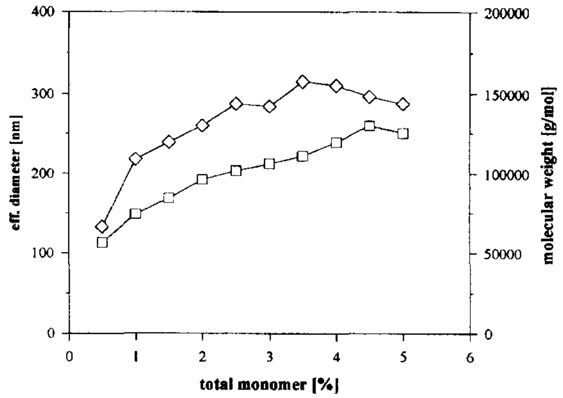

Fig. 5. Effect of the total monomer content on the effective diameter (–□–) and the molecular weight M_{GPC} (–◇–) of the resulting PMMA nanoparticles.

Attempts to employ a GPC method for the determination of the molecular weight of the copolymers using several solvents such as tetrahydrofuran, acetone, acetone-toluene mixtures, dichloromethane, isopropanol or N,N-dimethylformamide failed because of the poor solubility of the polymers or because the difference between refractive indexes of polymers in solution and the solvent was missing. Therefore, viscosity measurements in toluene were used to determine the copolymer molecular weights for nanoparticles prepared at a fixed SPM concentration of 5% w/w. In this case, too, the molecular weight of the polymer correlated with the size of the nanoparticles (Fig. 6).

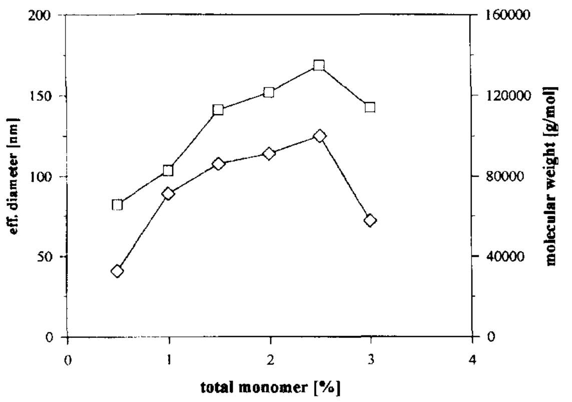

Fig. 6. Effect of the total monomer content on the effective diameter (–□–) and the viscosity average molecular weight (–◇–) of the resulting copolymer nanoparticles prepared at a SPM concentration of 5% w/w.

The results of this study demonstrated the feasibility of preparing copolymer nanoparticles of defined characteristics with a highly negative surface charge. The size of the resulting particles was mainly influenced by the amount of charged comonomer SPM used in the reaction. The surface charge of the comonomer nanoparticles was constant over a pH range between 3.0 and 9.0 due to the acid strength of the comonomer SPM. Assuming that drug binding to the SPM system is of ionic nature, further studies will have to be done to characterize the prepared formulations with regard to the loading behavior for ionic drugs, to the time course of drug release, and with the objective to study the influence of the ionic composition of the release medium on the release rate. In addition to other factors such as particle size and conformation of molecules adsorbed to the surface of colloidal carriers, particle charge and surface hydrophilicity are important parameters governing uptake in organs of the reticuloendothelial system (RES). The proposed SPM/MMA nanoparticles, therefore, hold promise for obtaining a different body distribution after intravenous injection compared to pure PMMA carriers.

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