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SHORT COMMUNICATION

Preparation and characterization of poly(acrylic acid)-based nanoparticles

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Abstract The present investigation describes the synthesis and characterization of nanoparticles based on poly(acrylic acid) (PAA) intramolecularly cross-linked with diamine, 2,2′-(ethylenedioxy)bis(ethylamine), using water-soluble carbodiimide. The aqueous colloid dispersions of nanoparticles were clear or mildly opalescent depending on the ratio of cross-linking, pH of the solution, and the molecular weight of PAA, finding consistent with values of transmittance between 3% and 99%. The structure was determined by nuclear magnetic resonance spectroscopy, and the particle size was identified by dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements. It was found that particle size depends on the pH, and at a given pH, it was caused by the ratio of cross-linking and the molecular weight of PAA. Particle size measured by TEM varied in the range of 20 and 80 nm. In the swollen state, the average size of the particles measured by DLS was in the range of 35–160 nm.

Keywords Poly(acrylic acid) · Nanoparticles · Cross-linking

Introduction

Recently, many researchers have investigated synthetic polymers as drug delivery carriers since they have compositional diversity and can be prepared in a variety of forms, and many have good compatibility [1, 2].

The interest of the researcher is turned to synthesis, characterization, and application of microscopic polymeric gels [3, 4], microgels [5, 6], and nanogels [7, 8] of poly (acrylic acid) (PAA), which is a biocompatible hydrophilic polymer and can easily be modified chemically through the free carboxyl groups [9]. The microgel and nanogel particles are of great interest because they exhibit properties of gels combined with the properties of colloids and can be employed for several biomedical applications, such as drug- or gene-delivery systems [5, 6].

Many recent attempts have been made to create particulate systems based on PAA. The nano- and micro-systems can be prepared in a wide range of methods: solvent evaporation [6], suspension [10], or template polymerization [11] are well-known techniques to produce suspension performed in inverse emulsion [5]. By emulsion methods, the size of the particles can be controlled by controlling the size of droplets; however, the emulsion systems contain at least two phases and surfactant.

Several studies report on the preparation of modified PAA using carbodiimide technique [12, 13]. Thiol-functionalized PAA [14], covalent conjugates with bovine serum albumin [15] or with cysteine [16, 17], were produced based on the formation amide bonds of functional amino and carboxyl groups. These results are devoted to interactions of micro and nanogels with drug molecules and proteins [18, 19].

The present investigation reports a method for the preparation of nano-sized particulate systems based on

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PAA by covalently cross-linking via carboxyl groups of the polymer chain with diamine in aqueous media at room temperature using carbodiimide technique. The resulting PAA nanoparticles are more compact, smaller, and have different properties compared to the PAA. Cross-linked PAA nanoparticles form stable colloid systems in aqueous media.

The correlation of size, ratio of cross-linking, and the molecular weight of PAA was investigated. The solubility, structure, and size of these nanoparticles in the dried and swollen states will be described and discussed. They are nano-sized and can be an attractive candidate as delivery biosystems for a variety of biomedical applications.

Experimental section

Materials

PAAs with M_w 100.000 Da (PAA1), 450.000 Da (PAA2), and 750.000 Da (PAA3) were obtained from Sigma-Aldrich Co., Hungary. 2,2′-(ethylenedioxy)bis(ethylamine) (EDBEA) and water-soluble 1- [3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride (CDI) were purchased from Sigma-Aldrich Co., Hungary and were used as received without further purification. Dialysis tubing cellulose membrane (Mw=12,400) was bought from Sigma-Aldrich Co., Hungary, and it was used for purification of aqueous colloid systems containing cross-linked PAA nanoparticles.

Poly(acrylic acid) cross-linking

Cross-linked PAA nanoparticles were prepared by the CDI technique, using EDBEA as the cross-linking agent. PAA with different molecular weight was modified: M_w: 100.000 Da (PAA1), 450.000 Da (PAA2), and 750.000 Da (PAA3).

Synthesis of cross-linked PAA nanoparticles

PAA was dissolved in water to produce a solution with a concentration of 1 mg/ml, and then the diamine was added to the PAA solution and mixed for 30 min at room temperature. After the addition solution of water-soluble carbodiimide dropwise, the reaction was stirred at room temperature for 24 h. The solution containing PAA nanoparticles was purified by dialysis for 7 days against distilled water and freeze-dried.

Synthesis of cross-linked PAA nanoparticles with EDBEA at diverse stoichiometric cross-linking ratios was accomplished according to the described reaction conditions. The data of the synthesis are summarized in Table 1.

Table 1 Reaction conditions of the synthesis of cross-linked poly (acrylic acid) nanoparticles

Poly(acrylic acid) (mg)	Stoichiometric ratio of cross-linking[a]	Quantity of EDBEA (μL)	Quantity of CDI (mg)
100	25%	26	103
100	50%	51	206
100	100%	101	412

The quantity of poly(acrylic acid) is independent of molecular weight

[a] Molar ratio of cross-linker and polymer units

Characterization

NMR Spectroscopy

PAA and its cross-linked derivatives were analyzed structurally with nuclear magnetic resonance (NMR) spectroscopy. ^1H NMR and ^{13}C NMR spectra were obtained on a Bruker 200SY (200 MHz) and a Bruker DRX500 (500 MHz). The samples were dissolved in D_2O. The chemical shifts were represented in parts per million (ppm), based on the signal for sodium 3-(trimethylsilyl)-propionate-d_4 as a reference.

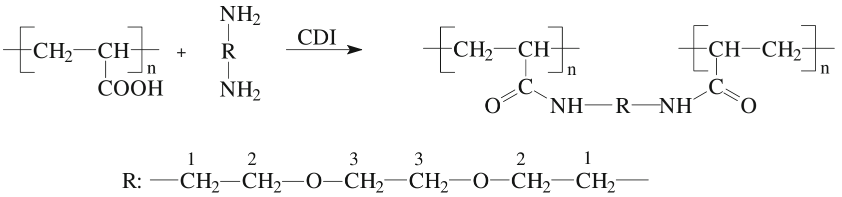
Transmittance

Transmittances of colloid systems containing PAA nanoparticles were measured by using Unicam SP 1800 ultraviolet spectrophotometer at an operating wavelength of $\lambda=500$ nm in optically homogeneous quartz cuvettes. The samples were obtained from the reaction mixture after dialysis at 25°C. The concentration of the solutions was 0.70 mg/mL.

Dynamic laser light scattering

Dynamic light scattering (DLS) measurements were carried out by using a BI-200SM Brookhaven research laser light scattering photometer equipped with a 10 mW Nd:YAG vertically polarized laser light source at an operating wavelength of $\lambda_o=532$ nm. All measurements were taken at 25°C with an angle detection of 90° in optically homogeneous quartz cylinder cuvettes. The samples were prepared from the reaction mixture after dialysis. The concentration of the cross-linked PAA derivative solutions was 0.70 mg/ml. The DLS measurements were performed in 0.01 M NaCl solution to keep the ionic strength constant. The scattering intensity data were processed using the instrumental software to obtain the hydrodynamic diameter and the size distribution of scatterers in each sample, which was measured three times, and average serial data were calculated.

Fig. 1 Schematic representation of the synthesis and structure of cross-linked poly(acrylic acid) derivatives



Transmission electron microscopy

A JEOL2000 FX-II transmission electron microscope was used to characterize the size and morphology of the dried PAA nanoparticles. For transmission electron microscopy (TEM) observation, the PAA nanoparticles were prepared from the reaction mixture after dialysis at a concentration of 100 µg/ml. The sample for TEM analysis was obtained by placing a drop of the colloid dispersion containing the cross-linked nanoparticles onto a carbon-coated copper grid. It was dried at room temperature and then examined using a TEM without any further modification or coating.

Results and discussion

Formation of cross-linked nanoparticles

In aqueous medium, the carboxyl groups of PAA can be negatively charged resulting in a highly charged polyelectrolyte macromolecule depending on the pH of the solution. At low charge density, polyanion chains collapse into a compact globule; at high charge density, the PAA macromolecules have an extended coil conformation. The coil-globule transition was influenced by the attraction and repulsion interaction between the polymer segments. Chemical cross-linking of PAA linear chains using diamine as cross-linking agents at different stoichiometric ratios was carried out to produce polyanion cross-linked nanoparticles.

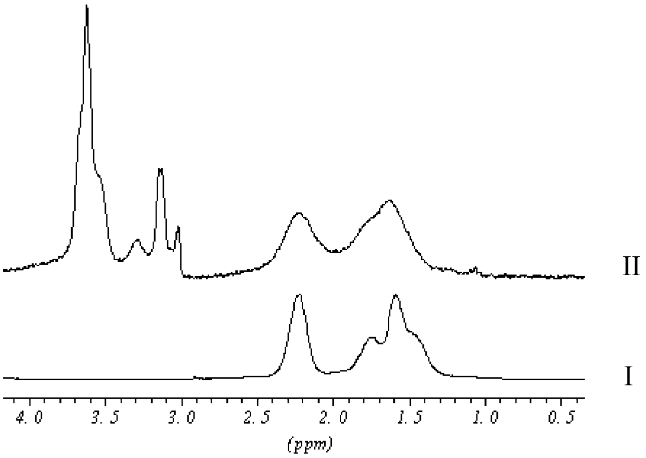


Fig. 2 ^{1}H NMR spectra of PAA2 (I) and poly(acrylic acid) nanoparticles based on PAA2 cross-linked with EDBEA at 50% (II)

Polyanions can be obtained by reacting PAA with diamine, if the ratio of cross-linking was less than 100%. In this case, the amino groups are bound covalently, and residual free carboxyl groups of the PAA chain are available. These free carboxyl groups can be deprotonated, forming polyanions. If each of the functional amino and carboxyl groups were covalently bound, PAA cross-linked with diamine results in uncharged nanoparticles in aqueous media.

NMR results

The structures of PAA used for the synthesis and the resulting cross-linked nanosystems (Fig. 1) were characterized by NMR spectroscopy. The assignments and chemical shifts of the ^{1}H and ^{13}C NMR signals of PAA and cross-linked PAA nanoparticles based on PAA2 were determined. The chemical shift values are in accordance with results published.

Proton chemical shift values are given in Fig. 2, which illustrates the difference between the signs of the PAA2 original material (I) and the nanoparticles based on PAA2 cross-linked with EDBEA at a stoichiometric ratio of 50% (II). The assignments and chemical shifts of the ^{1}H signals are given as follows: PAA2: ^{1}H NMR (D_2O): δ=1.3–1.9 (CH_2 of PAA), δ=2.1–2.4 (CH of PAA). PAA2 cross-linked with EDBEA at 50%: ^{1}H NMR (D_2O): δ=1.4–2.0 (CH_2 of PAA), δ=2.1–2.6 (CH of PAA), δ=3.26 (3-H of EDBEA), δ=3.68 (2-H of EDBEA), δ=3.76 (1-H of EDBEA).

The ^{13}C assignment was performed on the basis of ^{13}C projection. Figure 3 demonstrate the difference between the

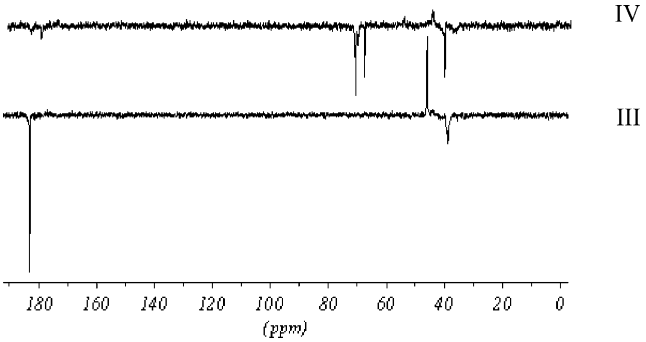


Fig. 3 ^{13}C NMR spectra of PAA2 (III) and poly(acrylic acid) nanoparticles based on PAA2 cross-linked with EDBEA at 50% (IV)

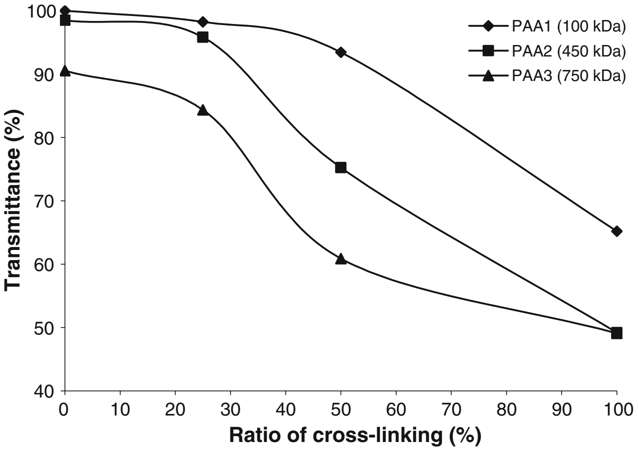


Fig. 4 Effect of ratio of cross-linking on the transmittance of nanoparticles (pH=6.5; *c*=0.7 mg/mL)

signs of original PAA2 material (III) and the nanoparticles based on PAA2 cross-linked with EDBEA at 50% (IV). The assignments and chemical shifts of the ^{13}C signals are given as follows: PAA2: ^{13}C NMR (D$_2$O): $\delta=38.00$ (CH$_2$ of PAA), $\delta=45.30$ (CH of PAA), $\delta=182.44$ (COOH of PAA). PAA2 cross-linked with EDBEA at 50%: ^{13}C NMR (D$_2$O): $\delta=36.70$ (CH$_2$ of PAA), $\delta=44.92$ (CH of PAA), $\delta=182.43$ (COOH of PAA), $\delta=178.85$ (CONH of PAA), and the signs of EDBEA $\delta=40.85$ (3-C), $\delta=68.17$ (1–C) and $\delta=71.31$ (2-C).

The degree of cross-linking was evaluated from the integral intensity of signs by using ^{1}H NMR spectra of cross-linked PAA nanoparticles. In case of 25% stoichiometric ratio, the degree of cross-linking was between 15% and 20%. Increasing the stoichiometric ratio, the degree of cross-linking increased. In the case of cross-linking at a stoichiometric ratio of 50%, it was between 30% and 45%, and it was 45–65% at a stoichiometric ratio of 100%. Based on these results, it can be concluded that polyanion cross-linked nanoparticles were prepared from PAA.

Transmittance

The transmittance of aqueous colloid systems containing cross-linked PAA nanoparticles was evaluated in deionized water at pH 6.5. Clear or opaque aqueous colloid systems were produced and were stable at room temperature for several days. Figure 4 summarizes the transmittance of the various nanoparticles composed of different molecular weight PAAs cross-linked with EDBEA at different cross-linking ratios. At lower ratio of the cross-linker, the swellability of the colloid dispersion was greater, and the aqueous systems were clear, caused by the deprotonation of free carboxyl groups of the PAA chain. An increasing ratio of cross-linker corresponds to decreasing the amount of residual carboxyl groups of PAA; the particles became more compact; therefore, the opalescence of solutions increased.

Considerable difference between the values of transmittance was found depending on the molecular weight of PAA. As the molecular weight of PAA increased, more opalescent nanosystems were performed. This trend has been observed, caused by the different swellability of nanoparticles depending on the permeability of cross-linked particles by the solvent molecules and the different commensurability of the size of nanoparticles with the operating wavelength.

Cross-linked PAA nanoparticles form colloid dispersions in neutral condition. These colloid dispersions are opalescent systems; the transmittance values vary between 45% and 95% at neutral pHs. It was observed that the transmittance of cross-linked particles increased by increasing the pH (Fig. 5). Residual carboxyl groups of PAA chains deprotonate by increasing the pH; the particles become more compact; therefore, the ratio of particle size and the wavelength changes caused rising transmittance values.

Particle size by DLS

Solution samples were prepared from the reaction mixture after dialysis. The concentration of the PAA solution was 0.70 mg/ml. The pH of the samples was adjusted by addition of hydrochloric acid solution in the presence of 0.01 M sodium chloride.

In polydispersed systems, the final results depend on the method of fitting. The average hydrodynamic diameters were calculated by non-negative least squares (NNLS) method, which separated the different peaks at multimodal distribution and provided more exact results at multimodal systems than obtained with other methods. The intensity-delay time correlation function was evaluated by means of NNLS fit; the called automatic routine was applied to

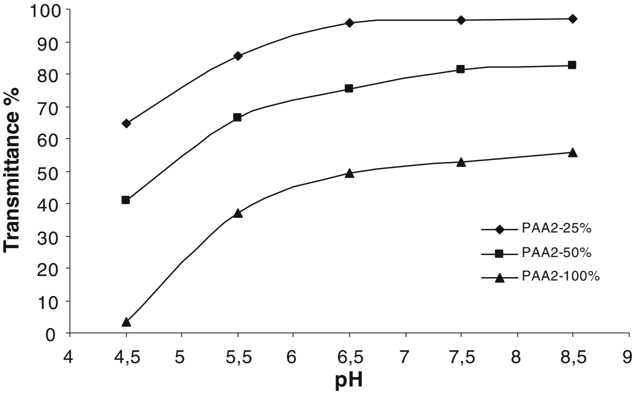


Fig. 5 Effect of pH on the transmittance of cross-linked nanoparticles (*c*=0.7 mg/mL)

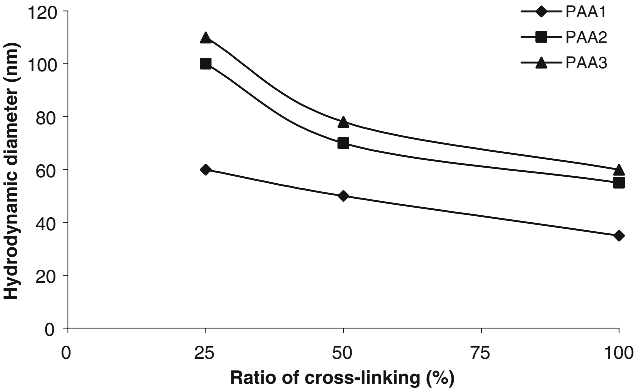


Fig. 6 Average hydrodynamic diameter of PAA nanoparticles measured at pH 6.5 in 0.01 M NaCl solution

determine the intensity diameter distribution. The effect of dust was cancelled by averaging of numerous simultaneous measurements. Figure 6 summarizes the average hydrodynamic diameters of swelled cross-linked PAA nanoparticles.

PAAs with different molecular weights were used for the cross-linking modification at different stoichiometric ratios. The cross-linked nanosystems were stable in aqueous media and can swell. It was found that the ratio of cross-linking and the molecular weight of PAA influence the hydrodynamic diameter of nanoparticles (Fig. 6). Compact particles with smaller size were produced by increasing the ratio of cross-linking independently of the molecular weight of PAA. The DLS measurements indicate that the average hydrodynamic diameter of nanoparticles is between 35 and 110 nm.

Direct but no typical relationship was observed between the size of spherical particles and the molecular weight. Considerable relationship was found between the values in the case of PAA1 and PAA3, but no significant differences were found between the sizes if the molecular weight of PAA is similar (PAA2 and PAA3). Therefore, the hydrodynamic size of cross-linked nanoparticles can be varied by changing the molecular weight of PAA: hydrodynamic diameter of particles can reduce decreasing the molecular weight of PAA.

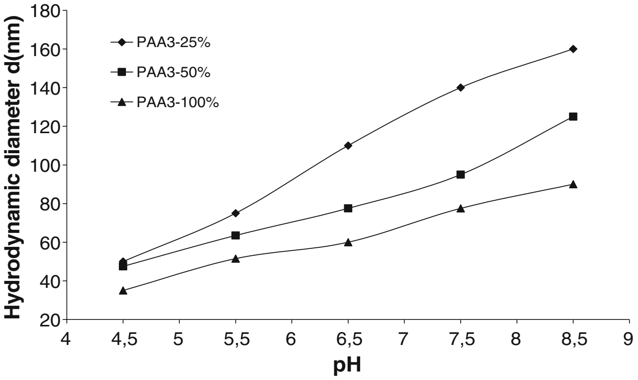


Fig. 7 Effect of pH on the hydrodynamic diameter of cross-linked PAA nanoparticles

Figure 7 shows the hydrodynamic diameters of cross-linked PAA nanoparticles measured at different pH. Average size of particles increased with increasing the pH, due to the charges of these macromolecules influencing the size of nanoparticles. The residual carboxyl groups of PAA chains can be deprotonated, and the repulsive interaction between the negative ions raise the degree of swelling and the size of nanoparticles. The amount of residual carboxyl groups decreased with increasing the ratio of cross-linking; therefore, the hydrodynamic diameter of cross-linked nanoparticles increased by decreasing the ratio of cross-linking.

The pH dependence shows that the cross-linked PAA nanoparticles can swell in aqueous media. The size of nanoparticles increased by increasing the pH, depending on the ratio of cross-linking. In case of cross-linking, ratio was 25%; the increasing of particle size as well as their swelling was 320%. This trend was similar, namely, 260%, in the case of 50% and 100% cross-linking ratios.

In summary, the average hydrodynamic diameters of swelled cross-linked PAA nanoparticles varied between 35 and 160 nm. To all appearances, the nanoparticles swell in aqueous media, and the ratio of cross-linking, the molecular weight of PAA, and the pH influence the hydrodynamic size of the spherical nanoparticles.

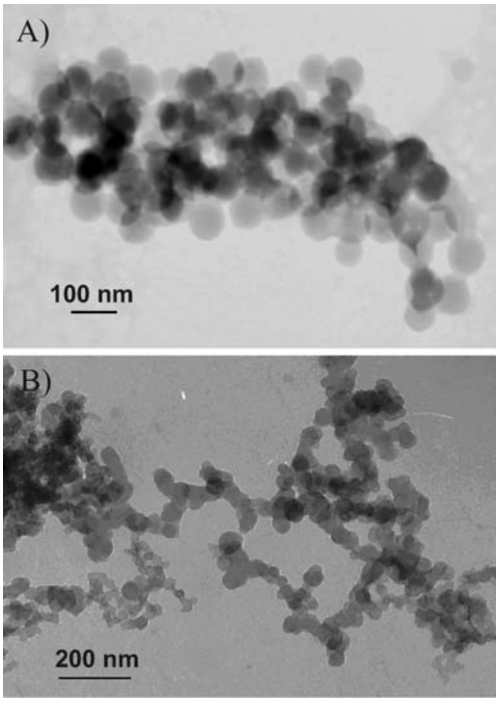


Fig. 8 TEM micrographs of poly(acrylic acid) nanoparticles cross-linked with EDBEA based on PAA1 at 25% (**a**) and PAA1 at 50% (**b**)

Particle size by TEM

The cross-linked PAA nanoparticles can be separated into spherical particles in an aqueous environment and in dried states. TEM micrographs (Fig. 8) confirmed the nanosize of dried, cross-linked PAA particles. The size of the dried particles varied in the range of 20 and 80 nm. The diameters of cross-linked PAA resulting from the TEM experiments were smaller than the swollen particles obtained from DLS. This result supports the establishment that these nanoparticles can swell in aqueous media. The difference between the size of nanoparticles in swollen and in dried state cannot be resolvable, because TEM micrographs show only two dimensions and the plane image cannot be comparable with the stereoscopic hydrodynamic size.

Conclusions

We have shown that nanoparticles based on biocompatible PAA has been successfully cross-linked by condensation reaction using EDBEA as a cross-linking agent. Cross-linked nano-sized particles were prepared and characterized from PAA with different M_w at different ratios of cross-linking. Clear or opalescent stable colloid systems were fabricated in aqueous medium at room temperature. Transmittance values of aqueous solutions containing PAA nanoparticles varied between 3% and 99% depending on the pH of the solution, the molecular weight of PAA, and ratio of cross-linking. Average hydrodynamic diameters of particles ranged from 35 to 160 nm. There was a direct but weak correlation between the particle size and the ratio of cross-linking as well as the molecular weight of PAA.

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