



Carboxyl functionalized poly(methyl methacrylate-acrylic acid-ethylene glycol dimethacrylate) copolymer particles and their amination with amine-nucleophiles

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Abstract: Poly(methyl methacrylate-acrylic acid-ethylene glycol dimethacrylate) copolymer particles abbreviated as P(MMA-AA-EGDM) copolymer particles were prepared by soap-free emulsion copolymerization. The concentration of carboxyl group on/near the surface of particles increased with the increase in acrylic acid (AA) content in the recipe. The carboxyl groups were reacted with amine nucleophiles such ethylene diamine and 1,6-diaminohexane through preactivation with dicyclohexyl carbodiimide as a coupling agent. The modified particles were characterized by electron micrographs and FTIR spectral analysis. Adsorption behaviours of some biomolecules were measured to have idea about the polarity of the particles surface.

Introduction

Monodispersed polymer particles in the submicron-size range bearing reactive functional groups have been widely studied and used in biomedical and biochemical fields, material science, calibration standards and information industries [1-4]. Leigh B. Bangs reported the usefulness of polystyrene type particles bearing aldehyde, carboxyl and to a lesser extent, amino groups, for the covalent binding of proteins [5, 6]. However, since many of these applications are influenced by morphology, surface properties and functional group density, attention has been focused on the proper designing of such polymer particles. Emulsion polymerization has been widely used to produce submicron-sized monodisperse polymer particles with various functional groups [7-9]. Considering the advantages of emulsion polymerization over other preparation techniques, from the beginning of the 1990's, researchers have concentrated on the preparation of carboxylated polymer microspheres as the surface carboxyl groups can be used for the covalent attachment of biomolecules [10-13]. Additionally the presence of carboxyl groups on/near the surface of particles is believed to improve properties like colloidal stability, mechanical and freeze thaw stability, rheology and adhesion [14, 15]. Polpanich et al. reported the polymerization kinetics of acrylic acid (AA) with relatively hydrophobic styrene [16]. The rate of polymerization was directly proportional to the AA content and the average particle size decreased with the increase in AA. The structural ordering and dynamics of 97±7 nm-sized charged poly(styrene-AA) latex particles were investigated as a function of volume fraction of the particles [17]. However, since these particles were

prepared in presence of anionic sodium dodecyl sulphate, the particles surface is expected to be not cleaned. In some recent works we reported the preparation of micron-sized carboxylated polymer particles mainly by seeded copolymerization in presence of polystyrene seed particles [18-20] and studied their usefulness as carriers for biomolecules.

In the present investigation submicron-sized crosslinked carboxyl functionalized copolymer particles were prepared by soap-free emulsion copolymerization of methyl methacrylate (MMA), AA and ethylene glycol dimethacrylate (EGDM). The carboxyl groups were derivatized by reacting with amine-nucleophiles to produce aminated particles. Physical attachment of three different biomolecules was then studied to understand the hydrophobicity of particles surface. The objective of this work is to diversify the applications of carboxylated polymer microspheres particularly in biological field. This work may open the possibility to design novel materials by incorporating new functional group on the surface of carboxylated particles via derivatization and thus controlling surface polarity and biocompatibility. Depending on the requirements and purposes, multifunctional polymer particles can also be prepared from carboxylated particles by partial or controlled reaction. In a related work, we reported the preparation of two different slow-release fertilizers from the respective homopolymer of AA and methacrylic acid (MAA) by covalent fixation with urea [21, 22].

Results and discussion

P(MMA-AA-EGDM) copolymer particles were prepared with varying proportions of AA by soap-free emulsion copolymerization. The influence of AA content on the percent conversion of polymerization reaction is shown in Figure 1. In each copolymerization reaction conversion increased with polymerization time and reached more than 90% within an hour. The final conversion did not go beyond 95% for P(MMA-AA-EGDM) copolymer particles prepared with 10 and 15% (w/w) AA contents whereas that prepared with 20% (w/w) AA content it reached 100%. At lower AA content (10 and 15%) colloidal stability possibly affected the final conversion as copolymer particles were partially coagulated.

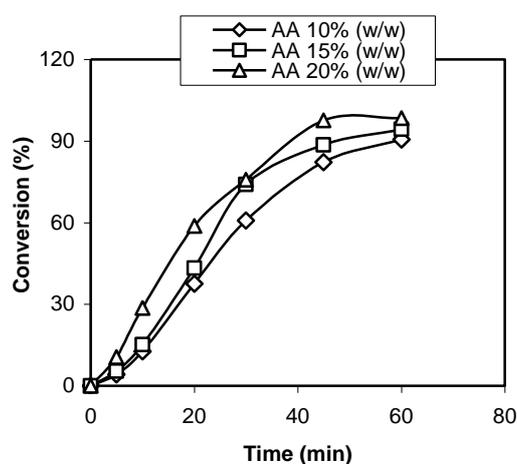


Fig. 1. Conversion-time curves in dependence on AA content at 70 °C in soap-free emulsion copolymerization of MMA, AA and EGDM.

Tab. 1. Rate of Polymerization (R_p).

AA content % (w/w)	R_p (%min ⁻¹)
10	2.1065
15	2.5394
20	2.635

The presence of higher number of carboxyl groups on the particle surface derived from higher AA content (20%) increased the stability of the nucleated primary particles. The initial conversion rate (R_p) seems to be a good measure to compare the polymerization reaction with varying AA contents. R_p was obtained from the least-square-best-fitted slope at the linear portion of the conversion-time curves in the period of 0 to 75% conversion. The dependence of R_p on AA content is shown in Table 1.

R_p increased with the increase in AA content. This behaviour can be explained by considering the homogeneous nucleation mechanism in which the increase in R_p is based on the increase in the number of primary particles formed in the aqueous phase during the early step of polymerization [16, 23]. It is already known that persulfate radicals start polymerization of monomers dissolved in the aqueous phase, producing oligomer radicals [16]. As the oligoradicals grow to a critical chain length, they precipitate and coagulate to form primary particles. An increase in AA content in the recipe at the expense of relatively hydrophobic MMA is therefore expected to increase the rate of formation of oligoradicals or oligomers and hence to some extent more primary particles are produced.

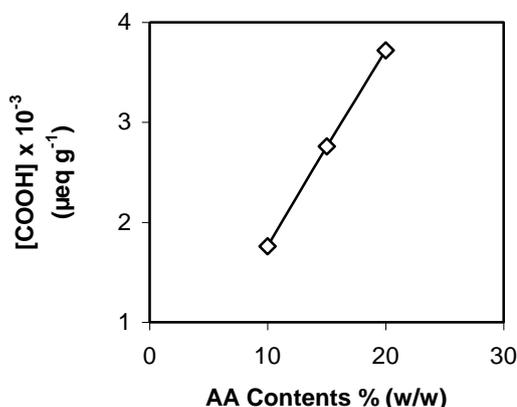


Fig. 2. Carboxyl group density on the surface of P(MMA-AA-EGDM) copolymer particles prepared with various AA contents.

Figure 2 shows the change in carboxyl concentration on or near the surface of washed P(MMA-AA-EGDM) copolymer particles prepared with varying proportions of AA. The carboxyl group density increased linearly with the increase in AA content. This behaviour suggests that as the AA content increased most of the AA participated in the copolymerization reaction with MMA and EGDM rather than in homo-polymerization. It is to be mentioned that only trace amount (<1%) of

copolymer particles which were coagulated during copolymerization, prepared with 10 and 15% AA contents, were removed before the analysis.

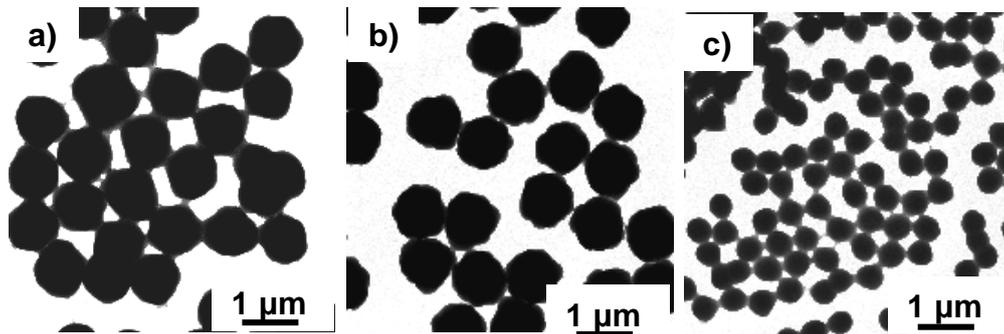


Fig. 3. TEM photographs of P(MMA-AA-EGDM) copolymer particles prepared with AA contents (w/w): 10% a); 15% b) and 20% C).

Figure 3 shows the TEM photographs of P(MMA-AA-EGDM) copolymer particles prepared with various AA contents. The particles are non-spherical and largely monodispersed as coefficient of variations measured from TEM photographs were in the range of 4-6%. The average hydrodynamic diameters measured by NICOMP particle sizer were 1.12 μm, 0.95 μm and 0.55 μm for copolymer particles prepared with 10, 15 and 20% (w/w) AA contents, respectively. In the dried state the average diameters measured from TEM photographs were much lower than those of hydrodynamic diameters and observed as 0.83 μm, 0.80 μm and 0.43 μm respectively.

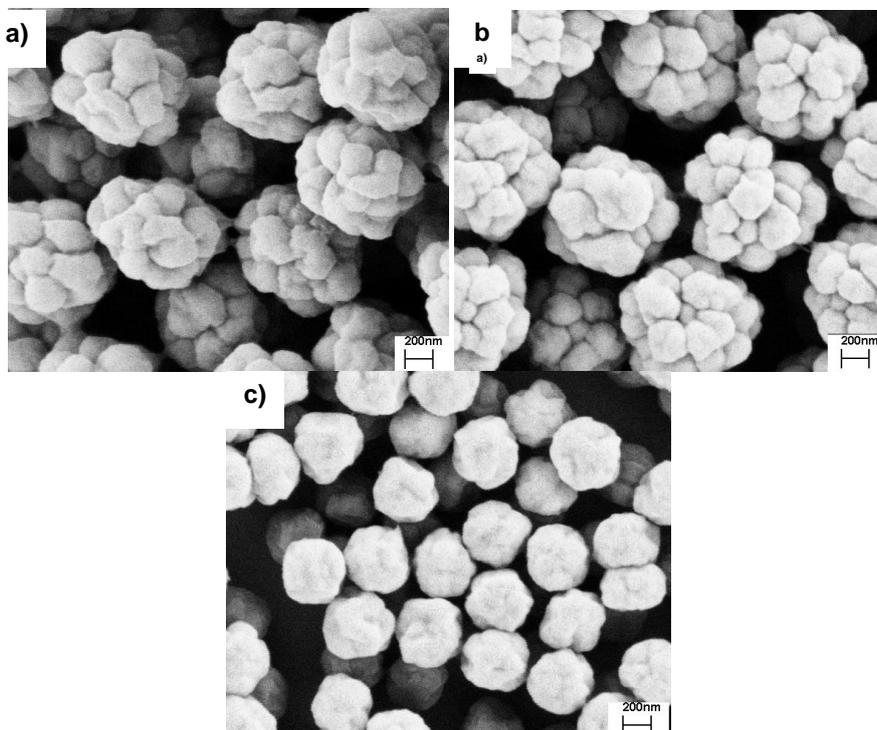


Fig. 4. SEM photographs of P(MMA-AA-EGDM) copolymer particles prepared with AA contents (w/w): 10% a); 15% b) and 20% C).

The average particle size decreased with the increase in AA content in P(MMA-AA-EGDM) copolymer particles i.e. the average size is inversely proportional to the AA content. The presence of higher amount of ionic functional AA monomer produced greater number of particle centre for polymer growth. Since the total amount of monomers remained constant in all formulations, the greater the number of primary particles formed the lower was the average particle size.

The surface morphology is best observed with SEM photographs as shown in Figure 4. The surface of the particles is not smooth and the appearance is independent of the AA content. Some authors reported similar observations in crosslinked particles prepared by radical polymerization in non-aqueous media [24, 25]. The dented surface in P(MMA-AA-EGDM) particles is possibly attributed to the non-homogeneous swelling and growth of comparatively rigid primary particles formed due to the inclusion of EGDM as cross-linking agent. The introduction of AA as a comonomer may also be partly responsible for the formation of such dented surface as the interfacial tension between the particles and continuous phase was reduced [26].

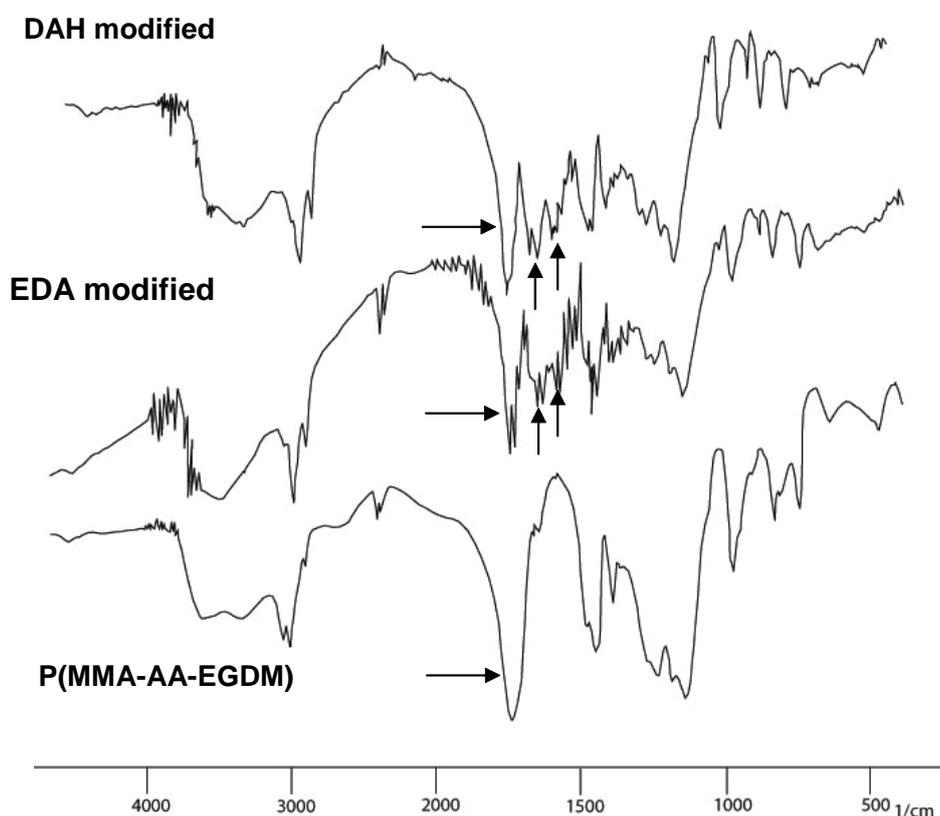


Fig. 5. FTIR spectra of P(MMA-AA-EGDM), EDA and DAH modified particles.

The amination of carboxyl functionalized copolymer particles was carried out with diamines such as ethylene diamine (EDA) and 1,6 diaminohexane (DAH). The copolymer particles containing the highest carboxyl group density on/near the surface was used for this modification. Figure 5 shows the FTIR spectra of P(MMA-AA-EGDM) copolymer particles before and after amination with EDA and DAH, respectively. In the spectra of P(MMA-AA-EGDM) copolymer particles a broad O-H

stretching of carboxyl group appeared at 2600-3500 cm^{-1} . An intense signal due to superimposed C=O stretching for carboxyl and ester groups appeared at 1732 cm^{-1} . In the aminated particles the absorption bands due to C=O stretching for –CONH- linkage with EDA and DAH are shifted to 1685 and 1627 cm^{-1} , respectively and the respective N-H bending appeared near 1550 cm^{-1} . Additionally the signal due to C=O stretching for ester group of MMA remained at near 1720 cm^{-1} . The most characteristic absorption band due to N-H stretching of free primary amine is expected to be in the region 3200-3500 cm^{-1} . The shape of the broad signal due to O-H stretching of carboxyl group in P(MMA-AA-EGDM) copolymer particles has been changed in amine functionalized polymer particles and appeared almost in the same region. The inclusion of moisture and even the presence of trace residual carboxyl group in amine functionalized particles might affect the appearance of N-H stretching. The above results may suggest that the most of the carboxyl groups lying on/near the surface of copolymer particles were bonded with diamine, EDA/DAH, via substituted amide (-CONH-) linkage.

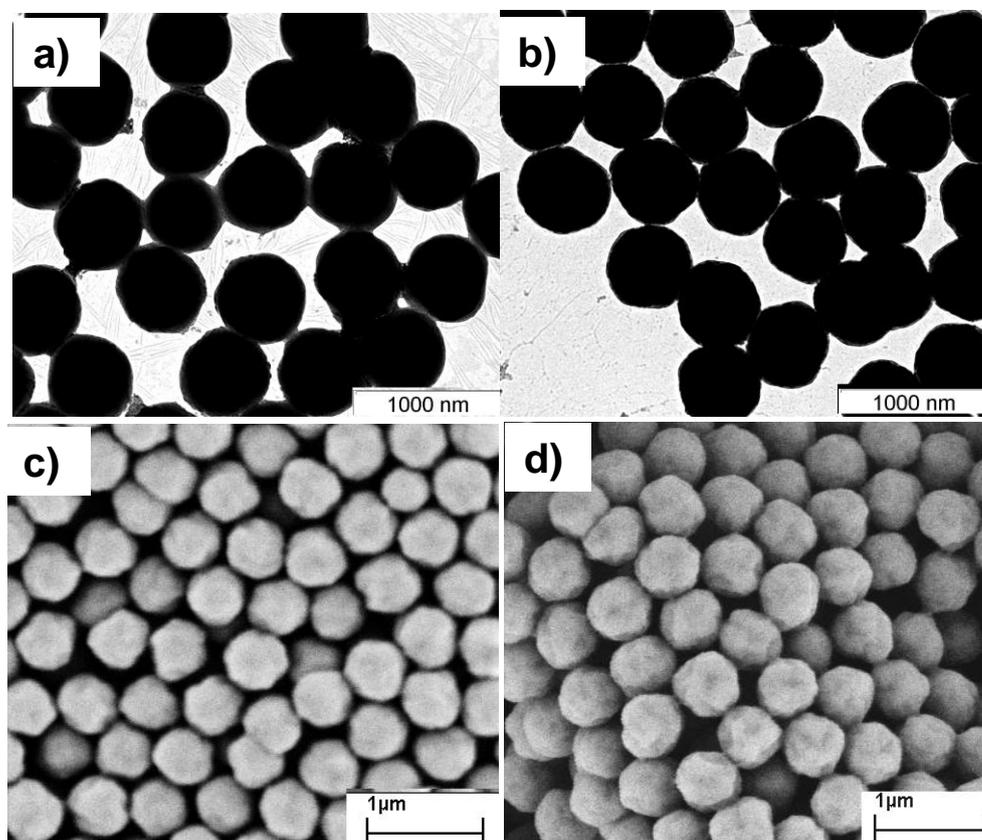


Fig. 6. TEM (a, b) and SEM (c, d) photographs of EDA (a, c) and DAH (b, d) modified P(MMA-AA-EGDM) copolymer particles.

Figure 6 shows the TEM and SEM photographs of EDA and DAH modified particles. The average diameters and coefficient of variations measured from TEM photographs are 0.65 μm and 3.16% for EDA; 0.7 μm and 2.77% for DAH modified particles, respectively. The average size of both aminated particles increased considerably as compared to that measured from the TEM photograph (0.43 μm) of P(MMA-AA-EGDM) particles before amination reaction. The surface morphology of both modified particles changed and became relatively smooth. Moreover, relative to

P(MMA-AA-EGDM) particles both aminated particles were stable under the electron beam. These results indicate that P(MMA-AA-EGDM) copolymer particles were mostly modified and covered with primary amine groups.

In the application phase, the adsorption of three different biomolecules such as trypsin (TR), albumin (AL) and casein (CS) were studied on the copolymer particles. It is well known that the magnitude of adsorption is largely dependent on the degree of hydrophobic interaction between the particles surface and biomolecules. Figure 7 shows the adsorption behaviours of biomolecules. Since the particles were not of same size the amounts of adsorption are presented in both, per unit mass (Figure 7A) and per unit surface area (Figure 7B) of the particles. This study was carried out at the respective isoelectric point of biomolecule to neutralize the effect of ionic interaction of the biomolecule with the particle surface. The magnitude adsorption of TR, AL and CS varied with the nature of copolymer particles. Irrespective of unit mass or unit surface area of the particles, the magnitude of adsorption on P(MMA-AA-EGDM) particles is the lowest followed by DAH and EDA modified particles. This behaviour is related to the relatively hydrophilic surface of P(MMA-AA-EGDM) particles due to the presence of polar carboxyl groups on/near the particles surface. The surface of DAH modified particles is expected to be more hydrophobic than EDA modified particles because the former has a longer hydrocarbon chain. Contrary to this, the magnitude of adsorption on DAH modified particles is slightly lower except for AL (Figure 7B) than that on EDA modified particles. This adsorption behaviour may suggest that carboxyl groups have partially been aminated by relatively hydrophobic DAH leaving some unreacted carboxyl groups. The longer hydrocarbon chain of DAH may have hindered the amination reaction to some extent. It is to be mentioned that different amounts of adsorption of TR, AL and CS on the same polymer particles was affected by the variations in molecular weight, size, shape, chain flexibility and hydrophobicity of biomolecules.

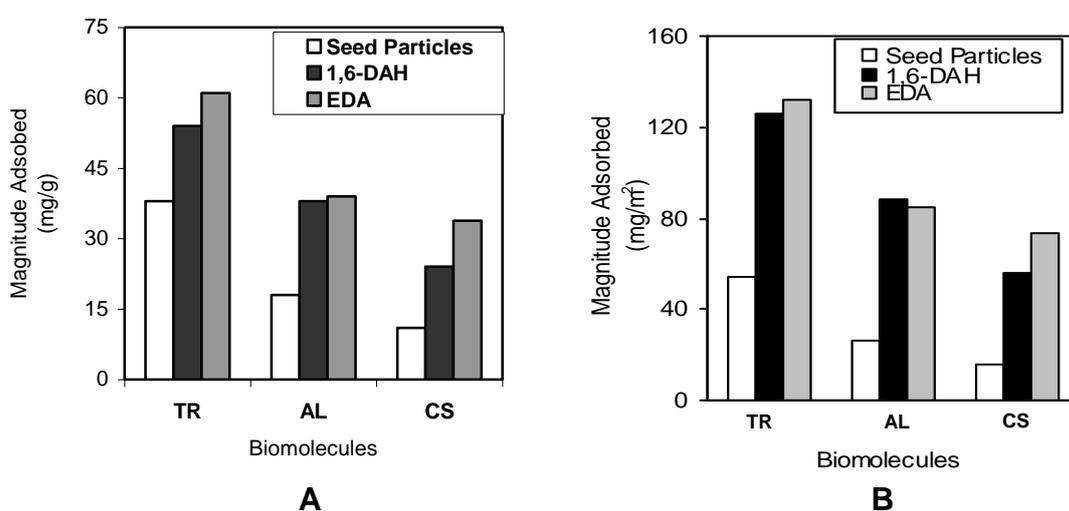


Fig. 7. Magnitude of adsorption of TR, AL and CS as biomolecules on P(MMA-AA-EGDM), DAH and EDA modified particles under constant concentration against the total solid content at 25 °C. Biomolecules immobilized: 200 mg/g of particles; polymer solid 0.1 g; immobilization time 60 min.; pH, respective isoelectric point (TR, pH 10; AL, pH 6; CS, pH 7.5). Magnitude of adsorption is expressed in mg/g (A), and in mg/m² (B) of particles.

Conclusions

The amination of carboxyl functionalized P(MMA-AA-EGDM) particles was successfully carried out with diamine nucleophiles. The surface homogeneity of the cross-linked particles improved after amination as observed from the electron micrographs. The modified particles showed variable hydrophobicity. This research opened up the possibility of derivatizing the functional polymer particles according to the needs without disturbing the colloidal stability.

Experimental parts

Materials and techniques

MMA and AA of monomer grade, purchased from Fluka Chemika, Switzerland, were distilled under reduced pressure and preserved in the refrigerator until use. EGDM from Fluka Chemika, Switzerland was used without purification. KPS of LOBA Chem, India, was recrystallized from water at low temperature. Dicyclohexyl carbodiimide (DCC), EDA and DAH all from LOBA Chem. India, were used as received. TR, CS and AL were used as biomolecules without purification. Other chemicals were of reagent grade. Deionized water was distilled using a glass (Pyrex) distillation apparatus.

Copolymerizations

P(MMA-AA-EGDM) copolymer particles with varying amounts of AA in the recipe were prepared by soap-free emulsion copolymerization using KPS as initiator. Polymerizations were carried out in a three-necked round bottom flask under a nitrogen atmosphere at 70 °C. Polymerization conditions are shown in Table 2.

Tab. 2. Preparation of cross linked P(MMA-AA-EGDM) particles by soap free emulsion copolymerization^{a)}.

Latex	H ₂ O (g)	AA (g)	MMA (g)	EGDM (g)	KPS (g)
1	100	1.5	13.05	0.45	0.15
2	100	2.25	12.30	0.45	0.15
3	100	3.00	11.55	0.45	0.15

^{a)}70°C, 12 h, N₂, 120 rpm

The intensity weighed average hydrodynamic diameters of the latex particles were measured with a NICOMP 380 particle sizer (Santa Barbara, California, USA). In this case the polymer samples were diluted with distilled water. The error in the reproducibility of the size measurements was around $\pm 4\%$. For transmission electron microscopy (TEM) observations the latex samples were diluted to about 0.1% solid by distilled water and then a drop was placed on a carbon coated copper grid. The samples were dried at ambient temperature before observation by a Zeiss EM 912 Omega microscope. Scanning electron microscope or SEM (LEO Electron Microscopy Ltd, UK) was also used to see the images of PS particles.

Overall monomer conversion and rate of polymerization

Polymer samples were withdrawn from the reactor at definite time interval and placed in a pre-weighed dried ceramic Petri dish. To prevent further polymerization the ceramic dish contained a known amount of 1% hydroquinone solution and was

immediately quenched in an ice water bath. Samples were kept in an oven at around 80 °C until a constant weight was reached. Then the percentage of overall monomer conversion was calculated from the solids content by taking into account the amount of auxiliary materials. The procedure as applied was quite reproducible as the conversion data could be reproduced with an accuracy of \pm (4-6)%. The rate of polymerization, R_p , was obtained from the least-squares-best-fitted slope at the linear portion of the conversion-time curves in the period of 0 to 70% conversion.

Measurement of Carboxyl value

The number of microequivalents of acid groups (-COOH) per unit mass of P(MMA-AA-EGDM) latex particles was measured by conductometric back titration on 0.1 g latex particles dispersed in 50 mL distilled deionized water at 20 °C. Excess amount of 0.01 M NaOH solution was first added into the diluted latex. Back titration was then started with 0.01 M HCL under mild stirring. The change in conductivity value of the latex was measured by a conductometer. The number of microequivalents of acid groups was calculated by using the following relationship:

$$[COOH] = \frac{V_{HCL} \cdot M_{HCL} 10^{-3}}{\text{Mass of latex (gm)} \times \text{Solid content}} \text{ (}\mu\text{eg/g)}$$

Amination of polymer particles

P(MMA-AA-EGDM) copolymer particles prepared with 20% (w/w) AA content were transferred from water medium to ethanol medium by ultra-centrifugal washing at 12,000 rpm and the solid content of the latex was adjusted to about 2%. EDA and DAH were covalently bonded to the carboxyl groups of copolymer particles by the following method.

100 g of latex dispersion was taken with 1.25 gm coupling agent (DCC) in a reagent bottle, magnetically stirred at 25 °C for 30 min. The activated carboxyl group was then reacted with 0.35 g EDA or 0.69 g DAH at 25 °C for 3 hr and then again at 0 °C for 24 h. The particles after modification were characterized by FTIR and electron micrographs.

Adsorption of biomolecules

A mixture of 20 mL was prepared from each purified polymer dispersion (polymer solid 0.1 g) and biomolecule (20 mg). Phosphate buffer was used to adjust the pH value of the mixture at the respective isoelectric point (TR, pH 10; AL, pH 6; CS, pH 7.5). The mixture was allowed to stand at 25 °C for 60 min, and then centrifuged at 10,000 rpm for 15 minutes. In order to remove the dust particles completely, the supernatant was centrifuged once more at 10,000 rpm. The concentration of the biomolecule in the supernatant was determined by UV-visible spectrophotometer at 280 nm. The magnitude of adsorption was calculated by subtracting concentration of biomolecule in the medium from that of the initial concentration. Calibration curve was used for this purpose.

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