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SHORT COMMUNICATION

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A study on the production of PMMA/P(MMA-AAm) composite polymer particles and the effect of acrylamide content on the adsorption behaviors of biomolecules

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Abstract A series of poly(methyl methacrylate)/poly(methyl methacrylate-acrylamide) composite polymer particles was prepared with varying proportions of acrylamide in the copolymer shell layer. Adsorption behaviors of some biomolecules and specific activity of adsorbed trypsin were studied. The hydrophobic interaction between the composite polymer particle surfaces and biomolecules decreased with increasing acrylamide content.

Key words Composite polymer · Adsorption behavior · Acrylamide · Biomolecules · Hydrophobic interaction

Introduction

Polymer particles [1–4] with well-defined colloidal and surface properties can be useful in designing bioreactors, biosensors and bioseparators. However, strong hydrophobic interactions between the polymer particle' surfaces and biomolecules often lead to their denaturation [5, 6] with loss of activity. Sugiyama et al. [7] studied the surface modification of polymer microspheres prepared by the emulsion copolymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC) with various vinyl monomers. The localization of the MPC moiety on the copolymer particles decreased the amount of adsorption of bovine serum albumin. Okubo [8] reported that the composite microsphere, having a hydrophilic-hydrophobic heterogeneous surface structure, was an excellent carrier for adsorption immobilization without a reduction in enzymatic activity.

So far most studies have been based on the interaction of copolymer microspheres with biomolecules. Composite polymer particles having core-shell morphology rather than copolymer microspheres were used in this study, as particle size, monodispersity and surface characteristics can be controlled more precisely. Here we report the preparation of a series of poly(methyl methacrylate) (PMMA)/poly(methyl methacrylate-acrylamide) [P(MMA-AAm)] composite polymer particles of different acrylamide contents. The surface characteristics of the prepared composite polymer particles, their relationship with the adsorption behaviors of a macromolecule, different biomolecules and the activities of the adsorbed trypsin (TR) were studied and compared.

Materials and methods

Materials

Methyl methacrylate (MMA) of monomer grade, purchased from BDH, England, was distilled under reduced pressure. Acrylamide (AAm) also of monomer grade and purchased from BDH, was purified by recrystallization from water. Potassium persulfate (KPS) was recrystallized from water before use. The biomolecules used were TR from Merck, Germany, albumin (AL) from LOBA, India, casein (CS) from Matheson Coleman & Bell, USA, and lysozyme (LZ) from Fluka, Switzerland. Emulsifier, polyoxyethylene sorbitan monooleate (Tween-80) (Fluka), was used as a macromolecule. L-Lysine monohydrochloride purchased from BDH was used for the preparation of lysine methyl ester hydrochloride (LME). Other chemicals used were of reagent grade. Deionized water was distilled using a glass (Pyrex) distillation apparatus.

Preparation of PMMA seed particles

PMMA seed particles were prepared by soap-free emulsion polymerization of 30 g MMA using 0.3 g KPS as a water-soluble initiator. Polymerization was carried out in a three-necked round bottom flask under a nitrogen atmosphere at 70 °C for 12 h.

Preparation of PMMA/P(MMA-AAm) composite polymer particles

PMMA/P(MMA-AAm) composite polymer particles were prepared by seeded emulsion copolymerization of MMA and AAm with PMMA seed particles using water-soluble KPS as initiator under a nitrogen atmosphere in a round bottom three-necked flask. Preparation was carried out at various AAm contents with a constant core/shell ratio of 1/0.8. The preparation conditions are detailed in Table 1.

Adsorption of emulsifier and biomolecules

A mixture of 20 ml was prepared from each purified emulsion (polymer solid, 0.1 g) and biomolecule (50 mg)/emulsifier (40 mg) aqueous solution. In the case of emulsifier the concentration was kept below the critical micelle concentration. For biomolecules, the pH value of the mixture was immediately adjusted to the respective isoelectric point (TR, pH 10.00; AL, pH 6.00; CS, pH 7.50; LZ, pH 10.50) and for the emulsifier Tween-80, the pH value was adjusted to 7.00. The mixture was allowed to stand at 20 °C for 45 ± 5 min, and then centrifuged at 10,000 rpm for 10 min. In order to remove wafting particles completely, the supernatant was centrifuged once more at 10,000 rpm. The concentration of the emulsifier and biomolecule in the supernatant was determined by ultraviolet spectrophotometer at 250 and 280 nm respectively. The magnitude of adsorption was calculated by subtracting concentration in the medium from that of the initial concentration. A calibration curve was used for this purpose.

Preparation of LME

LME was prepared by following the conventional procedure [9] of ester preparation with minor modifications. The ester obtained was dried in a vacuum desiccator over anhydrous calcium chloride and characterized as LME from its sharp melting point (196.5 °C), thin layer chromatography measurement and NMR spectra.

Measurement of specific activity of adsorbed TR

The enzymatic activities of adsorbed TR were determined at 20 °C by pH stat. method [10], using LME as a substrate according to the following procedure.

Purified seed or composite emulsion was mixed with a known amount of TR aqueous solution and the pH was immediately adjusted to 10.00 using 0.02 M KOH. In each case, TR concentration (50 mg/g of particles) was kept constant and maintained well below the minimum amount (115.50 mg/g of particles) adsorbed by composite particles prepared with 70% (w/w) AAm content. The emulsion/TR mixture after being maintained at 20 °C for 45 ± 5 min was mixed with 100 ml of 10^{-3} mol/l aqueous LME. Then the pH was immediately adjusted to 10.00 and maintained there with 0.02 M KOH under constant stirring at the respective temperature for 3 min. The activity of the adsorbed TR was calculated from the amount of KOH consumed to neutralize the acid liberated from the hydrolysis of LME. The unit of specific activity was expressed as micromoles per minute per milligram of TR adsorbed.

Table 1 Preparation of poly(methyl methacrylate)(*PMMA*)/poly(methyl methacrylate-acrylamide [*P(MMA-AAm)*] composite polymer particles with a constant core/shell ratio of, 1/0.8, carried out at 70 °C, for 12 h, at 100 rpm

AAm content (%, w/w)		10	30	50	70
PMMA emulsion[a]	g	31.44	31.44	31.44	31.44
MMA	g	2.16	1.68	1.2	0.72
AAm	g	0.24	0.72	1.2	1.68
Potassium persulfate	g	0.024	0.024	0.024	0.024
Water	g	170	170	170	170

[a] Solid content 95.4 g/l

Results and discussion

Figure 1 shows the transmission electron microscopic (TEM) photographs of unwashed PMMA seed and various PMMA/P(MMA-AAm) composite polymer particles prepared with different AAm contents. Both seed and composite polymer particles were spherical at the beginning of TEM observation, but since the taking of TEM photographs is a time consuming process, and PMMA is weak to electron beams, the particles were deformed and observed as non-spherical in the photograph. The diameters and the coefficients of variation of PMMA seed and various composites prepared with AAm contents of 10% (w/w), 30% (w/w), 50% (w/w) and 70% (w/w) were respectively 0.27 μm and 6.78%, 0.28 μm and 2.36%, 0.29 μm and 2.36%, 0.29 μm and 2.59%, and 0.293 μm and 2.43%. It is apparent that the sizes of composite polymer particles were slightly larger than that of seed particles. The coefficients of variation show that both the seed and the composite particles were monodispersed. The absence of any tiny polymer particles smaller than PMMA seed particles, indicates that no P(MMA-AAm) copolymer particle was produced during seeded emulsion copolymerization. Moreover, the absorbance of the supernatant separated from each composite emulsion was nearly zero. These results suggest that seeded emulsion copolymerization proceeded mainly in the PMMA seed particles.

Figure 2 shows the bar diagram of the magnitude of adsorption of Tween-80 as macromolecule and LZ, AL, CS and TR as biomolecules on the seed and various composite polymer particles prepared with different AAm contents. The adsorption of biomolecules was measured at the isoelectric point of the respective species, to eliminate the effects of ionic interaction between the particle surface and biomolecule. The magnitude of adsorption decreased with increasing AAm content in the composite polymer particles. In the case of seed particles, the magnitude of adsorption was always higher than that of composite polymer particles. These results suggest that the introduction of hydrophilic AAm in the composite polymer particles decreases the hydrophobic interaction between the composite particle surface and biomolecules. The magnitudes of adsorption of Tween-80, LZ, AL, CS, and TR on the same type of polymer particles were not identical and decreased in the order of TR > AL > CS > LZ >

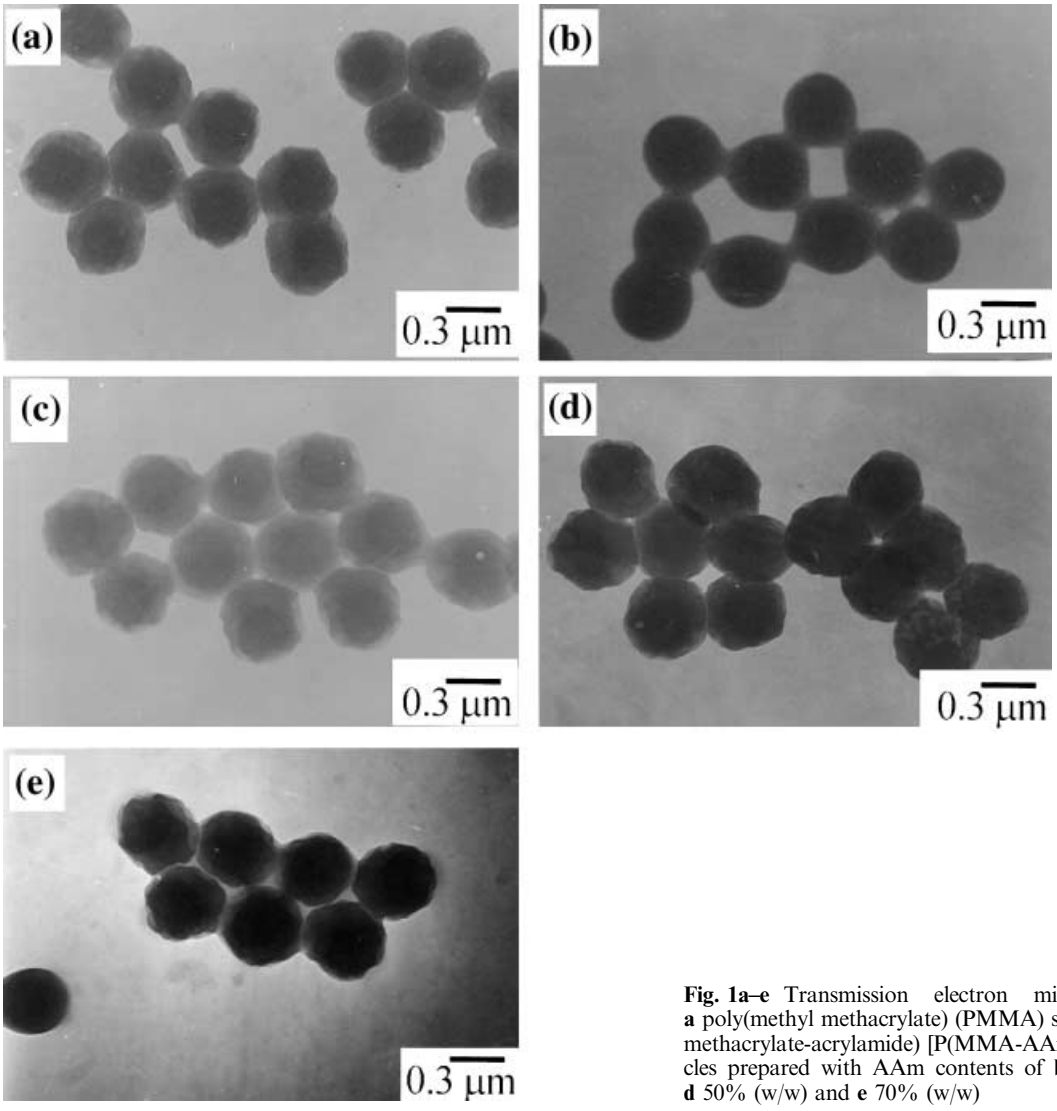

Fig. 1a–e Transmission electron microscopic photographs of **a** poly(methyl methacrylate) (PMMA) seed and PMMA/poly(methyl methacrylate-acrylamide) [P(MMA-AAm)] composite polymer particles prepared with AAm contents of **b** 10% (w/w), **c** 30% (w/w), **d** 50% (w/w) and **e** 70% (w/w)

Tween-80. This behavior is usually influenced by molecular characteristics, particularly molecular weight, size, shape, hydrophobicity and flexibility of the macromolecule and biomolecules [2, 3]. Since little information on the macromolecule and proteins is available, we can only assume that this difference in adsorption behavior on the same type of polymer particles is based on differences in molecular characteristics.

Figure 3 shows the variations of specific activities of adsorbed TR on PMMA seed and various PMMA/P(MMA-AAm) composite polymer particles prepared with different AAm contents. Specific activities of adsorbed TR on composite particles were always higher than that of adsorbed TR on seed particles. Specific activities of adsorbed TR also increased gradually with increasing AAm content in the composite polymer particles. This result suggests that the introduction of AAm makes the composite polymer particle surface increasingly hydrophilic.

Figure 4 shows the variations of specific activities of free TR (aqueous solution of TR) and adsorbed TR on PMMA seed and different PMMA/P(MMA-AAm) composite polymer particles prepared with AAm contents of 10% (w/w), 30% (w/w), 50% (w/w) and 70% (w/w) against time. This has been measured to observe the stability of the adsorbed TR with time. The activity of the adsorbed TR on composite polymer particles decreased slowly with time as compared to free TR. This is because, in the case of free TR, self-digestion occurred rapidly due to the frequent contact between active TR sites [1] whereas in the case of the adsorbed TR, such frequent contact among the active sites of TR was inhibited by the shielding effect of the polymer particles. In comparison with adsorbed TR on the PMMA seed,

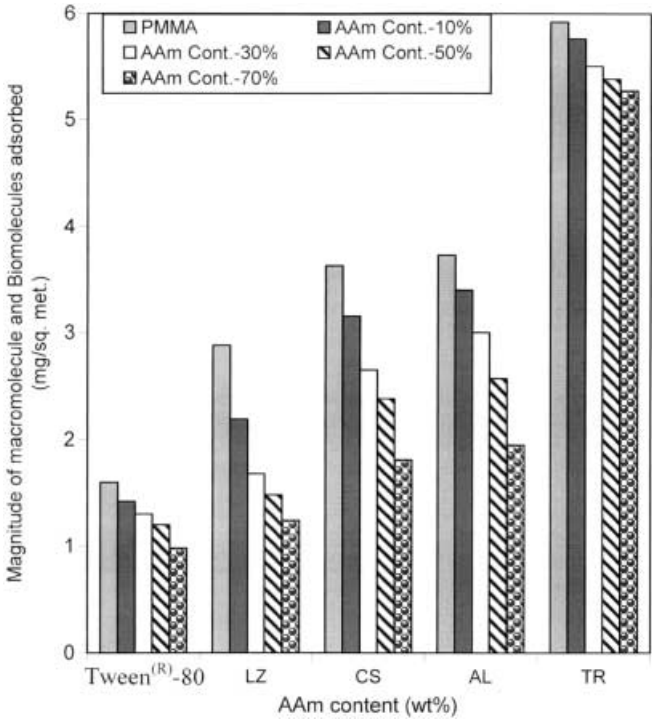

Fig. 2 Magnitude of adsorption of emulsifier Tween-80 as macromolecule and lysozyme (*LZ*), casein (*CS*), albumin (*AL*), and trypsin (*TR*) as biomolecules on PMMA seed and PMMA/P(MMA-AAm) composite polymer particles measured at 20 °C. Emulsifier: Tween-80, 400 mg/g of particles; polymer solid, 0.1 g; immobilization time, 45 ± 5 min; pH 7.00. Biomolecules: LZ, CS, AL, TR, 500 mg/g of particles; polymer solid, 0.1 g; immobilization time, 45 ± 5 min; pH, respective isoelectric point (LZ, pH 10.50; CS, pH 7.50; AL, pH 6.00; TR, pH 10.00)

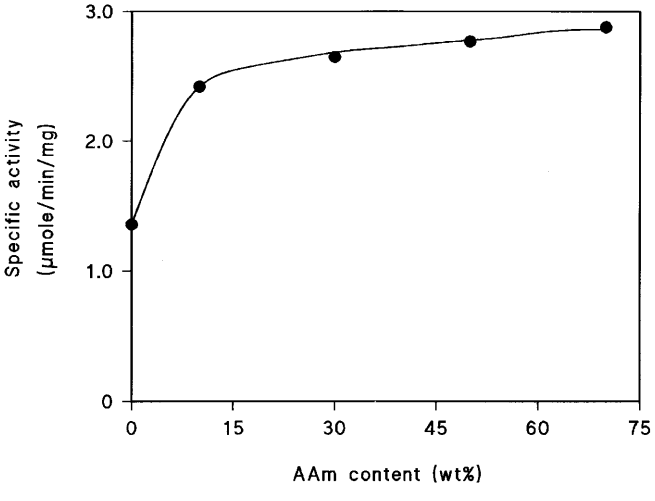

Fig. 3 Specific activities of adsorbed TR on PMMA seed and different PMMA/P(MMA-AAm) composite polymer particles measured at constant concentration at 20 °C. TR, 50 mg/g of particles; polymer solid, 0.1 g; immobilization time, 45 ± 5 min; pH 10.00

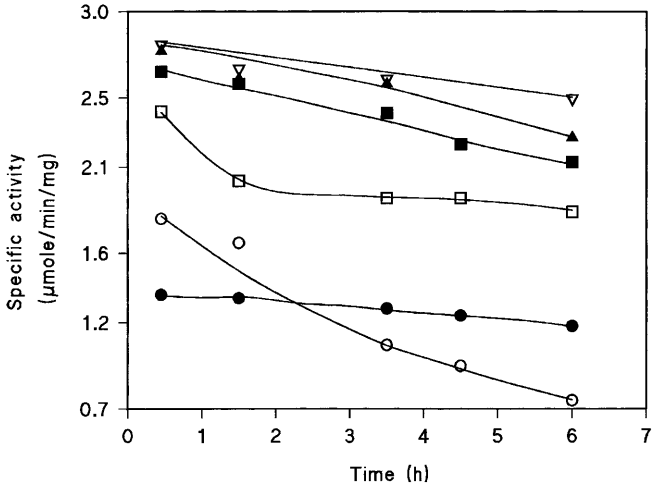

Fig. 4 Variations of specific activities of free TR (○) and adsorbed TR on PMMA seed (●) and PMMA/P(MMA-AAm) composite polymer particles prepared with AAm contents of 10% (w/w) (□), 30% (w/w) (■), 50% (w/w) (▲) and 70% (w/w) (▽) as a function of time measured at constant concentration at 20 °C. TR, 50 mg/g of particles; polymer solid, 0.1 g; immobilization time, 45 ± 5 min; pH 10.00

the specific activity of free TR was initially higher and then decreased gradually. This indicates that the native conformation of adsorbed TR collapsed immediately after the adsorption on the PMMA seed due to the relatively strong hydrophobic interaction with the particle surface rather than due to the gradual self-digestion which occurred with free TR. Moreover, regarding the specific activity, the composite polymer particles always exhibited higher efficiency as compared to seed particles. Figure 4 also shows the highest specific activity of adsorbed TR on composite polymer particles prepared with AAm content of 70% (w/w). It is generally believed that the efficiency of any adsorbed biological catalyst like TR is decreased with the increase in hydrophobic interaction between biomolecules and the substrate, and that this effect is more pronounced with increase of adsorption time [11, 12]. The higher activity of the adsorbed TR on composite polymer particles is due to the relatively weak hydrophobic interaction with the composite particle surface resulting from the incorporation of hydrophilic AAm.

These results suggest that the particle surface of PMMA/P(MMA-AAm) composite polymer particles prepared with AAm content of 70% (w/w) is sufficiently hydrophilic with a limited amount of hydrophobicity, can be useful as a carrier for biomolecules in designing bioreactors and bioseparators.

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