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Preparation of reactive polymeric microspheres by seeded copolymerization using a polymerizable surfactant bearing an active ester group

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Micron-size reactive polymeric microspheres with active ester groups on their surfaces were prepared by the seeded copolymerization of a polymerizable surfactant bearing an active ester group, namely *p*-10-undecenoyloxyphenyldimethylsulfonium methylsulfate (UPDS), and various dialkyl fumarates (DRFs), using 2,2′-azobis(2-amidinopropane)dihydrochloride (AIBA) and 2,2′-azobisisobutyronitrile (AIBN) as the initiators in aqueous systems. The DRFs were incorporated into monodisperse polystyrene (PS) microspheres of 4.1 μm diameter, and UPDS was then adsorbed on to the surface of the microspheres. When diethyl fumarate (DEF) was used as the comonomer, a bridging flocculation occurred even at a concentration of UPDS below the critical micelle concentration (c.m.c.), because the copolymer of UPDS with DEF is soluble in water. On the other hand, when di-n-butyl fumarate (DBF) or dioctyl fumarate (DOF), which both form water-insoluble copolymers with UPDS, were used as comonomers, and the concentrations of UPDS were lower than its c.m.c., the copolymerization of the adsorbed UPDS with, for example, DBF inside the particles, gave microspheres with active ester groups on their surfaces, without the formation of any new particles. The concentration of the active ester groups on the surface of the microspheres was controlled by keeping the initial particle size constant. The resulting microspheres modified with UPDS units on their surfaces were found to be highly reactive with the primary amine group in an aqueous medium.

(Keywords: polymerizable surfactant; active ester groups; reactive polymeric microspheres)

INTRODUCTION

Much attention has been denoted to polymeric microspheres because of their widespread application in biomedical and biochemical fields and microelectronics. The preparation of monodisperse microspheres has been established over the past few decades by emulsifier-free emulsion polymerization[1,2], dispersion polymerization[3,4] and seeded polymerization[5–7]. In particular, dispersion polymerization is known to be the most effective method for preparing monodisperse particles with sizes ranging from submicrons to several microns in diameter[4,8]. However, it is still difficult to produce monodisperse polymeric particles with reactive groups on their surfaces by dispersion copolymerization when using a reactive monomer as the monomer component.

Monodisperse polystyrene (PS) microspheres of several microns in size can be synthesized with relative ease by dispersion polymerization. Furthermore, the seeded copolymerization method is utilized not only to increase the particle size[6,9] and to prepare particles of the core–shell type[10], but also to introduce functional groups on to the particle surfaces[11–19]. Therefore, it is expected that monodisperse reactive particles of a relatively large size can be obtained by seeded copolymerization on the surfaces of PS particles, prepared by dispersion polymerization, where the latter act as seed particles.

Surfactant molecules are well adsorbed from aqueous solutions on to PS particles with a highly hydrophobic surface, with their hydrophobic tails and polar heads oriented toward the particle surface and the aqueous phase, respectively[20–22]. Thus, it would seem to be possible to modify the surfaces of seeded particles through the adsorption of polymerizable surfactants, followed by their polymerization or copolymerization, without changing the size of the seed particles. Such an approach has been used by several groups to produce particles with ionic groups[12] and sucrose units[16] on their surfaces. However, polymerizable surfactants with homopolymerizability were employed in these studies and hence seeded copolymerizations were always accompanied by the formation of homopolymers.

The concurrent formation of homopolymers in seeded copolymerizations may be prevented by using polymerizable surfactants with no homopolymerizability. We have recently developed a novel polymerizable surfactant with an active ester group, namely *p*-10-undecenoyloxyphenyldimethylsulfonium methylsulfate (UPDS), which has an allyl group as the polymerizable group[23]. UPDS was found to have a high adsorption property at the

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water–air interface, as evidenced by the high surface-tension reduction in water[23]. It was only slightly radically homopolymerized but well copolymerized with dialkyl fumarates (DRFs) to yield alternating copolymers with a relatively high molecular weight, particularly in aqueous micellar solutions of UPDS[24]. Furthermore, both UPDS and its copolymer with diethyl fumarate were highly reactive with some primary amines in an aqueous medium under mild conditions, giving the corresponding amides in high yields[23,24].

From the above viewpoints, UPDS seems to be suited as a polymerizable surfactant for the seeded copolymerization with DRFs which are easily absorbed into PS particles. Furthermore, its immobilization has the advantage of being able to introduce active ester groups on to the surface of seed particles, which are useful for directly binding various functional molecules through amidation. This present paper deals with the adsorption of UPDS on to monodisperse PS particles of 4.1 μm diameter produced by dispersion polymerization from aqueous solution, and the seeded radical copolymerization of UPDS adsorbed on the particles with diethyl, dibutyl and dioctyl fumarates incorporated into the particles in aqueous systems. The particles obtained by the seeded copolymerization were characterized with respect to the amount of UPDS units introduced and their reactivity toward a primary amine.

EXPERIMENTAL

Materials

The polymerizable surfactant having an active ester group (UPDS) was synthesized through the condensation reaction of 10-undecenoic acid with *p*-hydroxyphenyldimethylsulfonium methylsulfate in the presence of dicyclohexylcarbodiimide in acetonitrile, according to the method described elsewhere[23]. UPDS forms micelles in water with a c.m.c. value of $(3.0–6.5) \times 10^{-3}$ at 25°C[23].

Diethyl fumarate (DEF), di-n-butyl fumarate (DBF) and dioctyl fumarate (DOF) were purchased from Tokyo Kasei Kogyo Co. Ltd and purified by distillation under vacuum. 2,2′-Azobisisobuthyronitrile (AIBN), obtained from Wako Pure Chemical Industries Ltd (Wako) was purified by recrystallization from methanol. A water-soluble radical initiator, 2,2′-azobis(2-amidinopropane)dihydrochloride (AIBA, reagent grade from Wako) was employed as received. Sodium dodecylsulfate (SDS, Tokyo Kasei Kogyo Co. Ltd) was repeatedly recrystallized from ethanol containing 5% water. The sodium salt of 8-anilino-1-naphthalenesulfonic acid (ANS, Tokyo Kasei Kogyo Co. Ltd), and acridine orange (AO, Wako) were used without further purification. N/400-Potassium polyvinylsulfate solution (N/400-PVSK) for colloid titration was purchased from Wako and used at a dilution of N/1000. 2-Methoxyethylamine (MOEA) was received from Tokyo Kasei Kogyo Co. Ltd and distilled before use. A Millipore LGC filter was used in ultrafiltrations to rinse the microspheres. Water was repeatedly deionized until it had a specific conductivity of 1×10^{-6} S cm^{-1} or lower, and was then distilled in a nitrogen atmosphere.

Preparation of polystyrene seed particles

Micron-size polystyrene (PS) seed particles were prepared by dispersion polymerization[8]. The polymerization of styrene was carried out with AIBN in ethanol containing 10 wt% of methyl cellosolve in the presence of 2.5 wt% (based on the total) of poly(*N*-vinylpyrrolidone) K30 as stabilizer and 0.6 wt% (based on the total) of Aerosol OT as the costabilizer, with stirring at 200 r.p.m., at 70°C. Monodisperse particles of 4.1 μm average diameter were used for the experiments. The seed particles were rinsed several times with water and methanol before use.

Determination of adsorption isotherms on seed particles

UPDS solutions of different concentrations were added to 1.0 g of PS seed particles to prepare dispersions with a solids content of 5.0 wt%. The dispersions were equilibrated in a thermostated bath at 25°C for a set period of time. The microspheres were separated by centrifugation at 3000 r.p.m. for 30 min, followed by filtration through a membrane filter of 0.2 μm pore diameter. The concentration of UPDS in the supernatant liquid was determined by titration with SDS solution using methylene blue as an indicator[25].

Seeded copolymerization

The DRF was first incorporated into the PS seed particles by using the following method. An aqueous dispersion of seed particles was stirred at 200 r.p.m. in a four-necked, round-bottomed flask at 5°C, and a set amount of the DRF was added dropwise to the dispersion at a rate of 1.0 ml h^{-1}. UPDS was added to the dispersion, and then kept at 25°C for 5 h to allow adsorption on to the seed particles to take place. After this period the dispersion was stirred at 60°C under a nitrogen atmosphere after adding an aqueous solution containing an appropriate amount of AIBA as the radical initiator. After 24 h, a small portion of *p*-methoxyphenol was added and the latex formed was then filtered through a sieve (200 mesh). Ultrafiltration was carried out in order to separate the modified particles from the unreacted monomers and the water-soluble polymer in the serum. When AIBN was used as the initiator, the DRF containing an appropriate amount of AIBN was incorporated into the PS seed particles at 5°C. The seeded copolymerization of UPDS with the DRF was then carried out in the same manner as described above.

The amount of UPDS immobilized on the seed particles was estimated both from the residual UPDS in the serum, determined by titration with SDS solution as described above, and by colloid titration of the dispersion using a fluorescent indicator. A N/1000-PVSK aqueous solution was added to the latex dispersion, which included a small amount of fluorescent probe, and the fluorescence intensity was measured at 25°C. The excitation and emission wavelengths were 380 and 480 nm, respectively, for ANS, and 495 and 530 nm for AO. The inflection point in the titration curve of the fluorescence intensity against the amount of titrant was taken as the end point of the titration.

Reaction of active ester groups with amine

The modified particles with UPDS units on their surfaces were allowed to react with MOEA in water at 25°C for 24 h. The particles were rinsed out with water several times using ultrafiltration. Surface species were examined by X-ray photoelectron spectroscopy (X.p.s.) at a potential of 10 kV and an X-ray current of 30 mA.

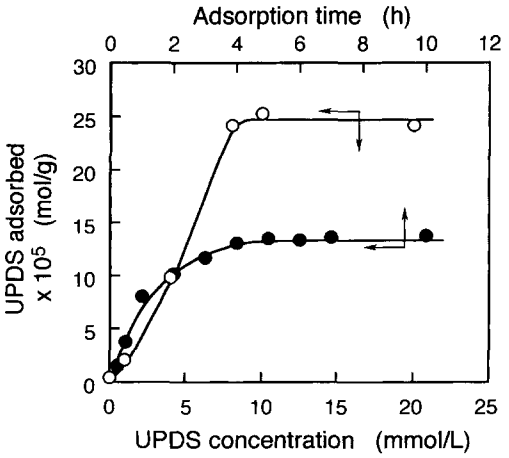

Figure 1 The adsorption isotherm of UPDS on to PS seed particles (○), and the amount of UPDS adsorbed on to PS seed particles against adsorption time (●), at [UPDS] of 5.0 mmol l^{-1} at 25°C in water; [seed particles] = 5 wt% in suspension

Measurements

The average diameter of the particles was determined by a Horiba CAPA-500 particle size analyser and by measuring the diameters of more than 100 particles on scanning electron micrographs taken by using a Hitachi S-415 scanning electron microscope. ^1H nuclear magnetic resonance (n.m.r.) spectra were obtained by a JEOL JNM-EX270 Fourier transform (*FT*) n.m.r. spectrometer. Fluorescence spectra were obtained using a Hitachi F-4010 fluorospectrometer, and X.p.s. spectra were obtained on a Shimazu ESCA-1000 electron spectrometer.

RESULTS AND DISCUSSION

Adsorption of UPDS on to PS seed particles

Figure 1 shows the amount of UPDS adsorbed on to PS seed particles as a function of the adsorption time, at a UPDS concentration of 5 mmol l^{-1}, and the adsorption isotherm for UPDS, which is of the Langmuir type. The adsorption was fast, with the adsorption amount reaching a constant value after about 4 h. Therefore, the adsorption isotherm was determined after incubation of the latex dispersions for more than 5 h at 25°C. The UPDS concentration on the abscissa in the adsorption isotherm is expressed as the feed concentration, rather than the equilibrium concentration, so that one can monitor the amount of adsorbed UPDS under the seeded copolymerization conditions. The plateau corresponds to the maximum amount of adsorption, which appears at concentrations above the c.m.c. value for UPDS[20].

Seeded copolymerization using a water-soluble initiator

UPDS does not radically homopolymerize at a concentration below the c.m.c. but copolymerizes well

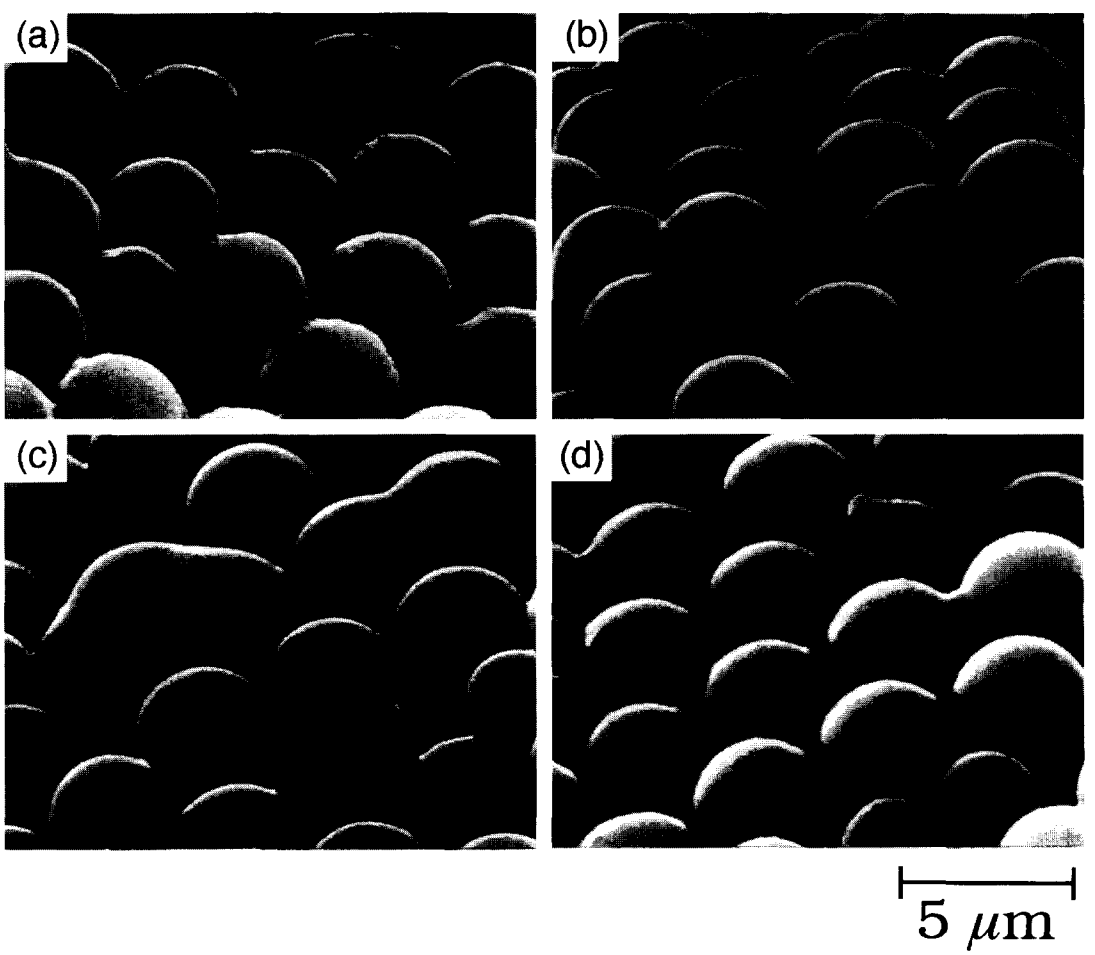

Figure 2 Scanning electron micrographs of the microspheres produced by the seeded copolymerization of UPDS with DEF using AIBA ([seed particles] = 5 wt% in suspension; [AIBA] = 2.0 mol%). [UPDS] = [DEF] in mmol l^{-1}: (a) 1.5; (b) 3.0; (c) 6.0; (d) 12.0

with DRFs to yield alternating copolymers, as reported in our previous paper[24]. DRFs are almost insoluble in water but are easily absorbed into PS particles, and hence they were used as comonomers for seeded copolymerization with UPDS. This seeded copolymerization of UPDS with equimolar amounts of DRFs was carried out using the water-soluble initiator AIBA. *Figure 2* shows scanning electron micrographs of the microspheres obtained by seeded copolymerization with DEF. The particles adhere to each other irrespective of the monomer concentrations used. This is caused by the copolymer of UPDS with DEF being soluble in water. Thus, copolymerization appears to take place mainly in the water phase for the system which uses DEF as the comonomer.

Figures 3 and *4* show micrographs of the microspheres produced by the seeded copolymerization reactions with DBF and DOF, respectively. It can be seen that the seed particles remain virtually unchanged at a feed concentration of UPDS of 3.0 mmol l^{-1} or lower. The loci for seeded copolymerization would be mainly at the surfaces of the seed particles at these concentrations, because the copolymers of UPDS with DBF and DOF are insoluble in water. However, the seed particles obtained at a feed concentration of 12 mmol l^{-1} are clearly observed to adhere to each other. This is probably due to the copolymers formed by the emulsion copolymerization of UPDS with DBF and DOF in the aqueous phase. In *Figure 3d*, the formation of particles of ~1–2 μm in diameter is observed at a feed concentration of UPDS of 12.0 mmol l^{-1}. This might result from the fact that PS particles plasticized with DBF were broken up by agitation during the copolymerization reaction.

Figure 5 shows the amount of UPDS copolymerized on the surfaces of the microspheres, against the UPDS concentration in the feed, obtained by seeded copolymerization under the conditions mentioned above. The amounts of UPDS units were estimated from the residual amounts of unreacted UPDS and UPDS units of the copolymers in the serum after the seeded copolymerization reactions. When DEF was used as the comonomer, the extent of immobilization of UPDS is considerably low. This is probably due to dissolution of the copolymer formed at the particle surfaces into the aqueous phase. On the other hand, the immobilized amounts of UPDS units on the seed particles obtained for the systems using DBF and DOF as comonomers increase with increasing UPDS concentration. *Figure 5* indicates that DOF is more effective in immobilizing UPDS than DBF, which may be associated with the hydrophobicity of the resulting copolymers.

Seeded copolymerization using a water-insoluble initiator

Effect of type of DRF. Seeded copolymerization of UPDS with DEF and DBF was carried out using the

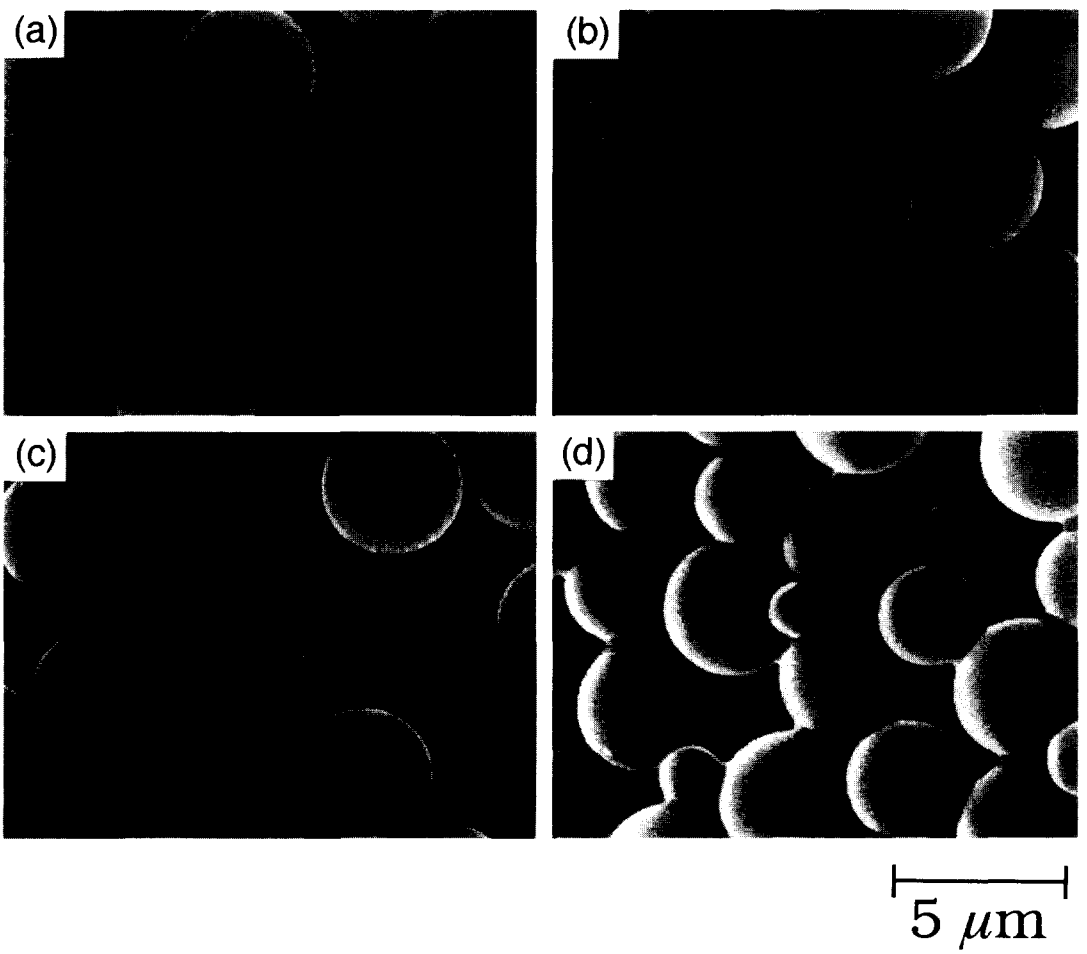

Figure 3 Scanning electron micrographs of the microspheres produced by the seeded copolymerization of UPDS with DBF using AIBA ([seed particles] = 5 wt% in suspension; [AIBA] = 2.0 mol%). [UPDS] = [DBF] in mmol l^{-1}: (a) 1.5; (b) 3.0; (c) 6.0; (d) 12.0

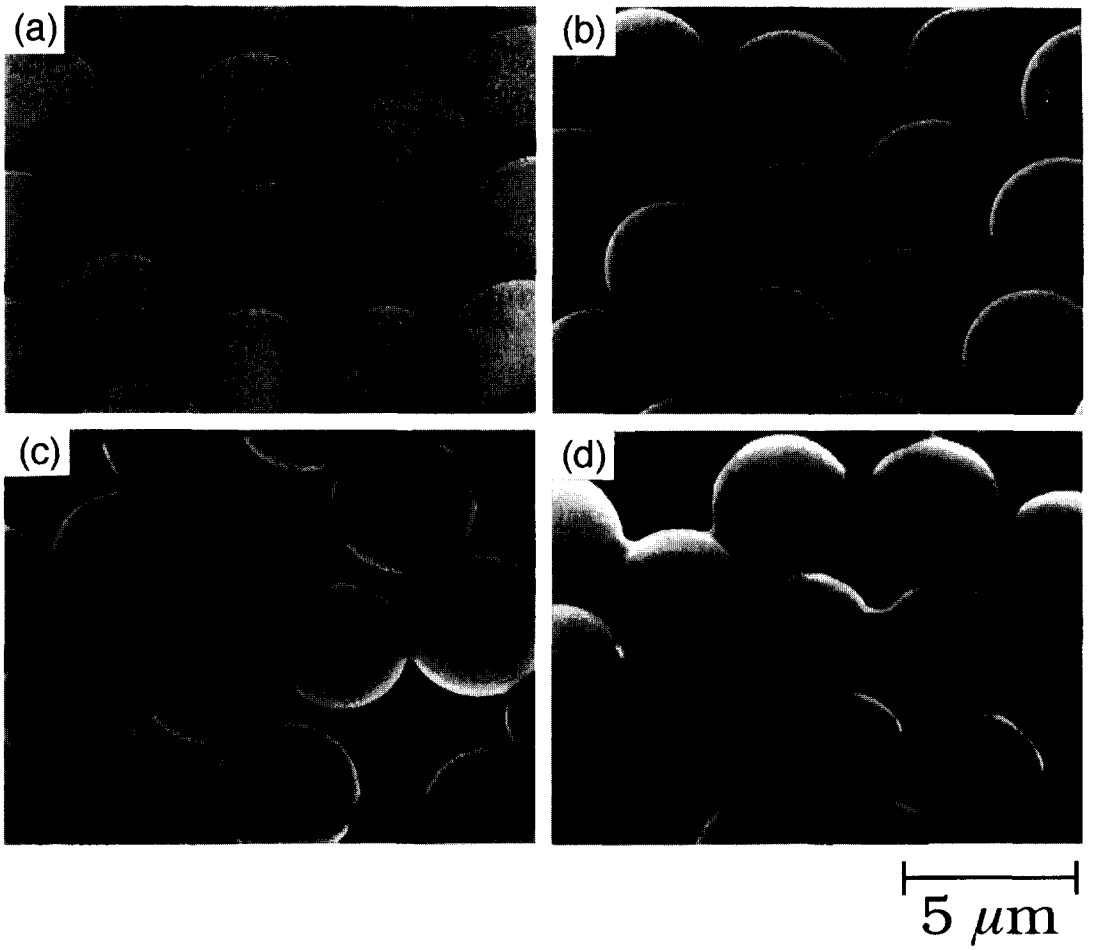

Figure 4 Scanning electron micrographs of the microspheres produced by the seeded copolymerization of UPDS with DOF using AIBA ([seed particles] = 5 wt% in suspension; [AIBA] = 2.0 mol%). [UPDS] = [DOF] in mmol l^{-1}: (a) 1.5; (b) 3.0; (c) 6.0; (d) 12.0

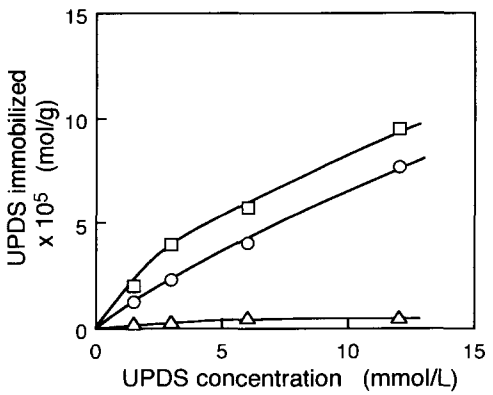

Figure 5 The amount of UPDS units immobilized on the PS particles, determined from the amount of residual UPDS in the serum, against the UPDS concentration in the feed for seeded copolymerization with DRFs using AIBA: (△) DEF; (○) DBF; (□) DOF

water-insoluble initiator AIBN. *Figure 6* shows scanning electron micrographs of the microspheres obtained before and after the copolymerization with DEF and DBF. When the copolymerization was carried out using DEF as the comonomer (over a period of 24 h) aggregation of the microspheres took place. This presumably arises from a bridging flocculation by the resulting copolymer of UPDS with DEF, which is soluble in water, as can be seen in *Figure 6b*. On the other hand, the corresponding micrograph of the microspheres obtained by copolymerization with DBF as the comonomer shows that the monodisperse microspheres used apparently remain intact without the concurrent formation of secondary particles or aggregation. This is because the copolymer of UPDS with DBF is insoluble in water, as mentioned above, and copolymerization mainly takes place at the surface of the seed particles. Therefore, DBF was used hereafter as the comonomer in these studies.

Effect of DBF concentration. *Figure 7* shows the amount of DBF incorporated into the PS seed particles against that in the feed solution (in water) at 5°C. DBF is nearly quantitatively absorbed up to an amount which is equal to that of the seeded particles by weight, while above this the incorporated amount levels off at amounts over 1.5 times this value. The PS seed particles were found to swell as the added amount of DBF is increased up to about twice that of the PS seed particles by weight. The average diameter of the particles increased from 4.1 to 5.4 μm with increasing DBF concentration. When the amounts of DBF added reached over twice that of the seed particles in weight no further variation in particle size was observed, and some drops of DBF remained unabsorbed in the aqueous phase.

Figure 8 shows scanning electron micrographs of the resulting microspheres obtained by seeded copolymerization with varying amounts of DBF. The seed particles

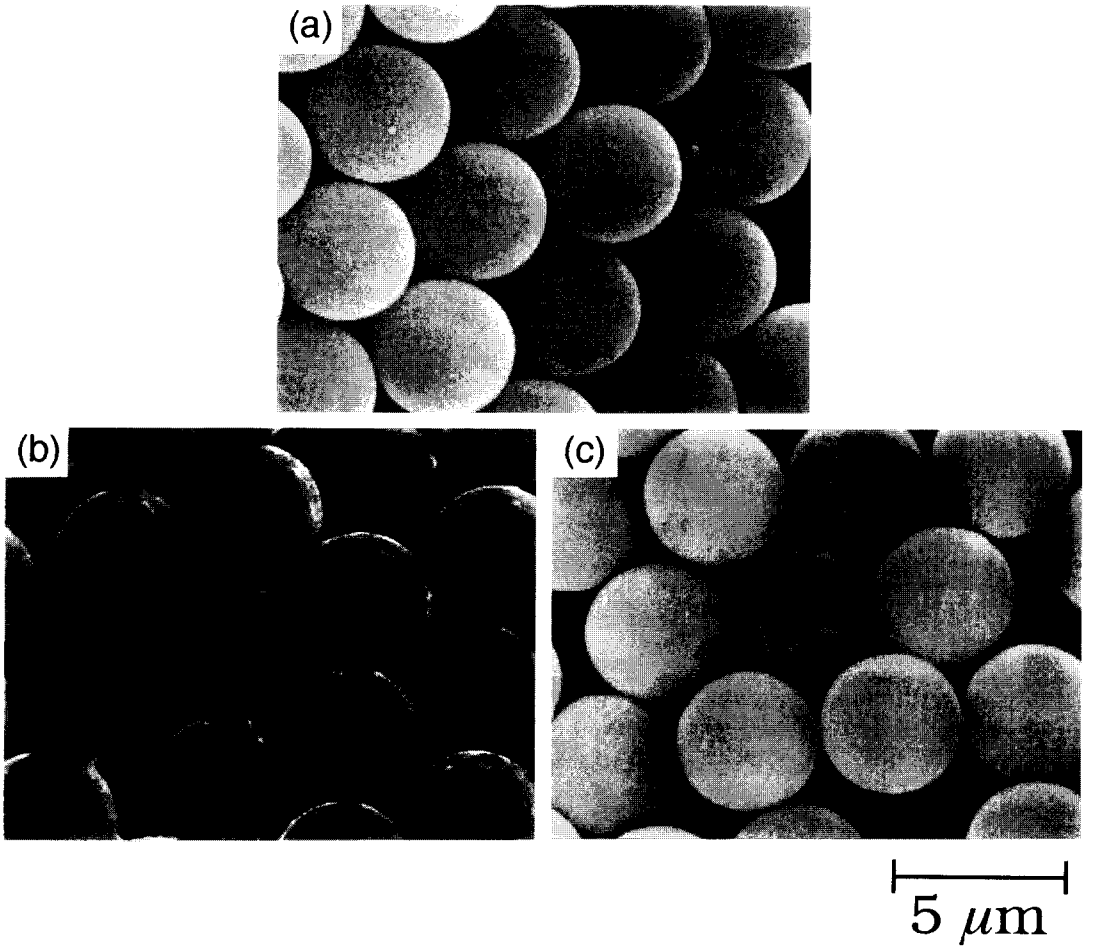

Figure 6 Scanning electron micrographs of the microspheres before and after the seeded copolymerization of UPDS with DEF and DBF using AIBN at 60°C for 24 h: (a) PS seed particles; (b) the particles obtained by the seeded copolymerization with DEF at [UPDS] = [DEF] = 3.0 mmol l^{-1}; (c) the particles obtained by the seeded copolymerization with DBF at [UPDS] = [DBF] = 3.0 mmol l^{-1} ([AIBN] = 2.0 mol%)

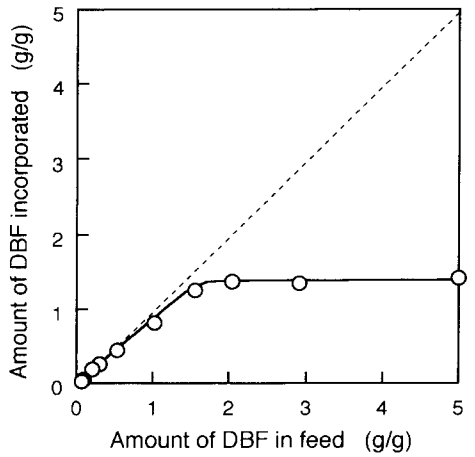

Figure 7 The amount of DBF incorporated into the PS seed particles against the amount in the feed in water at 5°C, with [seed particles] = 10 wt% in suspension

remain virtually unchanged for amounts of DBF of 10 and 50 wt% added to the seed particles. However, the seed particles obtained when amounts of 100 wt% or more are added to the particles are clearly observed to adhere to each other. This is probably due to copolymer being formed by the copolymerization of UPDS with amounts of DBF present in the aqueous phase.

Effect of UPDS concentration. Seeded copolymerization with DBF as the comonomer was carried out at varying concentrations of UPDS, while keeping the concentration of DBF constant at 10 wt% (based on the seed particles). *Figure 9* shows micrographs of the microspheres obtained by this seeded copolymerization. The resulting particles remain practically unchanged at feed concentrations of 3.0 mmol l^{-1} or less. On the other hand, the formation of a copolymer between the particle surfaces is definitely observed at feed concentrations of UPDS of 12 mmol l^{-1}, where the equilibrium concentration in the aqueous phase exceeds the c.m.c.

Characterization of the modified microspheres

UPDS units on the particle surfaces. The microspheres obtained by seeded copolymerization with DBF as the comonomer when using the water-insoluble initiator AIBN were characterized with respect to the charges resulting from the UPDS units on their surfaces. The amounts of UPDS units on the surfaces of the microspheres were determined by colloid titration using the fluorescent indicators, ANS and AO, according to the method for the quantitative determination of polyelectrolytes reported by Tanaka and Sakamoto[26,27]. ANS and AO behave as anionic and cationic fluorescence indicators, respectively. The former emits a strong

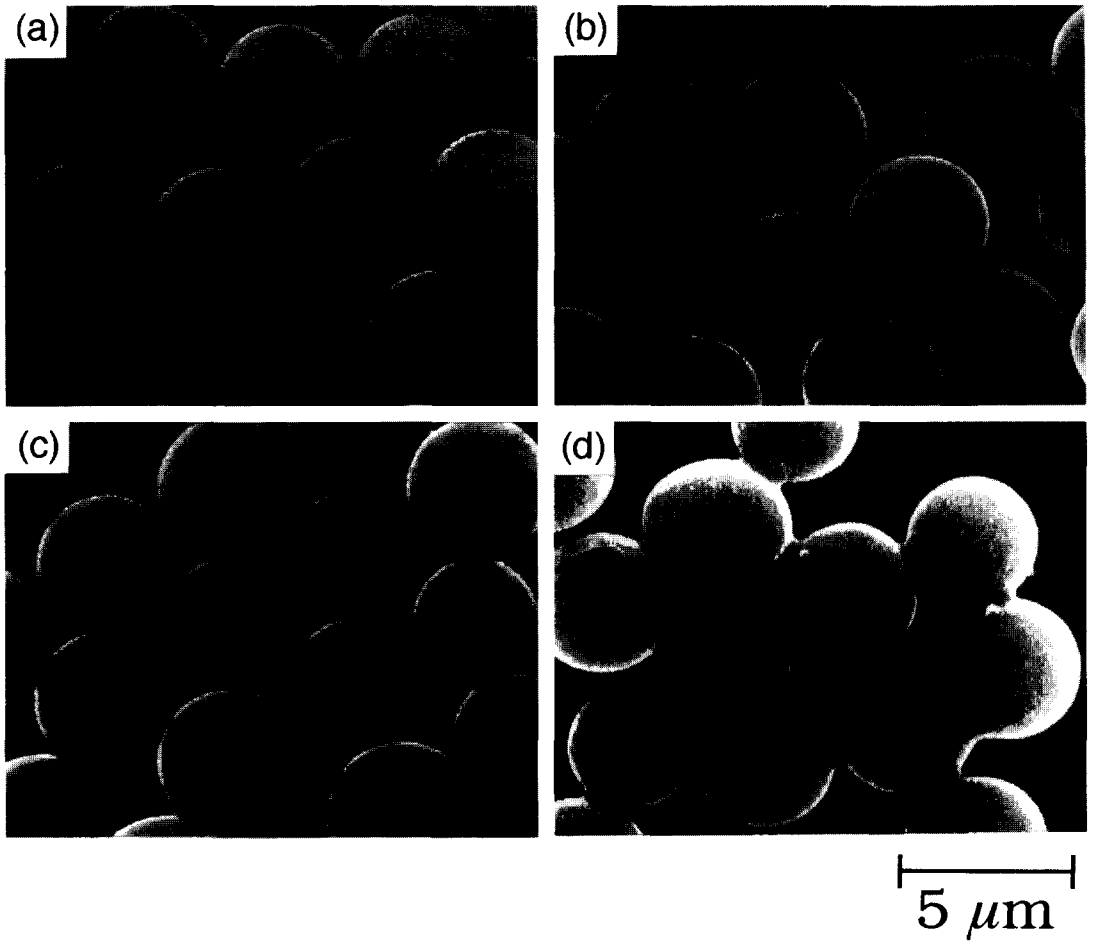

Figure 8 Scanning electron micrographs of the microspheres produced by the seeded copolymerization of UPDS with DBF using AIBN ([seed particles] = 10 wt% in suspension; [UPDS] = 3.0 mmol l^{-1}; [AIBN] = 2.0 mol%). [DBF] in wt% based on the seed particles: (a) 10; (b) 50; (c) 100; (d) 200

fluorescence when bound to a cationic charge; on the other hand, the strong fluorescence of free AO is quenched when bound to an anionic charge. *Figure 10* shows the titration curves obtained for colloidal solutions of the PS seed particles and microspheres modified by the seeded copolymerization of UPDS with DBF when using PVSK solution as the titrant and ANS and AO as indicators. For the titration carried out with the former indicator, the ANS ions bound to the UPDS units on the PS particles are released on adding the PVSK solution. As shown in *Figure 10*, the fluorescence intensity decreases linearly and then levels off with increasing the added amount of PVSK solution. On the other hand, for the titration with AO as the indicator, PVSK is first bound to the UPDS units on the PS particles and then AO is bound to the excess PVSK. The fluorescence intensity in the titration curve with AO as the indicator remains nearly constant up to an added amount of PVSK solution corresponding to the inflection point, which is in fairly good agreement with that observed in the titration with ANS as the indicator. From the inflection point, the surface concentration of UPDS units was calculated to be 71.4 μmol m^{-2}.

Figure 11 shows the amounts of UPDS units immobilized on the surfaces of the microspheres, determined from the inflection points in the titration curves when using PVSK solutions and from the residual amounts of UPDS in the serum, against the UPDS concentration in the feed at an added amount of 10 wt% DBF to the seeded particles. The amounts of UPDS units determined by the PVSK titration method are slightly larger than those estimated from the residual amounts of UPDS in the serum. These differences may arise from slight overestimations in the former method because the cationic charges are fixed at the particle surfaces. The immobilized amounts of UPDS units on the PS particles increase with increasing the UPDS concentration.

Reactivity of UPDS units on the particle surfaces

The reaction of the modified microspheres with 2-methoxyethylamine (MOEA), a water-soluble amine, was carried out in water at room temperature for 24 h. UPDS and its copolymers with DEF readily react with primary amines in water at room temperature to give the corresponding amides in high yields[24]. The reaction of the microspheres with MOEA was examined by using X.p.s. *Figure 12* shows the spectra obtained for the microspheres before and after the reaction with MOEA. For the microspheres modified by the seeded copolymerization of UPDS and DBF, the pair of peaks at 533.9 and 531.6 eV is assigned to carboxyl groups in the O_{1s} spectrum. The peak at 535.7 eV is characterized as the methylsulfate of the UPDS units. In the S_{2p} spectrum, the peaks assigned to dimethylsulfonium methylsulfate,

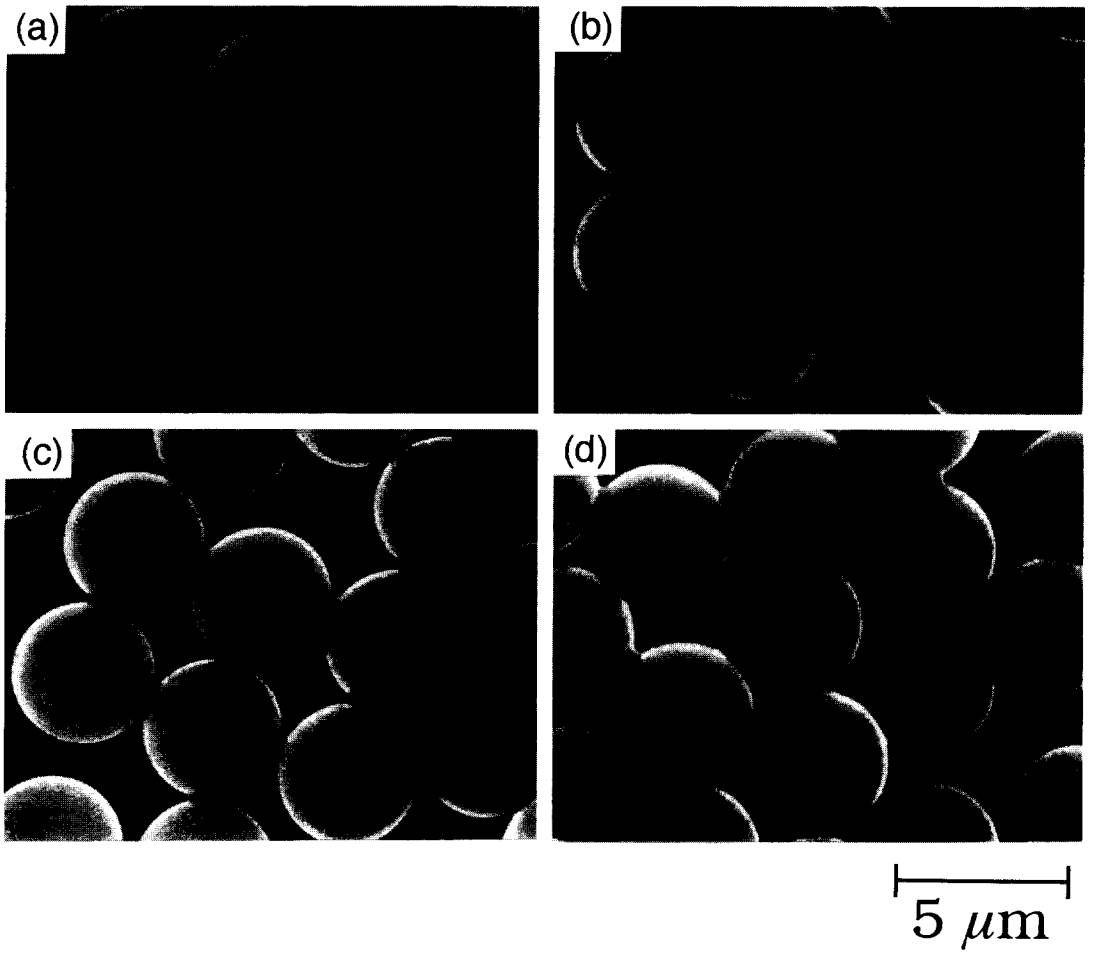

Figure 9 Scanning electron micrographs of the microspheres produced by the seeded copolymerization of UPDS with DBF using AIBN ([seed particles] = 10 wt% in suspension; [DBF] = 10 wt% based on the seed particles; [AIBN] = 2.0 mol%). [UPDS] in mmol l^{-1}: (a) 1.5; (b) 3.0; (c) 6.0; (d) 12.0

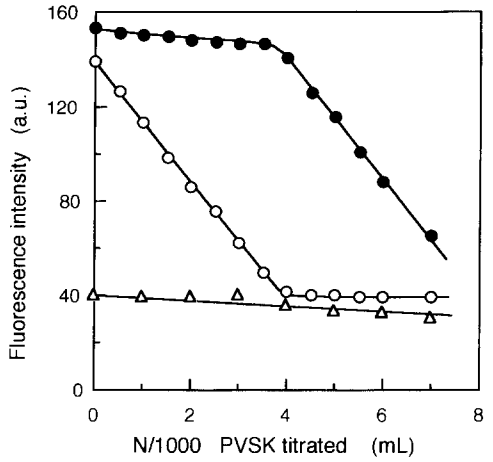

Figure 10 PVSK titration curves for colloidal solutions of the microspheres obtained by the seeded copolymerization of UPDS with DBF where [UPDS] = 6.26 mmol l^{-1}, [DBF] = 10 wt%, based on the seed particles, and [AIBN] = 2.0 mol%, based on the monomers. Fluorescent indicator: (○) ANS; (●) AO; (△) PS seed particles with ANS ([PVSK] = N/1000, [particles] = 0.5 wt% in suspension)

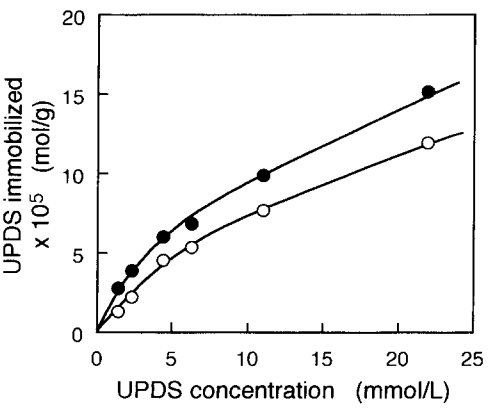

Figure 11 The amount of UPDS units immobilized on the PS particles against the UPDS concentration in the feed for seeded copolymerization with DBF where [DBF] = 10 wt%, based on the seed particles, and [AIBN] = 2.0 mol%, based on the monomers. The amounts of UPDS units were determined from the residual UPDS in the serum (○) and by the PVSK titration method (●)

the head group of the UDPS units, are also observed at 165.8 and 170.6 eV. Furthermore, a peak at 401.5 eV in the N_{1s} spectrum is also observed. Nitrogen atoms on the surfaces of the microspheres could arise from initiator fragments and the poly(*N*-vinylpyrrolidone) (PVP) used as the steric stabilizer for preparing the seed particles. The latter probably stems from graft copolymerization taking place during the dispersion polymerization reaction[28]. The binding energy of N_{1s} at 401.5 eV is usually assigned to the ammonium ion. The binding

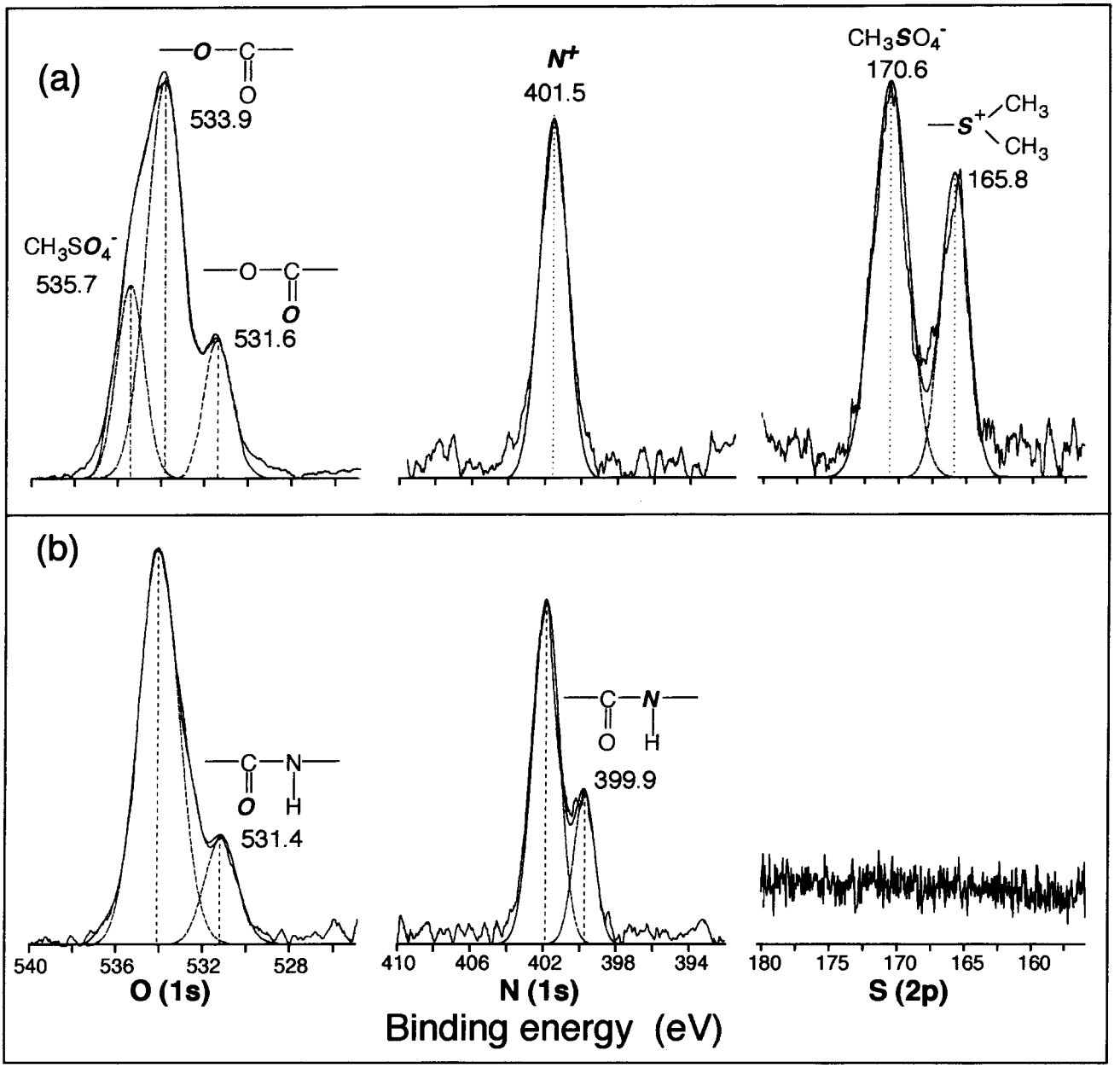

Figure 12 X-ray photoelectron spectra of: (a) the modified microspheres produced by the seeded copolymerization of UPDS with DBF where [UPDS] = 11.0 mmol l^{-1}, [DBF] = 10 wt%, based on the seed particles, and [AIBN] = 2.0 mol%, based on monomers; (b) the microspheres after reaction with 2-methoxyethylamine, where MOEA/UPDS (units on the surface) = 3/1

energy of N_{1s} in PVP was found to shift from 400.3 to 401.5 eV after dissolving in water. PVP is known to strongly interact with anionic dyes in aqueous solution[29]. Therefore, the shift seems to be due to the cationic nature of the nitrogen atom in the pyrrolidone ring of PVP. In the X.p.s. spectra of the microspheres that have reacted with MOEA, the peaks at 531.4 and 399.9 eV due to O_{1s} and N_{1s}, respectively, are assigned to amide groups[30]. Moreover, the peaks at 165.8, 170.6, and 535.7 eV, due to S_{2p} and O_{1s}, for the head group of the UPDS units, completely disappear after the reaction with MOEA. These points indicate that the microspheres bearing active ester groups on their surfaces are of high reactivity toward primary amines. Thus, these reactive polymeric microspheres could be used for immobilizing such bioactive substances as enzymes and antibodies.

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