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ORIGINAL CONTRIBUTION

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Preparation of monodisperse, reactive hydrogel microspheres and their amphoterization

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Abstract Monodisperse, reactive hydrogel microspheres were prepared by precipitation polymerization of *p*-nitrophenyl acrylate (NPA) with acrylamide, methacrylic acid, and methylenebisacrylamide in ethanol. The size of microspheres was controlled by the monomer ratio. Some fraction of reactive ester decomposed during the polymerization. The reactive hydrogel microspheres were converted to amphoteric ones by the reaction of NPA units with diamine. The isoelectric point of the amphoteric microspheres was around 4.0, but it was different from the pH at which the microspheres have the minimum size or the most shrunken state. This was attributed to the uneven distribution of induced amine groups.

Key words Hydrogen – microsphere – amphoterization – precipitation polymerization – reactive ester

Introduction

Reactive microgels have been extensively studied so far, but all of them were highly crosslinked, hard microspheres having average diameters of 0.01 μm order with wide distribution [1–3]. No monodisperse, swellable and soft hydrogel microspheres having 0.1 to 1 μm diameters have been developed yet. We aimed at preparing such microspheres and finding several applications of them.

In our previous study, we developed a method to prepare monodisperse hydrogel microspheres via precipitation polymerization [4, 5]. In this study, the method was applied for the preparation of the above-mentioned type of reactive hydrogel microspheres. Our object was attained by the precipitation polymerization of *p*-nitrophenyl acrylate (NPA), acrylamide (AAm), methacrylic acid (MAc), and methylenebisacrylamide (MBAAm) in alcohol.

The first trial to modify the microspheres using the reactivity of NPA was their conversion to amphoteric ones. Because the modified microspheres retain the unique properties of mother microspheres, they were expected to present some features different from existing amphoteric latex particles. pH-Sensitive change of microsphere size is one of the features and it was studied in reference to the distribution of ionic groups in the microspheres.

Experimentals

Materials

NPA was synthesized by adding acryloyl chloride gradually to an ethyl acetate solution of *p*-nitrophenol (HONP), and recrystallized from hexane. MAc was purified by distillation at 40 °C/3 mmHg. AAm was recrystallized from ethanol at 40 °C. MBAAm and azobis-isobutyronitrile (AIBN) were used without further purification.

Preparation of reactive hydrogel microspheres

Monomers were dissolved in ethanol or ispropanol in a 100 ml four-necked flask which was equipped with a stirrer, a nitrogen inlet tube, a condenser and a serum rubber. The flask was fixed in a water bath at 60 °C. After 1 h nitrogen-bubbling, an initiator solution was injected with a syringe. The polymerization was continued for 22 h. Conversion was calculated from the gas chromatographic analysis of remaining monomers. The product was purified by repetitive centrifugation, decantation, and redispersion.

The size and shape of particles were measured and observed on electron or optical micrographs. The hydrodynamic size was measured by photon correlation spectroscopy.

Amphoterization and characterization

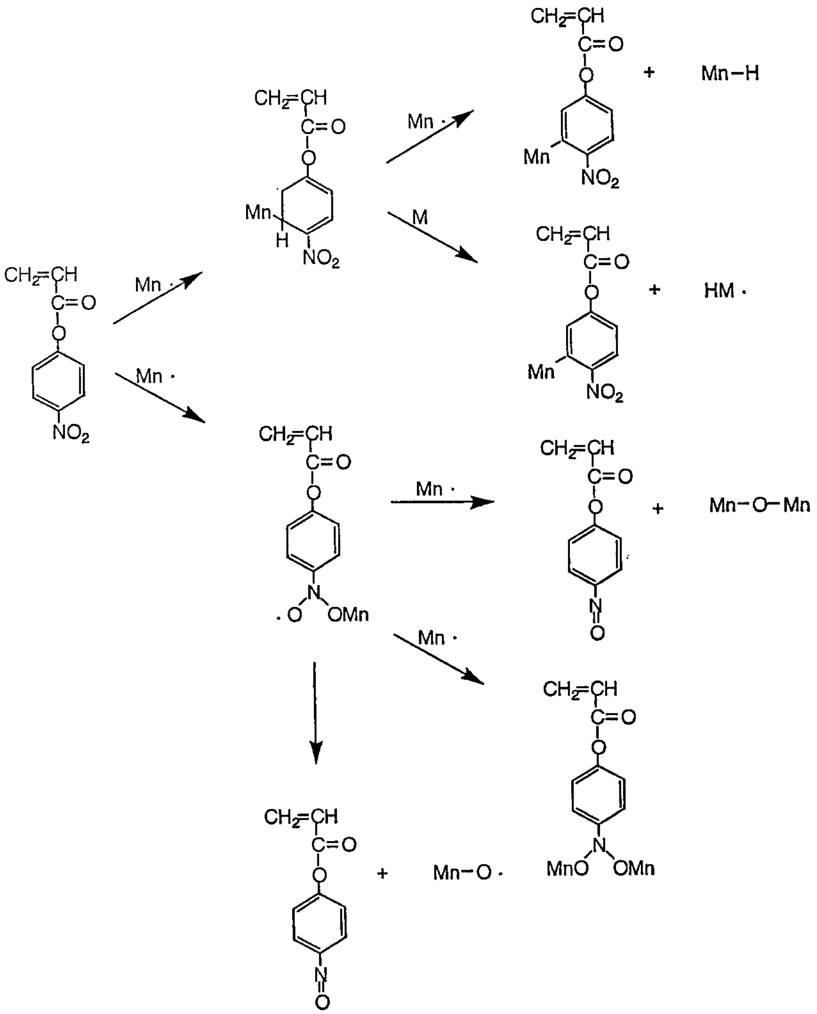
Reactive microspheres were mixed with 1 M ethylenediamine dihydrochloride at room temperature at pH 7.0. After a certain incubation time, the microspheres were centrifuged. The residual *p*-nitrophenyl esters were hydrolyzed with 1 N NaOH. The released HONP was determined by the absorbance at 400 nm in N/10 NaOH. Th electrophoretic mobility was measured at ionic strength 0.01 with an electrophoresis microscope system of Sugiura Lab. Co.

Results and discussion

Preparation of reactive hydrogel microspheres

NPA is an active ester monomer which easily decomposes to acrylic acid and *p*-nitrophenol (HONP) under alkaline condition [6–8]. It also forms amides in the reaction with amine releasing HONP [6]. Among several reactive monomers, NPA was chosen here as it can be easily prepared and can indicate the extent of reaction, release of HONP, with the increase in absorbance at 400 nm. Because we had found that the precipitation polymerization of AAm, MAc, and MBAAm in ethanol or isopropanol gave monodisperse hydrogel microspheres [4], we added NPA as the

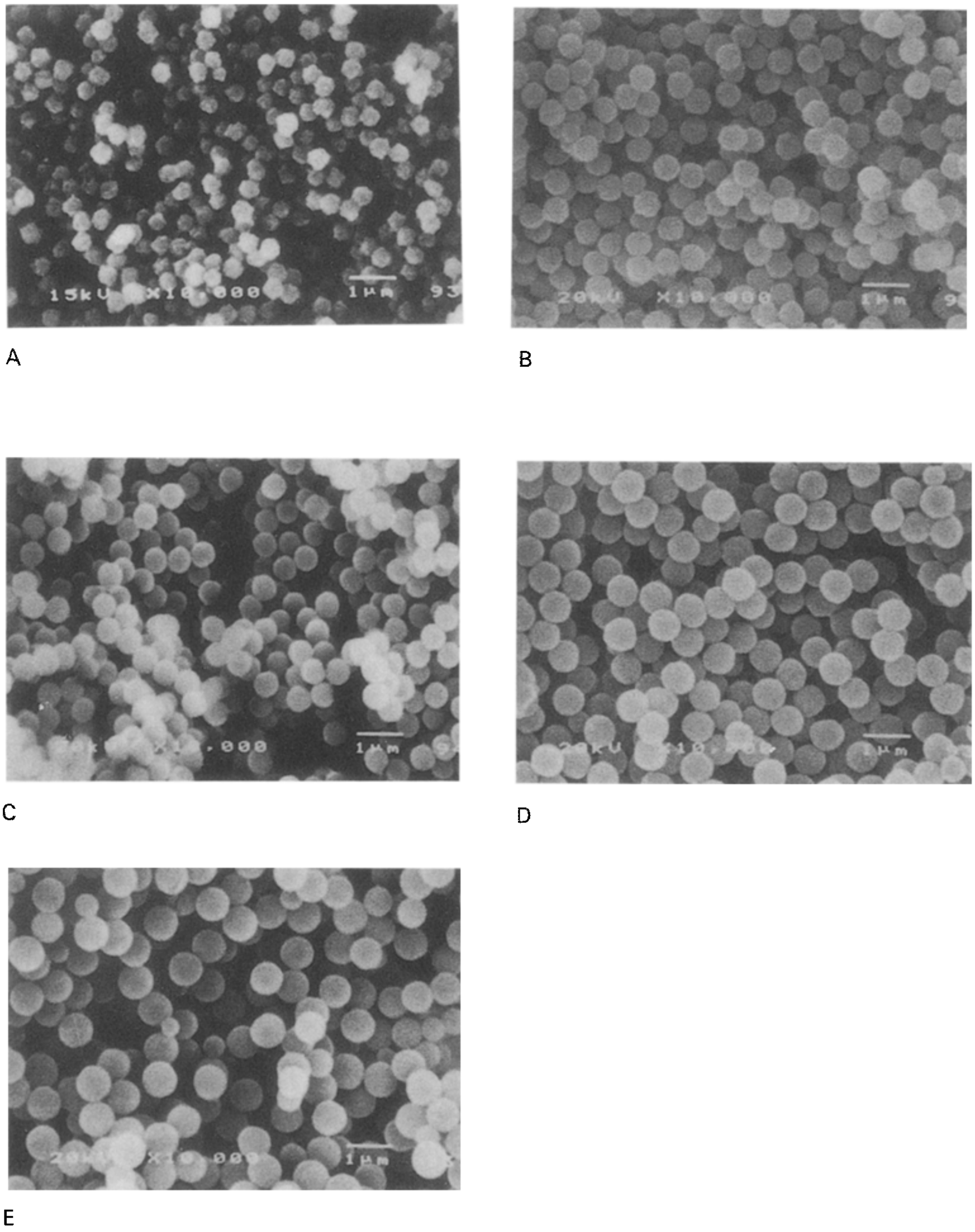
Scheme 1 Scavenge of radicals by NPA



fourth comonomer in the precipitation polymerization to obtain monodisperse reactive hydrogel microspheres.

The basis recipe was fixed to be MAc/AAm/NPA/MBAAm = 10/20/10/5 mmole in 40 g ethanol. Ethanol was better at retaining monodispersity of microspheres than isopropanol. Trial polymerization was carried out using 0.1 g of initiator at 60 °C but the conversion after 24 h polymerization was less than 10%. This means that an appreciable fraction of initiator was consumed by some undesirable interactions between the initiator and NPA [9]. The possible interactions are shown in Scheme 1 . A large amount of initiator was necessary to attain a high conversion. When more than 1.5 g AIBN was used, monodisperse microspheres were obtained with a high yield.

Then, the effect of MAc concentration in copolymerization systems on the size of microspheres was studied. The SEM views of microspheres formed at different MAc concentrations are shown in Fig. 1. As shown in the figure, a certain amount of MAc is necessary to form monodis-



A B C D E

Fig. 1 Scanning electron micrographs of reactive hydrogel microspheres having different content of methacrylic acid. MAc/AAm/NPA/MBAAm = x/20/10/5 (mmol) x: A 0, B 1.0, C 2.5, D 5.0, E 10.0 EtOH 40 g, AIBN 1.50 g perse microspheres. The size of microspheres increased with increasing MAc concentration. This is attributed to a solvency effect [10–12]. Namely, the increase in MAc concentration brings about the increase in the solubility of MAc-containing oligomers and consequently the decrease in the nuclei formation. As a results, a small number of large microspheres were obtained in the polymerization in which a large amount of MAc was employed. The solubility parameter (SP) is the key factor for this effect. The SP values of the concerned polymers were calculated by the Fedors method [13]. The SP of poly-MAc is 11.8 $(\mathrm{cal/cm^3})^{1/2}$, which is close to that of ethanol (SP = 12.7) $(\mathrm{cal/cm^3})^{1/2}$). Under such condition, MAc-containing oligomers do not easily precipitate to become nuclei of microspheres.

It is worth mentioning that MAc affected the particle size in a different manner when polymerization was carried out without NPA. In AAm, MAc and MBAAm ter-polymerization system, the particle size decreased with increasing MAc fraction in monomer [4, 5]. This was attributed to a stabilization effect by MAc. In the 3-monomers system, only MAc units or sequences contributed to the stabilization and the total surface area of microspheres was decided by the amount of MAc charged [4]. On the contrary, in the present 4-monomers system, NPA units or sequences whose SP is 12.2 $(\mathrm{cal/cm^3})^{1/2}$ seem to locate the microsphere/medium interface preferentially to stabilize the interface and share the role of stabilizer with MAc units. When NPA is used above a certain amount, the total surface area of microspheres composed of 4 monomers become insensitive to the amount of MAc. This speculation was supported by the appreciable stability of the microspheres composed of NPA, AAm and MBAAm.

The effect of AAm concentration on the size of microspheres formed was also studied. The three SEM views in Fig. 2 clearly show that the size of microspheres increased with decreasing amount of AAm employed in the polymerization. This change was again explained by SP, that is, poly-AAm has the highest SP (17.2 $(\mathrm{cal/cm^3})^{1/2}$) among the components in the polymerization system and plays an opposite role with MAc in terms of microsphere nucleation. Poly-AAm-rich growing chains easily precipitate from the medium during the polymerization and cause the formation of a large number of microspheres. In conclusion, the microspheres can be enlarged with increasing MAc fraction and/or decreasing AAm fraction in monomers in the present polymerization system. This conclusion will be expanded to a more general one indicating that, when a monomer which has a SP close to the medium's SP is used in a large amount, the polymerization results in the formation of larger particles. This may agree with Paine's prediction which pointed out that large particles are obtained in dispersion polymerization using stabilizers whose SPs are close to the media's one [4].

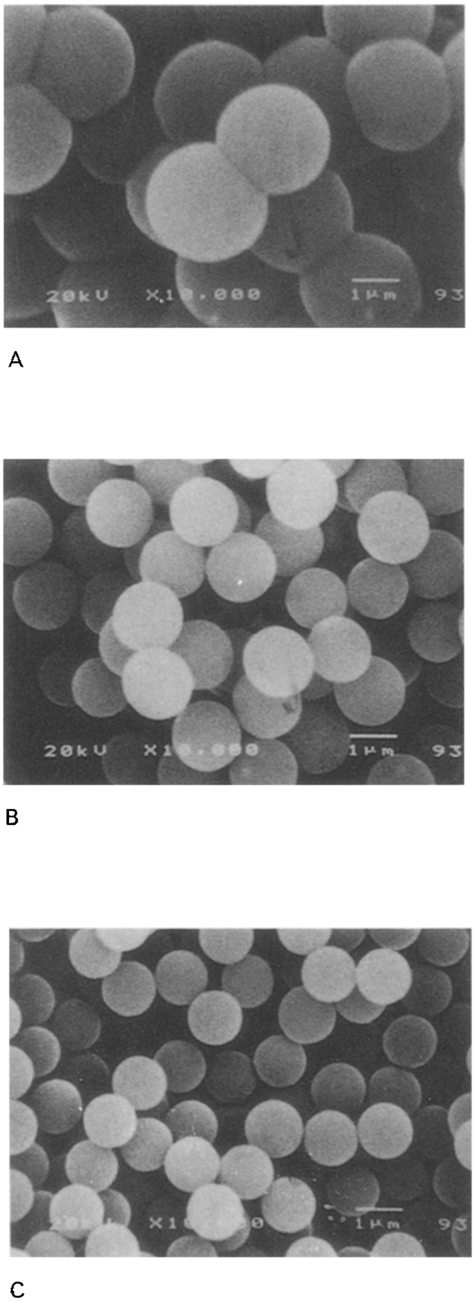


Fig. 2 Scanning electron micrographs of reactive hydrogel microspheres having different content of acrylamide. MAc/AAm/NPA/MBAAm = 10/y/10/5 (mmol) y: A 0, B 1.0, C 20 EtOH 40 g, AIBN 1.50 g.

As mentioned in the previous section, the decomposition of active ester NPA can be easily detected by the eye because of colorization of the solution. The absorption measurement gives quantitive data in terms of the NPA decomposition. The decomposition of NPA was measured in the course of polymerization and it was revealed that MAc catalyzes the decomposition. Figure 3 shows the dependence of decomposition of NPA during the 22 h polymerization on the amount of MAc. The decomposition of NPA is negligible in MAc-free polymerization system whereas more than 50% of NPA was decomposed during the polymerization with the basic recipe. Therefore, it was concluded that the amount of MAc must be carefully restrained to suppress the decomposition of NPA although a certain amount of MAC is necessary to form monodisperse microspheres.

MBAAms employed in this study were obtained from two sources, one from Wako Pure Chemicals Co. and the other from Tokyo Kasei Co. Both MBAAms gave monodisperse microspheres in the precipitation polymerization with NPA, AAm and MAc but the sizes were different between two systems. This is the reason for the different sizes between the two microspheres in Fig. 1 E and Fig. 2 C. The microspheres containing Tokyo Kasei's MBAAm (Fig. 2 C) are two to three times larger than those containing Wako Chemical's MBAAm (Fig. 1 E). This unexpected result would be caused by the difference in purity or the double bond content between the two kinds of MBAAms although the difference in the composition could not be detected by any spectroscopic measurements. MBAAm from Tokyo Kasei Co. was used when we needed microspheres having diameters of a few to several micron.

Conversion of reactive hydrogel microspheres to amphoteric ones

The most simple method to prepare amphoteric hydrogel microspheres would be to polymerize anionic and cationic monomers with hydrophilic monomer and crosslinker in heterogeneous systems. Hard amphoteric latex particles were prepared in such a manner by Homola [15]. We tried precipitation polymerization of MAc and methacrylamidopropyl trimethyl ammonium chloride (MAPTAC) with AAm and MBAAm in ethanol. MAPTAC had been used in the preparation of amphoteric gels [16]. The polymerization, however, resulted in the formation of coagulum. All alternations of cationic monomer or reaction conditions tried did not give good results. Therefore, we took another approach. This was a modification method in which existing microspheres are converted to amphoteric ones. Once we adopted a similar technique for the preparation of amphoteric latex from a styrene-AAm copolymer latex [17]. In this study, the reactive hydrogel microspheres obtained above were used as mother microspheres for the preparation of amphoteric ones. The mother microspheres are anionic ones and convertible to amphoteric ones by the reaction of NPA units with diamine. By the reaction with ethylenediamine (EDA) at pH 7 at room temperature, more than 90% of NPA units on/in the microspheres were amidated within 6 h. Excess amount of EDA (about 100 times of NPA units in molar basis) was used to prevent the amide formation at the both ends of each EDA molecule.

Characteristics of amphoteric hydrogel microspheres

Electrophoretic mobility (EPM) of microspheres was measured to confirm their amphotericity. Figure 4 shows the EPM of aminated hydrogel microspheres, which were prepared by the polymerization with the standard recipe

Fig. 3 Dependence of decomposition of NPA on the amount of MAc. MAc/AAm/NPA/MBAAm = x/20/10/5 (mmol) EtOH 40 g, AIBN 1.50 g

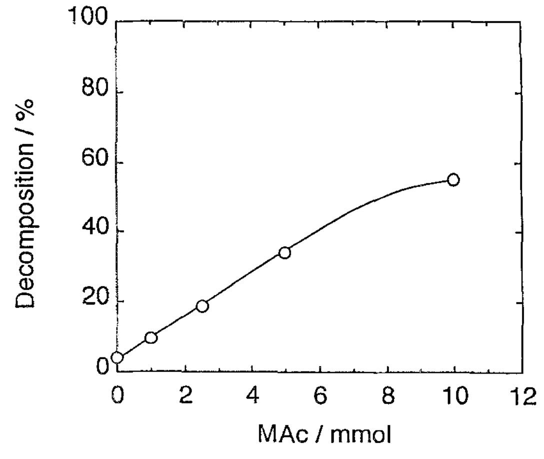
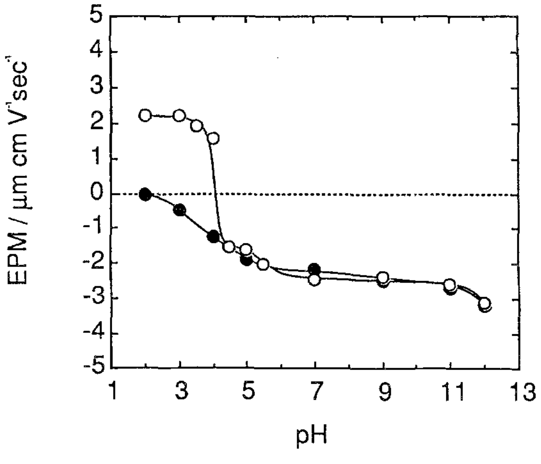


Fig. 4 Dependence of electrophoretic mobility of microspheres on pH. Electrophoretic measurement was carried out in 0.01 M KCl at 25 °C. ○ Aminolyzed microspheres ● Hydrolyzed microspheres Microspheres: MAc/AAm/NPA/MBAAm = 10/20/10/5 (mmol)



followed by the treatment with EDA, as a function of pH. The result in Fig. 4 clearly indicates that the anionic mother microspheres were converted to amphoteric ones having an isoelectric point (IEP) of 4.0. The microspheres aggregated between pH 3.5 and 4.5, that is, around the IEP. Interestingly, the IEPs of other microspheres prepared from different recipes were all around 4.0. Because IEP reflects the chemistry of surface layer, the small difference in IEPs among the microspheres would indicate that the surface chemistry is not affected sensitively by the monomer composition. Another curve in Fig. 4 shows the EPM of hydrolyzed microspheres in which NPA units were converted to carboxylic acid using 1 N NaOH. They had negative potentials regardless of pH, except for very low pH, as expected.

The IEP is decided by the ratio of cationic to anionic ionic groups of the surface layer of microsphere. The chemical composition on surface layer does not necessarily coincide with the composition of whole microsphere. Therefore, the neutralization pH at which the amount of dissociated cationic groups is equal to that of anionic ones does not necessarily coincide with the IEP. The neutralization pH can be obtained from the particle size-pH relation because amphoteric microspheres generally have the least volume at the neutralization pH. The change in size was observed with an optical microscope as a function of pH and the results are shown in Fig. 5. As can be see, the neutralization pH was lower than the IEP of microspheres. There are some possible reasons for this discrepancy. The most probable explanation would be the uneven distribution of ionic groups. Namely, in our microspheres, NPA units are supposed to have a graded distribution to increase its concentration toward the surface because of the high affinity with the medium and the low polymerization rate of NPA compared with other monomers. As a result, the ratio of amine to acid on surface becomes higher than that of whole particle. Under this situation, the IEP becomes higher than the pH at which dissociated carboxyl groups are balanced with cationic groups to give the microspheres the least volume. Topochemical reaction in aminolysis process might contribute to the formation of graded distribution of ionic groups.

We could not analyze more details of volume change as a function pH because of rough measurement of particle volume using an optical microscope. But these monodisperse amphoteric microspheres are expected to serve as a helpful model to examine phases of polymer gels [16].

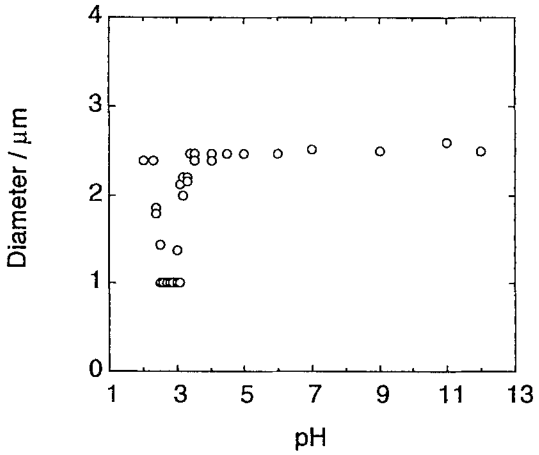


Fig. 5 Dependence of size of amphoteric microspheres on pH. Microspheres: MAc/AAm/NPA/MBAAm = 10/20/10/5 (mmol)

Conclusions

Monodisperse, reactive hydrogel microspheres were obtained by precipitation polymerization of acrylamide, methacrylic acid, p-nitrophenyl acrylate and methlenebisacrylamide in alcohols. The size of microspheres could be controlled with the monomer composition and initiator concentration. Amination of the microspheres resulted in the formation of amphoteric microspheres having micron-order diameters. The difference between the IEP and neutralization pH of the microspheres suggested an uneven structure in terms of the distribution of ionic groups.

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