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ORIGINAL CONTRIBUTION

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Suspension polymerization of poly(ethylene glycol) methacrylate: a route for swellable spherical gel beads with controlled hydrophilicity and functionality

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Abstract Spherical and swellable gel beads were obtained by the suspension polymerization of poly(ethylene glycol) methacrylate macromonomer (PEG-MA). The average size and size distribution properties, the equilibrium swelling behaviour and the protein adsorption characteristics of PEG-MA-based gel beads were determined. In the suspension polymerization system, the organic phase including monomer, cross-linker and diluent solution was dispersed in an aqueous medium by using poly(vinylpyrrolidone) as the stabilizer. The diluent solution was prepared by mixing cyclohexanol and octanol at different volume ratios. The suspension polymerization experiments were designed in two separate parts. In the first part, ethylene glycol dimethacrylate was selected as the cross-linker and swellable PEG-MA-based gel beads were obtained by changing the cross-linker concentration, the monomer/diluent ratio and the stirring rate. In the second part, a more hydrophobic structure, divinylbenzene (DVB) was tried as a cross-linker. In this part, PEG-MA-DVB copolymer beads were obtained by changing the DVB/PEG-MA feed ratio. Then, the hydrophicility of the resulting gel beads could be controlled by changing the feed ratio of hydrophilic macromonomer to hydrophobic cross-linker. This property was also used to control the extent of nonspecific protein adsorption onto the surface of the gel beads. The non specific albumin adsorption onto the gel beads decreased with increasing PEG-MA content. No significant nonspecific adsorption at the isoelectric point of albumin was detected onto the gel beads produced with the higher PEG-MA/DVB feed ratios. For specific albumin adsorption, a triazinyl dye (i.e., cibacron blue, CB F3G-A) was covalently attached onto the surface of the copolymer beads via terminal hydroxyl groups of PEG-MA. The results of albumin adsorption experiments with the CB F3G-A carrying beads indicated that an appreciable specific albumin adsorption capacity could be obtained with the gel beads produced with a PEG-MA/DVB feed ratio of 1.5/4.0.

Key words Poly(ethylene glycol) · Poly(ethylene glycol) methacrylate · Embolization · Protein adsorption · Affinity chromatography

Introduction

Spherical gel beads have attracted significant attention in a wide variety of biotechnological and medical applications. 2-Hydroxyethylmethacrylate (HEMA) is one of the most widely used monomers for the synthesis of spherical gel beads either in microporous or macroporous form. HEMA-based microbeads have been investigated in the fields of affinity chromatography, enzyme immobilization, drug delivery, cell culturing,

embolization and immunochemistry [1–14]. Mueller et al. [15] developed a method for the synthesis of spherical poly(HEMA) beads. Cross-linked macroreticular poly(HEMA) beads produced by suspension polymerization were used as sorbents in different chromatographic applications [1, 2, 16, 17]. Scranton and coworkers [6, 7] prepared spherical microporous poly(HEMA) beads by suspension polymerization and used these beads as a carrier matrix in the controlled release of pharmaceuticals. Macroporous spherical poly(HEMA) particles were also prepared by suspension polymerization in the presence of the polymeric porogens, by using magnesium hydroxide as a stabilizer in concentrated NaCl solution [16]. Horak et al. [17] proposed another method for the preparation of HEMA-based macroporous gel beads. In their study, spherical beads with a size of hundreds of microns and a porosity of up to 68% were prepared by the suspension copolymerization of HEMA with ethylene dimethacrylate in which cyclohexanol mixed with various alcohols, hydrocarbons, butyl acetate, or cyclohexanone was utilized as a porogen solution [17].

In our study, to prepare spherical and swellable gel beads as an alternative matrix to poly(HEMA)-based particles, a macromonomer with terminal hydroxyl functionality [i.e., poly(ethylene glycol) monomethacrylate, PEG-MA] was selected. Although the synthesis of latex beads by the emulsion polymerization processes conducted in the presence of PEG-MA has been extensively investigated [18–29], the studies on the synthesis of PEG-MA-based larger polydisperse particles – especially by suspension polymerization – were very limited. In this study, a PEG-MA macromonomer with a relatively shorter chain length ($M_n = 360$) was preferred to have higher number of terminal hydroxyl groups per unit volume in the resulting gel beads. The proposed suspension polymerization provided spherical PEG-MA-based gel particles in the size range 40–200 μm. The equilibrium swelling behaviour of the resulting particles was changed by adjusting the feed ratio of the hydrophilic macromonomer (i.e., PEG-MA) to the cross-linker (e.g., ethylene-glycol dimethacrylate, EGDMA, or divinylbenzene, DVB). Then, the protein adsorption characteristics of the particles (i.e., surface properties) could also be controlled. To exemplify the usability of gel beads in the specific separation of proteins, an albumin-specific dye (cibacron blue, CB F3G-A) was covalently attached onto the beads and the albumin adsorption capacities of CB F3G-A carrying gel beads were determined.

Experimental

Materials

PEG-MA (M_n:360, $n \approx 6$, Aldrich Chemical Co., Milwaukee, Wis. was used without removing the inhibitor. 1-Octanol (Oct-OH, BDH Chemicals, Poole, UK) and cyclohexanol (Cyc-OH, BDH Chemicals) were selected as the disperse phase components in the suspension polymerization. The polymerizations were initiated with benzoyl peroxide (BPO, 97% active compound, Aldrich Chemical Co.). Poly(vinylpyrrolidone) (PVP, M_r: 360,000, Sigma Chemical Co., St. Louis Mo) was used as the stabilizer. Two different cross-linkers, EGDMA(Aldrich Chemical Co.) and DVB (55%, Aldrich Chemical Co.) were utilized. All polymerizations were performed using distilled, deionized water.

Preparation of PEG-MA-based gel beads

Cross-linked PEG-MA-based gel beads were prepared by a suspension polymerization method. A typical procedure may be given as follows: The stabilizer, PVP, was dissolved in 40 ml distilled, deionized water for the preparation of the continuous phase. The disperse phase was prepared by mixing Cyc-OH (5.5 ml), Cyc-OH (2.0 ml), PEG-MA (4.0 ml) and EGDMA (0.6 ml) in a test tube. The initiator, BPO (0.12 g), was dissolved in this homogeneous solution. The disperse phase was added to the continuous medium in a glass-sealed polymerization reactor (125 ml) placed in a water bath equipped with a temperature-control system. The polymerization reactor was heated to 85 °C within about 30 min by stirring the polymerization medium at 450 rpm. The polymerization was conducted at 85 °C for 4 h and at 90 °C for 1 h. After completion of polymerization, the reactor content was cooled to room temperature. An extensive washing procedure was applied after polymerization to remove the diluent and any possible unreacted monomer from the product. The gel beads were filtered and resuspended in ethyl alcohol. The new dispersion was stirred for about 2 h at room temperature and the gel beads were isolated by decanting the liquid part. The beads were washed twice with ethyl alcohol and then three times with distilled, deionized water using the same procedure. In the suspension polymerizations, the Oct-OH/Cyc-OH volume ratio, the PEG-MA concentration, the type and concentration of the cross-linker and the stirring rate were changed. The effects of these conditions on the bead yield, the average size and the swellability of gel beads were investigated.

In the production of PEG-MA-based gel beads using DVB as the cross-linker, a similar procedure was also followed. In a typical experiment, the droplet phase was prepared by mixing PEG-MA (1.5 ml), DVB (4.0 ml), Cyc-OH (4.5 ml) and Oct-OH (1.0 ml). Then 0.10 g BPO was dissolved in the resulting homogeneous solution. The suspension copolymerization procedure after this step was identical with the previous one.

Yield of the gel beads

The washed polymer microbeads were dried in a vacuum oven at 60 °C for 48 h before weighing. The microbead yield was determined by the following expression:

$$\text{Microbead yield} = (W_p/W_m) \times 100 \ , \quad (1)$$

where W_p and W_m are the weight of dry microbeads and the total weight of the monomers initially charged in the reactor, respectively.

Average size and the size distribution of the gel beads

The biological applications usually involve the use of gel beads within aqueous media. For this reason, the average size and the size distribution in the swollen state of the gel beads were considered. The beads equilibriated in a phosphate buffer medium having a pH of 7.4 were observed with an optical microscope (Nikon Alphapot YS2, Japan) with 40× magnification. The photographs from the

optical microscopic examination were printed with 110× magnification. Approximately 200–400 microbeads were counted on the photograph of each sample. The number-average diameter (D_n) of the latex particles were calculated according to Eq. (2), where, N_i is the number of particles with diameter D_i (μm). The polydispersity index (U) was calculated using Eq. (3). Here, D_w is the weight-average diameter of the latex particles calculated using Eq. (4).

$$D_n = \sum N_i D_i / \sum N_i \quad (2)$$

$$U = D_w / D_n \quad (3)$$

$$D_w = \sum N_i D_i^4 / \sum N_i D_i^3 \quad (4)$$

The histograms indicating the size distribution of the gel beads were plotted by taking into account the size range values defined in the Tyler standards [30].

Swellability of the gel beads

In order to determine the equilibrium swelling ratio of the gel beads, approximately 5 g dry sample was put into a cylindrical tube. The apparent volume of the formed bed by the dry beads (V_d) was measured. Then, 50 ml phosphate buffer having a pH of 7.4 and a total ionic strength of 0.1 was added into the tube. The sealed tube was shaken on a rotator at 30 rpm for 24 h. At the end of this period, the apparent volume of the bed formed by the swollen beads (V_s) was recorded. The equilibrium swelling ratio was calculated based on the following expression:

$$\text{Equilibrium swelling ratio} = (V_s/V_d) \quad (5)$$

Morphology of the gel beads

To observe the surface morphology, the gel beads produced with different Oct-OH/Cyc-OH volume ratios were dried in vacuo at 60 °C. The beads were then coated with a thin layer of gold (about 100 Å thickness) in vacuo and the electron micrographs showing the surface and internal structures were obtained using a scanning electron microscope (JEOL, JEM 1200EX, Japan).

Protein adsorption experiments

First, the nonspecific bovine serum albumin (BSA) adsorption onto PEG-MA/DVB copolymer beads produced with different PEG-MA/DVB feed ratios was investigated. The equilibrium adsorption experiments were performed at the isoelectric point of BSA (i.e., pH 5.0) in batch fashion. In these experiments, the initial BSA concentration was changed between 0.5 and 5.0 g/l by using PEG-MA/DVB copolymer beads produced with a certain PEG-MA/DVB feed ratio. In a typical adsorption experiment, BSA was dissolved in 50 ml buffer solution (CH_3COOH/CH_3COONa) having a pH of 5.0 and a total ionic strength of 0.1. A certain amount of dry PEG-MA/DVB copolymer particles (1.0 g) was dispersed in the BSA solution and the adsorption was continued for 24 h at 25 °C with a 250-rpm stirring rate. The preliminary experiments indicated that the adsorption process reached equilibrium within 4–6 h depending on the type of gel beads. Then 24 h was used as a safety period for the establishment of equilibrium in the adsorption process. At the end of this period, the particles were separated from the adsorption medium by filtration. The initial and final BSA concentrations were determined by the Biuret method and the equilibrium BSA adsorption capacity was calculated from the difference [31].

To test the specific albumin adsorption ability of the gel beads, first an albumin-specific ligand (i.e., CB F3G-A) was covalently attached onto the gel beads via the terminal hydroxyl groups of the PEG chains. A typical procedure for the attachment of CB F3G-A may be given as follows. CB F3GA (300 mg) was dissolved in 10 ml water. The dye solution was added to a 90-ml aqueous dispersion including 3 g PEG-MA-*co*-DVB copolymer particles produced with a certain DVB/PEG-MA volume ratio. Then, NaOH (4 g) was added to the dispersion. The resulting mixture was kept at 80 °C in a sealed beaker for 4 h with a stirring rate of 400 rpm. The particles were filtered and washed extensively with distilled, deionized water until all unbound dye had been removed. After completion of the washing, the supernatant samples withdrawn from the aqueous dispersion of the dyed gel particles were observed using a UV-vis spectrophotometer at a wavelength of 540 nm. The washings were continued until no significant absorption due to the dye leakage from the particles was detected [31, 32].

The equilibrium BSA adsorption capacities of CB F3G-A attached PEG-MA/DVB copolymer beads produced with different PEG-MA/DVB feed ratios were also determined under conditions identical to those used in the nonspecific BSA adsorption experiments.

Results and discussion

First, to have an idea about the solubility behaviour of PEG-MA within water, the absorbances of PEG-MA water mixtures prepared with different PEG-MA concentrations were measured at a wavelength of 540 nm, using a UV-vis spectrophotometer. Before measurement of the absorption, each solution was sonicated at 200 W for 5 min. The variation of absorbance with PEG-MA concentration is given in Fig. 1. As seen here, no significant change was observed in the absorbance up to a PEG-MA concentration of 4% (v/v) since transparent PEG-MA water mixtures were obtained in this range of PEG-MA concentration. The absorbance sharply increased after 4.0% because turbid solutions

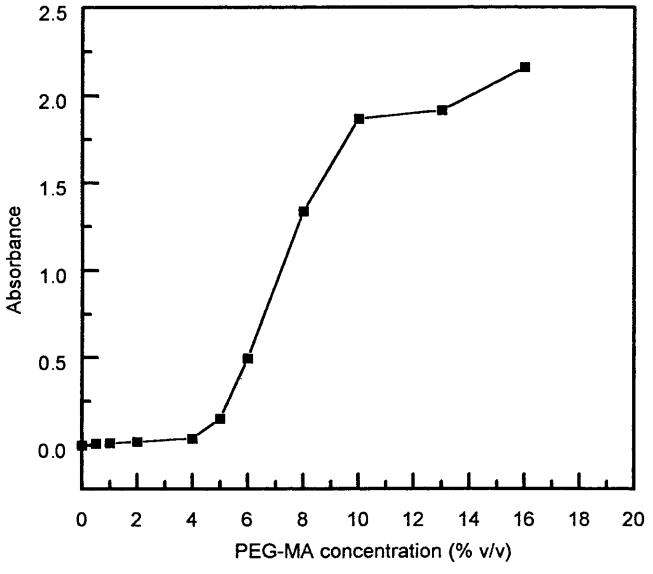

Fig. 1 The variation of absorbance with poly(ethylene glycol) methacrylate (*PEG-MA*) concentration in PEG-MA/water mixtures (λ = 540 nm)

Table 1 Typical suspension polymerization recipes for poly(ethylene glycol) methacrylate(*PEG-MA*)

Ingredient	Recipe I	Recipe II	Recipe III[a]
PEG-MA (ml)	4.0	–	4.0 (91.3)
Ethylene glycol dimethacrylate (ml)	0.6	4.6	0.6 (8.7)
Benzoyl peroxide (g)	0.12	0.12	0.12
Cyclohexanel (ml)	–	–	5.5
1-Octanol (ml)	–	–	2.0
Poly(vinylpyrrolidone) (g)	0.40	0.40	0.40
Water (ml)	40	40	40
Stirring rate (rpm)	450	450	450
Temperature (°C), time (h)	85.4/90.1	85.4/90.1	85.4/90.1
Product properties:			
Number-average size (D_n, μm)	112.8	~2500 (irregular)	97.7
Polydispersity index (D_w/D_n)	1.26	–	1.11
Bead yield (% w/w)	31.7	87.5	81.6
Glass-transition temperature (°C)	234	247	224
Swelling ratio	Nonswellable	Nonswellable	2.22

[a] The concentrations of monomeric ingredients based on the total moles of monomer and cross-linker are given within the parentheses

were obtained with the higher PEG-MA concentrations. Therefore, the maximum solubility of PEG-MA within water was estimated as approximately 4.0%.

To find an appropriate recipe for the suspension polymerization of PEG-MA performed using an aqueous phase as the continuous medium, the three different formulations given in Table 1 were tried. In the first recipe, a conventional suspension polymerization procedure was tried by using an oil-soluble initiator in the absence of an organic diluent; a reasonably low bead yield (i.e., 31.7% w/w) was obtained. The optical micrograph of the product obtained using the first recipe (Fig. 2A) showed that the gel particles in spherical form could be achieved in the absence of diluent. In the second recipe, only the cross-linker (i.e., EGDMA) was utilized as the monomer phase in the absence of diluent mixture. Note that this recipe was applied as a reference. As seen in Table 1, the second recipe provided a reasonably higher bead yield (i.e., 87.5%) relative to that of the first; however, reasonably large and irregular (i.e., shapeless) beads were produced using this recipe. The suspension polymerization of PEG-MA in the presence of a diluent phase comprised of Cyc-OH and Oct-OH was tried as the third recipe. It should be noted that similar diluent mixtures have been tried by others for the suspension polymerization of a highly water-soluble monomer (e.g., HEMA) by using an aqueous phase as the continuous medium [17]. By applying the third recipe, spherical gel beads could be obtained with a satisfactory yield (Fig. 2B, Table 1).

The differential scanning calorimetry thermograms of the beads produced with these recipes are given in Fig. 3. The glass-transition temperatures (T_g) of the gel beads determined by the evaluation of the differential scanning calorimetry thermograms are given in Table 1. The T_g values obeyed the following order: T_g (recipe II) > T_g (recipe I) > T_g (recipe III).

The highest T_g value (247 °C) was obtained for the gel beads produced by using only EGDMA as the monomer phase (i.e., recipe II). This is an expected result because the gel beads having the highest cross-linking density were probably obtained with this recipe. It should be noted that these beads were obtained in the nonswellable form. The T_g value of the poly(PEG-MA-*co*-EGDMA) gel beads produced in the absence of diluent (234 °C, recipe I) was higher than that of the gel beads obtained with the diluent (224 °C, recipe III). This result may be attributed to the cross-linking density of the gel beads produced in the absence of diluent being higher relative to those produced in the presence of diluent. This conclusion was also supported by the equilibrium swelling ratios of these beads (Table 1). While nonswellable gel beads were obtained in the absence of diluent, the equilibrium swelling ratio of the gel beads produced with the diluent was 2.22. This result probably indicated that the PEG-MA content of final gel beads was higher when the organic diluent was included in the suspension polymerization performed using water as the continuous medium.

To support this conclusion, the physical properties of the ingredients used in the suspension polymerization were determined and are given in Table 2. The monomeric ingredients (PEG-MA and EGDMA) were infinitely soluble within the selected diluent mixture (Cyc-OH and Oct-OH) because the solubility parameters were very close. As seen in Table 2, the solubilities of PEG-MA and EGDMA in water were 4.0 and less than 0.1%, respectively. When a 40 ml PEG-MA/water mixture including 10% PEG-MA was prepared as in recipe 1, most of the water-soluble macromonomer was probably

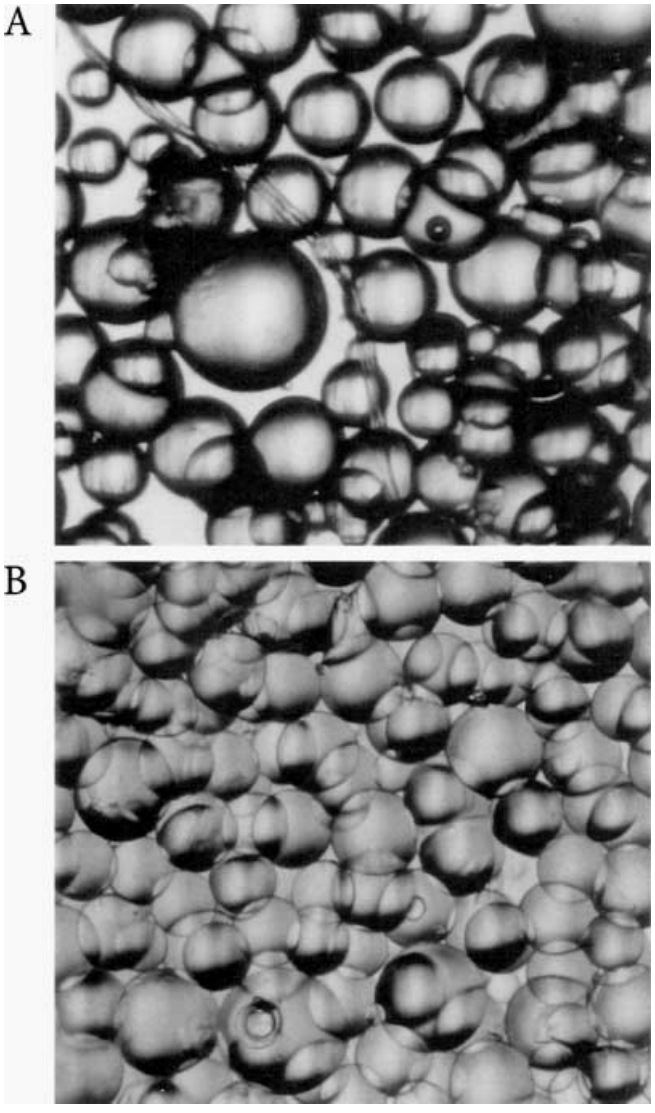

Fig. 2A, B Optical micrographs of the PEG-MA-based gel beads produced with different formulations. Magnification: ×110. **A** Recipe I, **B** recipe III

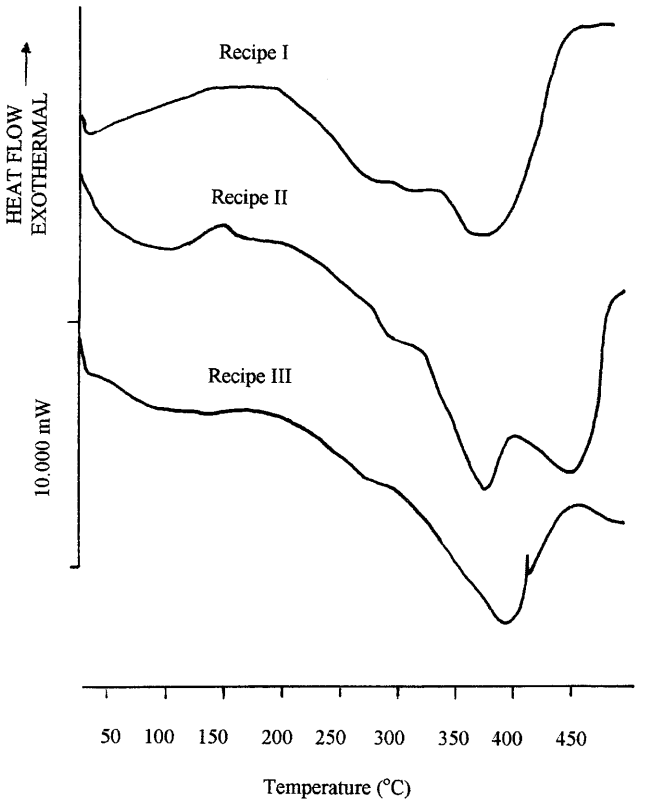

Fig. 3 Differential scanning calorimetry thermograms of gel beads produced with different formulations

not converted into the bead form in the suspension polymerization initiated by an oil-soluble initiator such as BPO. Actually, this conclusion was confirmed by the bead yield value obtained using the first recipe (i.e., 31.7%). The solubility behaviour of the selected macromonomer involved the introduction of a water-insoluble organic diluent phase having a solubility parameter similar to that of its own. By using such a diluent mixture, a significant fraction of loaded PEG-MA could be preferentially solubilized in the organic droplet phase dispersed in an aqueous continuous medium.

Additionally, the dispersion behaviour of PEG-MA in water is not convenient for the typical product range of suspension polymerization usually leading to spherical beads in the size range 50–1000 μm. The optical micrographs of PEG-MA/water and EGDMA/water dispersions are given in Fig. 4. Here, EGDMA was included as a reference material for comparison and both dispersions contained 10% monomeric ingredient (i.e., PEG-MA or EGDMA). These dispersions were prepared by mixing the corresponding ingredients at 300 rpm for 5 min at room temperature. Although large droplets (mostly in the size range 50–150 μm) were observed in the EGDMA/water mixture, the size of the PEG-MA droplets in the PEG-MA/water dispersion was between 2 and 20 μm. The droplet size observed in the PEG-MA/water dispersion was reasonably small relative to the usual range of suspension polymerization. The interfacial tension between PEG-MA and water is lower relative to that between EGDMA and water because PEG-MA has a surface tension closer to that of water relative to EGDMA (Table 2). As a result the low droplet size was probably obtained. To achieve larger final particle size (i.e., larger droplet size) values in the suspension copolymerization of PEG-MA and to obtain sufficiently large droplets in which PEG-MA was preferentially solubilized, water-insoluble organic agents having sufficiently

Table 2 Physical properties of ingredients used in suspension polymerization

Ingredient	Density[a] (g/ml)	Molecular weight[a] (g/gmol)	Surface tension (dyn cm^{-1})[b]	Viscosity[c] (cP)	Solubility parameter $(cal/cm^3)^{1/2}$	Solubility in 100 parts water
PEG-MA	1.105	360.0	42.95	34.6	9.0[d]	~4.0
Ethylene glycol dimethacrylate	1.051	198.2	33.52	3.1	9.2[e]	<0.1
Cyclohexanol	0.948	100.2	36.79	50.2	11.4[f]	3.6[g]
1-Octanol	0.827	130.2	31.46	5.9	10.3[f]	0.054[g]

[a] Taken from supplier catalogue
[b] Measured in a Traube stalogmometer at 20 °C using pure water as a reference liquid [33]
[c] Measured in an Ostwald viscometer at 20 °C using pure water as a reference liquid
[d] Calculated value [34]
[e] Taken from Ref. [33]
[f] Taken from Ref. [34]
[g] Taken from Ref. [30]

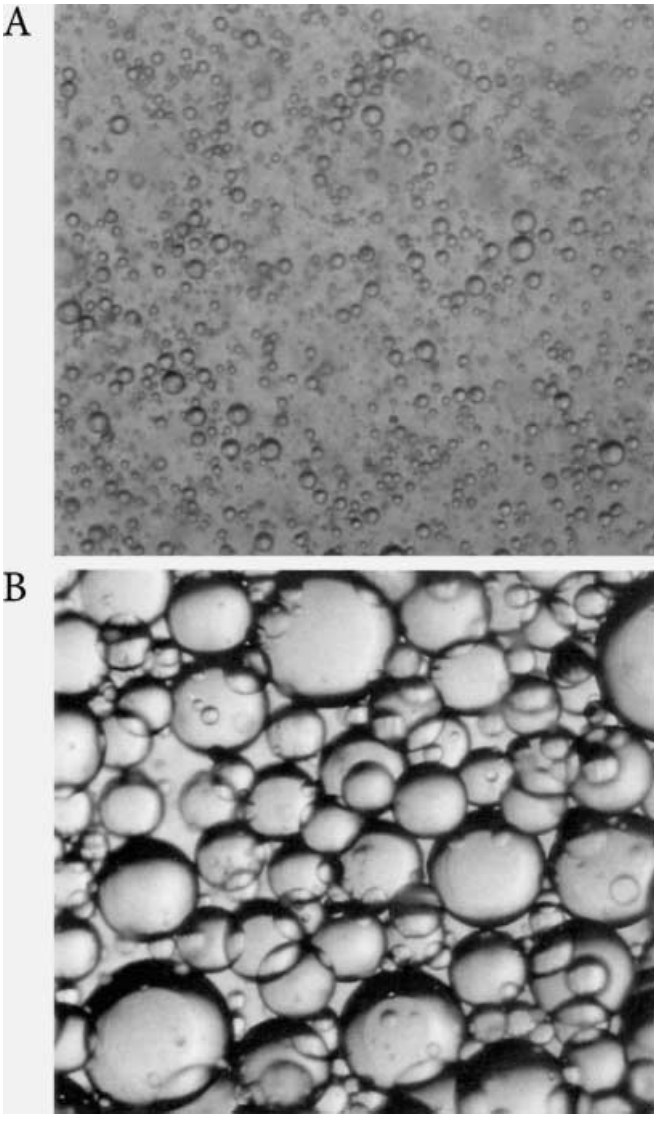

Fig. 4A, B Optical micrographs of PEG-MA/water and ethylene glycol dimethacrylate (*EGDMA*) water dispersions. Magnification: ×110. **A** PEG-MA/water, **B** EGDMA/water

Table 3 The variation of bead properties with the stirring rate

Stirring rate (rpm)	Bead yield (% wt)	Average size (D_n, μm)	Polydispersity index (U)	Swelling ratio
300	80.3	161.3	1.12	2.03
450	81.6	97.7	1.11	2.22
600	82.4	41.3	1.51	2.29

lower surface tension values relative to water (i.e., Cyc-OH and Oct-OH) were selected as the components of the diluent mixture (Table 2).

Unless stated otherwise, the conditions given for recipe III were utilized as the common conditions in the systematic suspension polymerization experiments on the effect of monomer/diluent ratio, composition of diluent solution, cross-linker concentration and stirring rate. The effects of polymerization conditions on the product properties are given in the following sections.

Stirring rate

In this set of experiments, the stirring rate was changed between 300 and 600 rpm. The properties of the gel beads obtained with different stirring rates are given in Table 3. As seen here, the yield of the gel beads did not change significantly with the stirring rate. The average size clearly decreased with increasing stirring rate as described in the literature for different suspension polymerization systems [35–40]. The histograms indicating the size distribution of the gel beads are exemplified in Fig. 5. The polydispersity index values calculated from the data given Fig. 5 indicated that the size distribution was relatively narrower with the lower stirring rates (Table 3). A relatively wide size distribution was obtained with the highest stirring rate (i.e., 600 rpm). It should be noted that no significant effect of stirring rate on the equilibrium swelling ratio was observed.

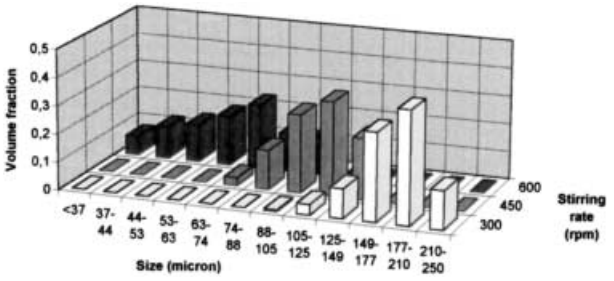

Fig. 5 The histograms indicating the size distribution of PEG-MA-based gel beads obtained with different stirring rates

Monomer/diluent ratio

The total monomer/diluent ratio was changed in the range 0.15–0.61 (i.e., 1.15/7.5–4.6/7.5). Note that, the cross-linker (i.e., EGDMA) feed concentration was fixed to 13.0% v/v based on total monomer. The bead yields and the average size values obtained with different monomer/diluent ratios are given in Table 4. As seen here, higher bead yields were observed with the higher monomer/diluent ratios. The higher monomer concentration in the organic phase possibly caused an increase in the rate of polymerization, which in turn provided higher bead yields for a constant reaction period. Only a slight increase was detected in the bead size with increasing monomer/diluent ratio. Each monomer/diluent ratio corresponded to a certain value of the monomer/water ratio since the diluent/water volume ratio was fixed at a constant value in this group of experiments (i.e., 7.5/40). According to the common mathematical model of suspension polymerization, the average size was directly proportional to the volume ratio of the monomer phase to the suspension medium [35–37]; however, this model was valid for the suspension polymerization systems containing a monomer phase which was completely immiscible with the continuous medium. Then, our system did not obey the usual tendency. Although the monomer/diluent ratio did not seem to have an effect on the bead size, the size distribution was strongly influenced. Reasonably narrower size distributions were obtained on increasing the monomer/diluent ratio.

Table 4 The variation of bead properties with the monomer/diluent ratio

Monomer/diluent ratio[a]	Bead yield (% wt)	Average size (D_n, μm)	Polydispersity index (U)	Swelling ratio
1.15/7.5 (1.0, 0.15)	68.2	90.4	1.49	2.08
2.3/7.5 (2.0, 0.3)	80.1	96.1	1.28	2.31
4.6/7.5 (4.0, 0.6)	81.6	97.7	1.11	2.22

[a] The volumes of PEG-MA and ethylene glycol dimethacrylate are given in parentheses

EGDMA feed concentration

The EGDMA concentration was changed between 7.0 and 37.5% based on the volume of the total monomer (i.e., PEG-MA and EGDMA). In this set of experiments, the volume ratio of the total monomer to the diluent was kept constant at 4.6/7.5 by using the common conditions given in the third recipe (Table 1). The properties of the gel beads obtained with different cross-linker concentrations are given in Table 5. The results indicated that the yield of the gel beads slightly increased with increasing cross-linker concentration. This is an expected result since the conversion of monomers into the cross-linked polymer form is higher in the presence of a higher amount of cross-linker. On the other hand, the highest average size was obtained with the lowest cross-linker concentration. The other cross-linker concentrations led to the lower size values. This result is probably related to the equilibrium swelling capacity of the gel beads because the average size was determined in the swollen form. As expected, a clear decrease was observed in the equilibrium swelling ratio of the gel beads with increasing cross-linker concentration (Table 5); therefore, the gel beads having higher swelling ratios exhibited higher average size values within the aqueous medium. Another reason explaining a decrease in the average size may be related to the droplet phase viscosity. On the basis of the viscosity values given in Table 2, the increase in the feed concentration of EGDMA causes a decrease in the droplet phase viscosity. This case leads to a decrease in the size of the gel beads according to the previously published models for different suspension polymerization systems [35–37].

Composition of diluent solution

In the first group of these experiments, the Oct-OH/Cyc-OH volume ratio was changed between 0.0/7.5 and 7.5/0.0 by fixing the EGDMA concentration at 13% by volume based on the total monomer (i.e., PEG-MA/

Table 5 The variation of bead properties with the cross-linker concentration

Ethylene glycol dimethacrylate conc. (%v/v)	Bead yield (% wt)	Average size (D_n, μm)	Polydispersity index (U)	Swelling ratio
7.0	84.6	131.7	1.11	2.65
13.0	81.6	97.7	1.11	2.22
23.1	84.9	91.2	1.16	1.49
37.5	90.4	92.6	1.20	1.38

Table 6 The variation of bead properties with the diluent composition. In the first five experiments, the ethylene glycol dimethacrylate PEG-MA volume ratio was fixed at 0.60/4.0

1-Octanol/ cyclohexanol ratio[a]	Bead yield (% wt)	Average size (D_n, μm)	Polydispersity index (U)	Swelling ratio
0.0/7.5 (0.0)	86.3	71.5	1.30	2.25
2.0/5.5 (26.7)	81.6	97.7	1.11	2.22
2.5/5.0 (33.3)	83.8	106.4	1.18	2.30
3.5/4.0 (46.7)	80.1	126.3	1.31	2.14
7.5/0.0 (100.0)	82.8	Broken beads	–	2.00
7.5/0.0 (100.0)[b]	86.4	205.4	1.11	1.21
10.5/0.0 (100)[b]	86.6	Broken beads	–	1.37
7.5[b,c]	81.9	372.7	1.14	1.28

[a] The 1-Octanol concentration (% v/v) in the diluent mixture is given in parantheses
[b] Ethylene glycol dimethacrylate PEG-MA volume ratio was fixed at 4.0/4.0
[c] Only toluene was used as the diluent instead of 1-Octanol

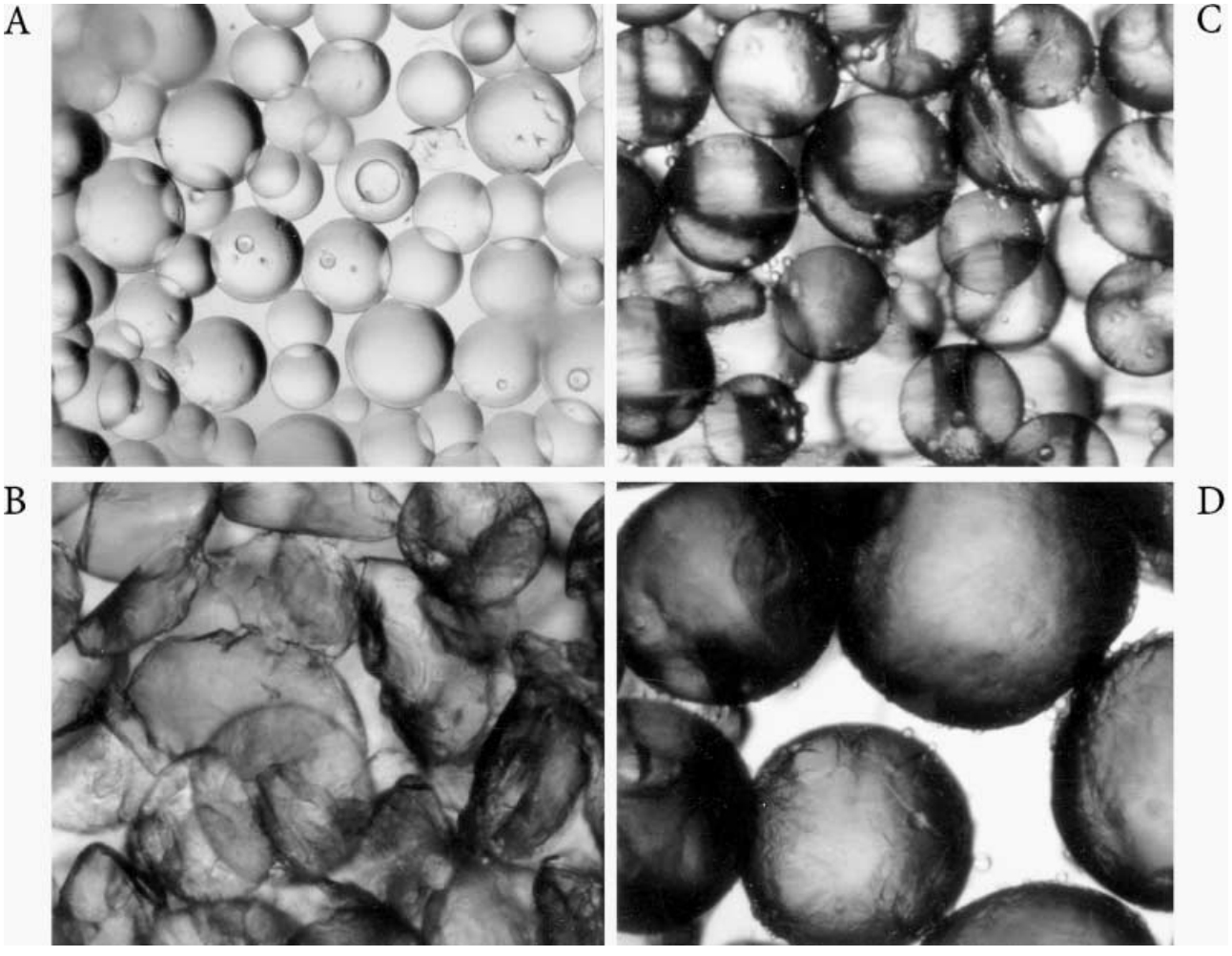

Fig. 6A–D Optical micrographs of PEG-MA-based gel beads produced with different 1-octanol(*Oct-OH*) concentrations. Magnification: ×110. Oct-OH concentration (%v/v) and PEG-MA/EGDMA ratio: **A** 33.3, 4.0/0.6; **B** 100, 4.0/0.6; **C** 100, 4.0/4.0; **D** pure toluene, 4.0/4.0

EGDMA volume ratio was 4.0/0.6). The yield of the gel beads did not change significantly with the diluent composition (Table 6). For constant EGDMA/PEG-MA volume ratio, a clear increase in the bead size was observed with increasing Oct-OH concentration in the diluent mixture. It should be noted that the surface tension of Oct-OH was reasonably low relative to Cyc-OH (Table 2). Then, the increase in the average size may be explained by the increasing interfacial tension between the droplet and the aqueous phases. A linear increase in the average size with Oct-OH concentration was also

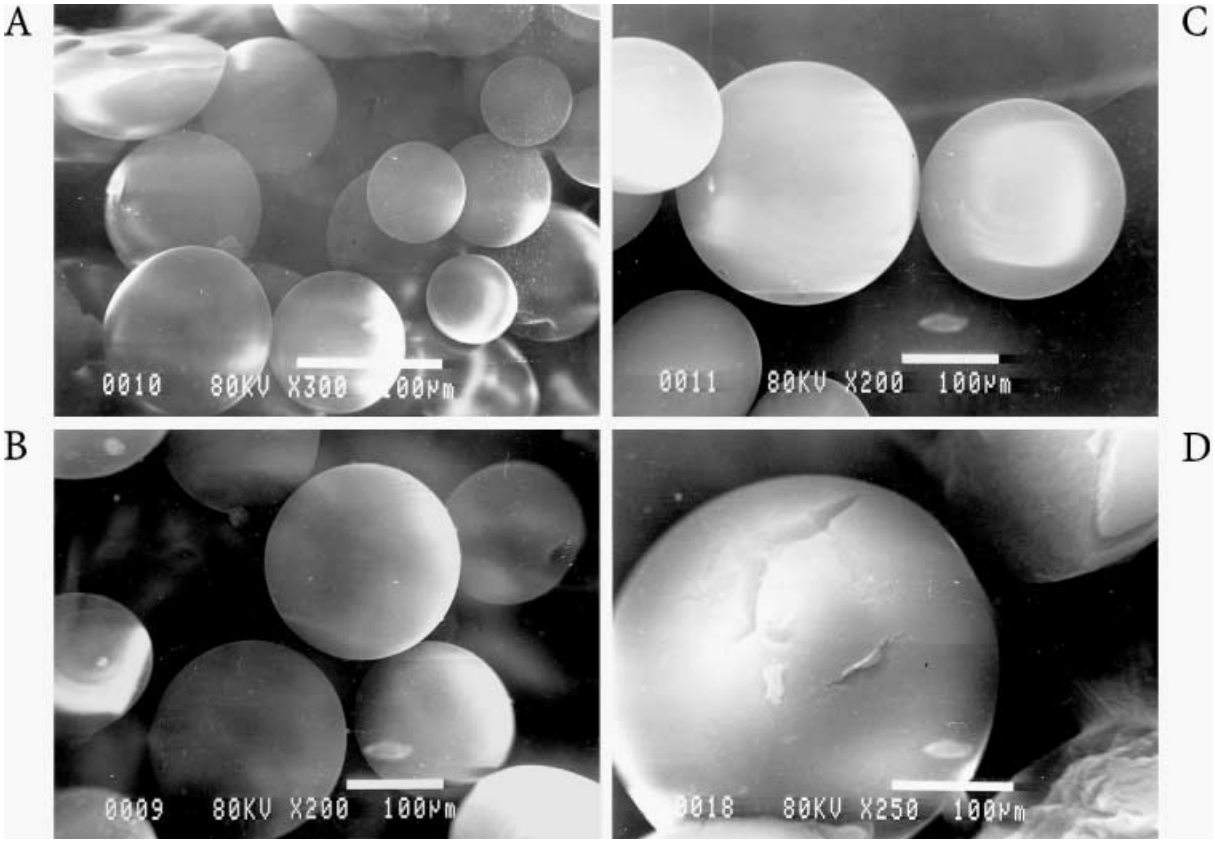

Fig. 7A–D Electron micrographs showing the surface morphology of PEG-MA-based gel beads produced with different Oct-OH concentrations. Oct-OH concentration (%v/v) and PEG-MA/EGDMA ratio: **A** No diluent, 4/0.6, magnification ×300; **B** 26.7, 4.0/0.6, magnification ×200; **C** 100, 4.0/4.0, magnification ×200; **D** pure toluene, 4.0/4.0, magnification ×250

reported for the suspension polymerization of HEMA conducted using a similar diluent mixture [17]. In that study, macroporous poly(HEMA) beads were obtained with Oct-OH concentrations of 20 and 40% (v/v), while the microporous gel beads were produced with lower values [17]. In most of the similar studies, the macroporous gel beads were visualized under an optical microscope in the form of opaque spheres [38–40]. As seen in Fig. 2B, the gel beads obtained with 26.7% Oct-OH concentration were in the transparent form. The gel beads produced with Oct-OH concentrations of 33.3 and 46.6% were also transparent (Fig. 6A). The transparency clearly indicated that these beads were not macroporous and that they possessed conventional gel-type microporosity in the swollen form. As seen in Fig. 6B, the use of pure Oct-OH as a diluent with the EGDMA/PEG-MA volume ratio of 0.6/4.0 provided broken gel beads; however, these gel beads were not transparent. To produce mechanically stable and macroporous gel beads, the volume ratio of EGDMA/PEG-MA was increased to 4.0/4.0 and the same amount of pure Oct-OH was again used as a diluent (Table 6). This modification led to reasonably opaque and spherical gel beads as given in Fig. 6C. Finally, toluene was used instead of pure Oct-OH as a diluent and the EGDMA/PEG-MA volume ratio was again fixed at 4.0/4.0 (Table 6). This combination also provided more opaque and spherical gel beads as given in Fig. 6D.

The surface and internal morphology of the produced beads were examined by scanning electron microscopy. The electron micrographs of the gel beads produced with different diluent compositions are given in Fig. 7. The surface of the nonswellable and nonporous beads produced in the absence of diluent (i.e., recipe I) was completely smooth (Fig. 7A). As expected, the swellable gel beads (i.e., the beads produced in the presence of diluent having 26.7% Oct-OH concentration) also had a smooth and nonporous surface in the dry state (Fig. 7B). Note that a similar surface structure was observed for the gel beads produced with the Oct-OH concentrations of 33.3 and 46.7%. Because these beads have no permanent porosity but possess gel-type microporosity generated by the expansion of the bead volume by the diffusion of water into the cross-linked PEG-MA chains (i.e., formation of swollen gel), the microporosity disappears due to the contraction of the gel structure by the removal of water from the beads [17]. Then, a smooth and nonporous surface structure was obtained in the dry state (Fig. 7). It was interesting that the gel

beads observed as opaque spheres under the optical microscope (i.e., the beads produced using pure Oct-OH or toluene as the diluent and with a EGDMA/PEG-MA ratio of 4.0/4.0) also had a nonporous surface in the dry state (Fig. 7C, D). These findings may be attributed to the fact that the gel beads produced with pure Oct-OH or toluene had a smooth surface – which was probably a microporous layer in the swollen state – covering the macroporous interior. To prove this conclusion, the broken microbeads were examined by electron microscopy. The electron micrographs of the gel beads produced using Oct-OH and toluene are exemplified in Fig. 8. In both photographs, the bead interior had a reasonably rough structure. These photographs show the macroporous character of the internal part of the gel beads obtained with a EGDMA/PEG-MA ratio of 4.0/4.0 and using pure Oct-OH or toluene as a diluent. The equilibrium swelling ratios of these beads were lower relative to those obtained with the first five runs because a higher cross-linker feed concentration in the monomer mixture was utilized (Table 6). An efficient phase separation leading to the formation of a macroporous structure can occur with a sufficiently high cross-linker concentration within the beads [17, 41–43]. Due to the low water solubility of EGDMA (below 0.1%, Table 2),

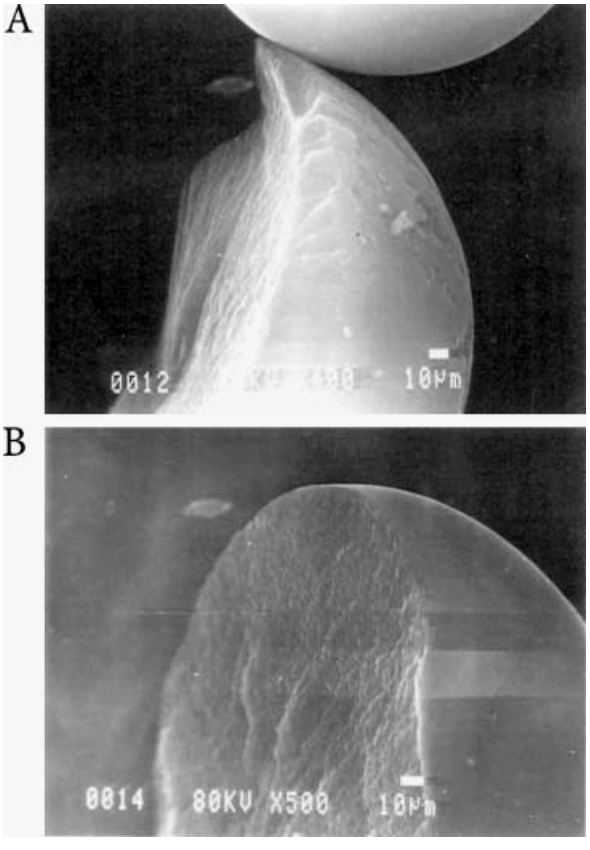

Fig. 8 Electron micrographs showing the internal structure of PEG-MA-based gel beads produced with different diluents. Diluent type and PEG-MA/EGDMA ratio: **A** Oct-OH, 4.0/4.0, magnification ×400; **B** pure toluene, 4.0/4.0, magnification ×500

Table 7 The variation of bead properties with the monomer composition with divinylbenzene (*DVB*) as the cross-linker. Polymerization conditions: benzoyl peroxide 0.12 g; cyclohexanol 4.5 ml, 1-Octanol 1.0 ml, poly(vinylpyrrolidone) 0.40 g, water 40 ml, stirring rate 450 rpm, temperature and time 85 °C, 4 h and 90 °C, 1 h

PEG-MA/DVB ratio	Bead yield (% wt)	Average size (D_n, μm)	Polydispersity index (U)	Swelling ratio
0.5/5.0	91.6	137.6	1.21	Nonswellable
1.5/4.0	83.7	144.7	1.13	1.06
2.5/3.0	83.0	122.1	1.13	1.11
5.0/0.5	81.4	109.7	1.12	2.03

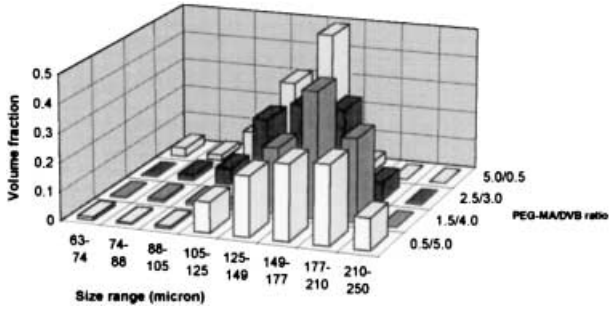

Fig. 9 The histograms indicating the size distributions of PEG-MA-based gel beads obtained with different PEG-MA/divinylbenzene(*DVB*) ratios

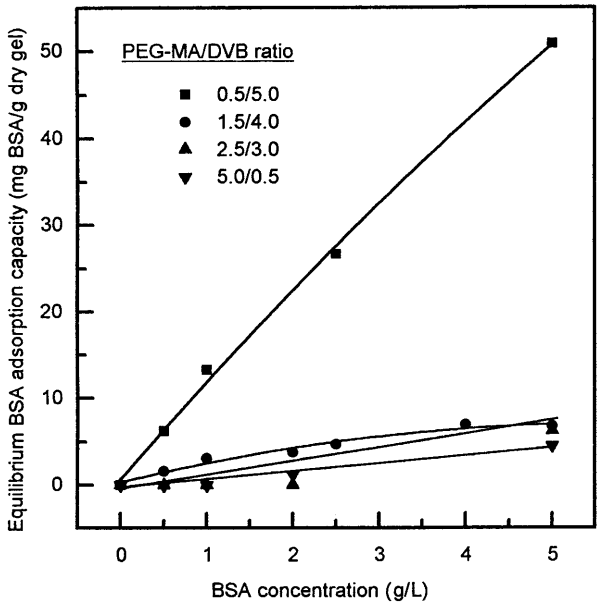

Fig. 10 The variation of nonspecific bovine serum albumin(*BSA*) adsorption capacity with the initial BSA concentration for the gel beads produced with different PEG-MA/DVB ratios

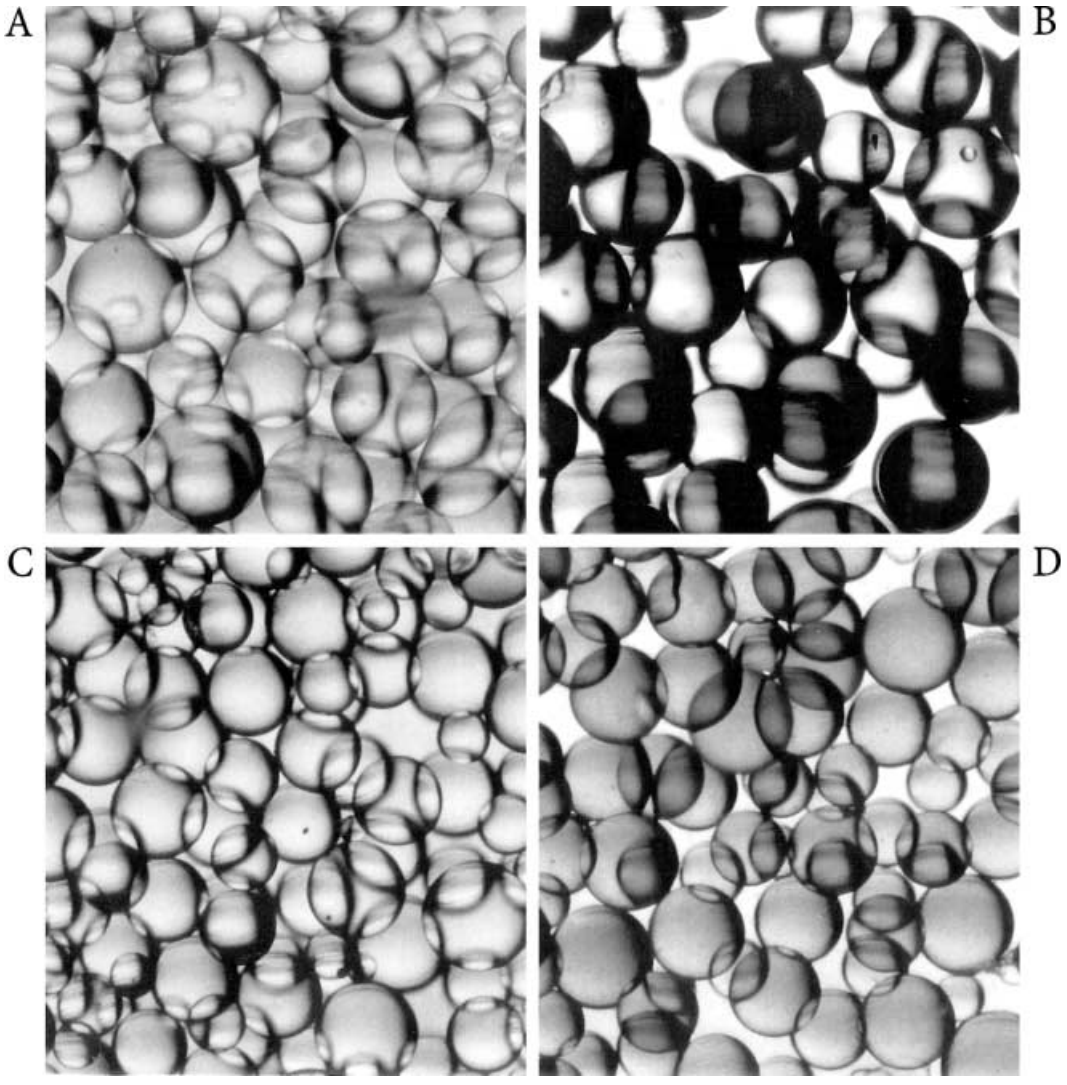

Fig. 11A–D Optical micrographs of plain and dyed PEG-MA-based gel beads obtained with different PEG-MA/DVB ratios, Magnification: ×110, PEG-MA/DVB ratio: **A** 1.5/4.0, plain beads; **B** 1.5/4.0, cibacron blue F3G-A(*CB F3G-A*) attached beads; **C** 5.0/0.5, plain beads; **D** 5.0/0.5, CB F3G-A attached

its concentration on the surface of the forming beads surrounded by an aqueous medium is probably low relative to that in the bead interior. This case may lead to a gel-type microporous surface with a low cross-linking density relative to the bead interior (Fig. 7C, D). The macroporous interior (observed in Fig. 8A, B) is probably formed by an efficient phase separation taking place with the higher cross-linker concentration in the bead interior.

PEG-MA-*co*-DVB beads

PEG is recognized as an effective material for low protein adsorption and low cell adhesion; therefore, it is one of the most widely used agents for the reduction or elimination of nonspecific protein adsorption and non-specific cell adhesion onto polymeric biomaterials [24, 44–45]. On the other hand, a widely accepted generalization is that the more hydrophobic the surface, the greater the extent of nonspecific protein adsorption [46]. By combining these two opposite cases, it is proposed that the copolymer beads with controlled hydrophobicity (i.e., controlled protein adsorption characteristics) can be prepared by the suspension copolymerization of hydrophilic PEG-MA with a reasonably hydrophobic comonomer. DVB was selected as the constituent which could act as both the hydrophobic comonomer and the cross-linker. In this set of experiments, a series of PEG-MA-*co*-DVB copolymer beads were prepared by changing the feed ratio of PEG-MA/DVB between 0.5/5.0 and 5.0/0.5. The synthesis conditions and the properties of the gel beads are given in Table 7. As seen here, the bead yield increased with increasing cross-linker (i.e., DVB)

concentration. It should be noted that similar behaviour to that observed with EGDMA was also obtained for the bead yield with DVB. The size distributions of the PEG-MA-*co*-DVB beads produced with different PEG-MA/DVB ratios are given in Fig. 9. The size polydispersity index values calculated based on the size distribution data in Fig. 9 indicated that the size distribution was reasonably wide for a low PEG-MA/DVB ratio (i.e., 0.5/5.0) (Table 7). Higher PEG-MA/DVB ratios led to a narrower size distribution. Then, the size distribution characteristics of the final beads could be improved by increasing the PEG-MA feed concentration. For a roughly constant polydispersity index value (i.e., 1.13), the average size decreased with increasing PEG-MA concentration. This result may be explained by the decreasing interfacial tension between the droplet phase and the continuous medium by the increasing PEG-MA concentration. As expected, the equilibrium swelling ratio of the gel beads clearly increased with decreasing DVB concentration.

BSA adsorption onto the PEG-MA-*co*-DVB gel beads

First, PEG-MA-*co*-DVB gel beads produced with different PEG-MA-*co*-DVB ratios were used as sorbents for the nonspecific BSA adsorption. Here, the initial BSA concentration was changed between 0.5 and 5.0 g/l for each type of gel bead. The effect of the initial BSA concentration on the equilibrium BSA adsorption capacities of the gel beads produced with different PEG-MA/DVB ratios is given in Fig. 10. These curves were obtained at the isoelectric point of BSA (i.e., pH 5.0) at 25 °C. At constant initial BSA concentration, the nonspecific BSA adsorption capacity of PEG-MA-*co*-DVB gel beads produced with the lowest PEG-MA/DVB feed ratio (i.e., 0.5/5.0) was reasonably high relative to those of the other gel beads (Fig. 10). As expected, an increase in the PEG-MA content of the gel beads resulted in a significant decrease in the nonspecific BSA adsorption. In the BSA concentration range 0–2 g/l, approximately zero nonspecific BSA adsorption was observed with the gel beads produced with the PEG-MA/DVB ratios of 2.5/3.0 and 5.0/0.5. One can conclude that the nonspecific BSA adsorption can be reduced by increasing the hydrophilicity of the copolymer structure produced with PEG-MA and DVB.

To test the usability of copolymer beads as a specific sorbent for BSA adsorption, an albumin-specific dye (i.e., CB F3G-A) was directly attached onto the beads via terminal hydroxyl groups of PEG-MA. The plain and dyed copolymer beads are exemplified in Fig. 11 by the optical micrographs taken with the swollen beads in phosphate buffer. As seen here, no deformation was observed in the spherical form due to the dye-binding reaction conducted at strong alkaline conditions at 80 °C. The transparent form of the plain gel beads indicated that no macroporosity was introduced into the bead structure by the proposed copolymerization method. It should be noted that the other copolymer beads had a structure similar to that given in Fig. 11. The swellable beads (except the nonswellable sample produced by the PEG-MA/DVB ratio of 0.5/5.0) possessed conventional gel-type microporosity. The variation of the equilibrium BSA adsorption capacity of CB F3G-A attached PEG-MA-*co*-DVB beads is given in Fig. 12. For constant initial BSA concentration, the highest BSA adsorption capacity was obtained with the CB F3G-A attached PEG-MA-*co*-DVB beads produced with the lowest PEG-MA/DVB ratio. It should be emphasized that reasonably high nonspecific BSA adsorption capacities were also obtained with the plain form of the same beads (Fig. 10). A comparison of the BSA adsorption capacities given in Figs. 10 and 12 indicated that the BSA adsorption capacity observed with the CB F3G-A attached PEG-MA-*co*-DVB beads produced with the lowest PEG-MA/DVB ratio included a considerable amount of nonspecific BSA adsorption. Reasonably high BSA adsorption capacities up to 32 mg/g could be achieved with the CB F3G-A attached PEG-MA-*co*-DVB beads produced with PEG-MA/DVB ratio of 1.5/4.0. The plain form of these beads also exhibited reasonably low nonspecific BSA adsorption (Fig. 10). One can conclude that most of the BSA adsorption onto the dyed form of the beads produced with a PEG-MA/DVB ratio of 1.5/4.0 occurred via the specific interaction between BSA molecules and immobilized CB F3G-A. Then, the PEG-MA/DVB ratio of 1.5/4.0 seems the

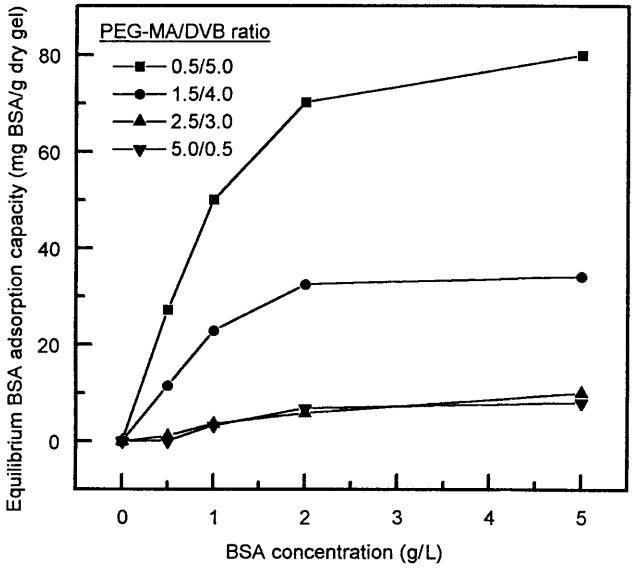

Fig. 12 The variation of equilibrium BSA adsorption capacity with the initial BSA concentration for CB F3G-A attached gel beads produced with different PEG-MA/DVB ratios

most appropriate feed ratio for the synthesis of copolymer beads exhibiting a maximum value for the ratio of specific to nonspecific BSA adsorption. The dyed form of the copolymer gel beads produced with the PEG-MA/DVB ratios of 2.5/3.0 and 5.0/0.5 exhibited reasonably low BSA adsorption capacities. The high PEG content (i.e., strong hydrophilic character) of these beads is probably responsible for preventing the interaction of albumin and CB F3G-A molecules immobilized on these beads.

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