Survey of Preparation Techniques of Monodispersed Microspheres of Glycidyl Methacrylate and Its Derivatives

MITSUTOSHI HOSHINO* and KOICHI ARISHIMA

NTT Opto-Electronics Laboratories, Tokai-mura, Ibaraki-ken, 319-11 Japan

SYNOPSIS

This article describes the preparation of monodispersed microspheres of glycidyl methacrylate, 2-hydroxyethyl methacrylate, and triethylene glycol dimethacrylate by radical polymerization. The initial stage of the polymerization reaction began in the liquid phase. As the polymerization proceeded, a nuclear polymer chain of microspheres was generated from soluble oligomeric radicals and then solidified from the liquid phase. The radius of the microspheres was controlled by polymerization parameters, such as monomer concentration, polymerization time, and the kind of polymerization solvent. A small number of thin platelike substances may be produced by the anisotropy of the two-dimensional monomer added to the oligomeric radicals. The monodispersed microspheres were achieved through the use of the following: (1) a fluorinated tube in which the polymerization reaction proceeded; (2) a monomer concentration of about 1 mol/L; (3) a higher concentration of crosslinking reagent than used commercially; (4) the prevention of the occurrence of polymer microsphere aggregation; (5) pouring the solution mixtures into a large amount of cooled acetone after the polymerization reaction; and (6) the use of a low-temperature purification process and centrifugation at low temperature. This article describes the mechanism of microsphere formation and growth by solution polymerization. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

We developed a very sensitive new assay,[1-3] i.e., the laser magnetic immunoassay (LMIA), several years ago. A feature of the method is the use of fine magnetite particles (particle diameter: about 100 Å) as a labeling reagent for capturing target viruses. The particles were connected by γ-globulin to microspheres (particle diameter: about 10,000 Å) with amino groups. The antibody-magnetite labeling conjugates were gathered in a small area on a water surface and detected by the magnetic driving force of a magnetic pole. The height of the microprotuberance that they formed above the water surface was proportional to the number of object viruses (diameter: about several 10 Å) labeled with magnetite. The polymer microspheres used for our new sensitive assay must meet the four criteria outlined below: First, the microspheres must have hydrophilic properties. Second, they must have no magnetic contamination. Third, they must be monodispersible. Fourth, their surface must include an amino group which will attach itself to the antigen IgG, which, in turn, attaches itself to the target virus.

Hosaka et al. described the preparation of microspheres of poly(glycidyl methacrylate) and its derivatives.[4-8] They reported that the advantage of precipitation polymerization was being able to preclude the possibility of epoxy group cleavage during polymerization. The way to immobilize γ-globulin on the microspheres was described in their paper.[4] Another advantage of this polymerization method is that the reaction system does not include the polymer dispersion stabilizer that is usually used in dispersion polymerization, so the resulting microspheres are produced without any contamination except for initiator fragments. In this article, we describe the preparation of monodispersed microspheres for LMIA by precipitation polymerization.

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 57, 921-930 (1995)
 CCC 0021-8995/95/080921-10

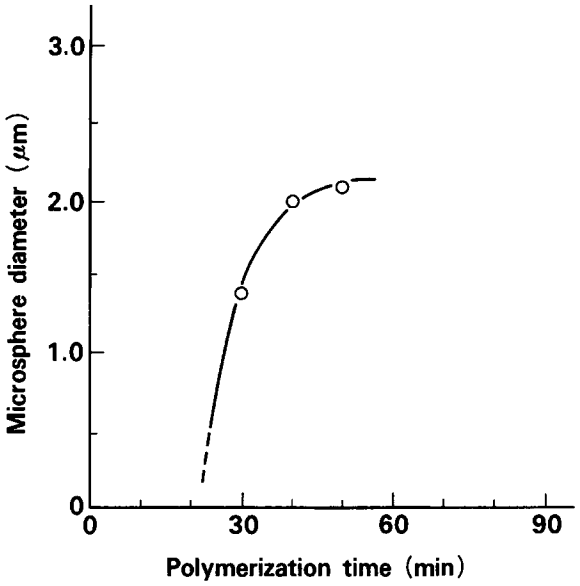

Figure 1 Relation between polymerization time and microsphere diameter. Total monomer concentration: 1.0 mol/L. Molar ratio: GMA:HEMA:3G = 85:10:5. Initiator: V-65. Initiator concentration: 10×10^{-3} mol/L. Polymerization temperature: 40°C. Total volume: 30 mL.

EXPERIMENTAL

Materials

Ethyl propionate was purified by distillation. Isobutyl propionate, phenyl propionate, and di-2-ethylhexyl phthalate were used without further purification. The above four reagents were guaranteed reagents supplied by Tokyo Kasei Inc. Glycidyl methacrylate (GMA), methacrylic acid 2-hydroxyethyl ester (HEMA), and triethylene glycol dimethacrylate (3G) were used without further purification. These three extra-pure reagents were supplied by Tokyo Kasei Inc. Two kinds of monomer and one kind of crosslinking reagent were stored at 4°C. They were used without further purification. The radical initiators that we used were as follows:

2,2′ - Azobis(2,4 - dimethyl - 4 - methoxyvaleronitril): $CH_3OC(CH_3)_2CH_2C(CN)(CH_3)N{=}NC(CN)(CH_3)CH_2C(CH_3)_2OCH_3$, MW = 308.42 (abbreviation: V-70, guaranteed reagent; Wako Chemicals Inc.).

2,2′-Azobis(2,4-dimethyl valeronitril): $(CH_3)_2CH\text{-}CH_2(CH_3)C(CN)N{=}NC(CN)(CH_3)CH_2\text{-}CH(CH_3)_2$, MW = 248.37 (abbreviation: V-65, guaranteed reagent; Wako Chemicals Inc.).

These initiators were used without further purification.

Polymerization Process

All polymerization reactions were carried out in polytetrafluoroethylene tubes with an inner diameter of 25 mm and 27 mm in length. Solvent, monomers, and initiator were introduced into the tube in this order with a pipette. The air in the tube was then immediately replaced with argon gas, and the tubes were sealed with rubber stoppers. The tubes were then placed in a water bath whose temperature was raised from room temperature to the polymerization temperature which was maintained for a given time. The polymerization solution was allowed to stand without any vibration or stirring. After a given polymerization time, the polymerization mixture in the tube was poured into five times its volume of cooled acetone at a temperature of 5°C.

Monodispersing Operations

The polymerization mixture was centrifuged at 4°C and 227g (1500 rpm) for 10 min. Polymer microspheres 2 μm in diameter were precipitated at 1500 rpm. After centrifugation, the upper part of the transparent liquid in the centrifuge tube was decanted and cooled acetone was poured into the tube which was shaken vigorously to achieve resuspension. The microspheres were then irradiated with

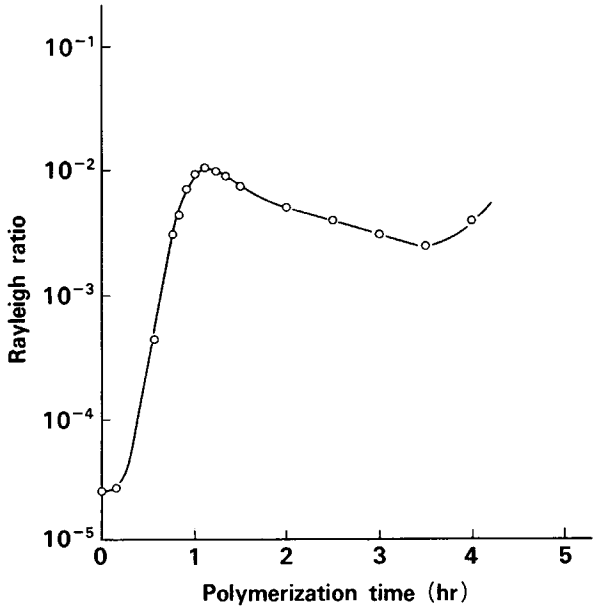

Figure 2 Relation between Rayleigh ratio and polymerization time in the polymerization of glycidyl methacrylate. Glycidyl methacrylate concentration: 1.6 mol/L. Initiator: V-70. Initiator concentration: 3.28×10^{-2} mol/L.

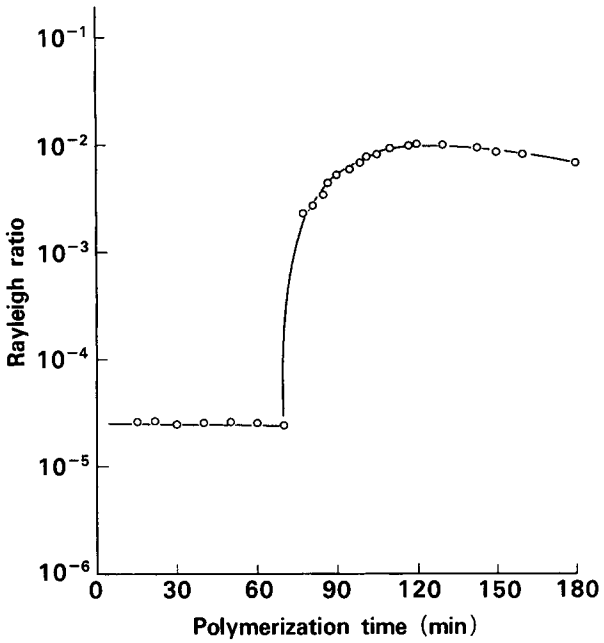

Figure 3 Relation between Rayleigh ratio and polymerization time in the polymerization of glycidyl methacrylate and 2-hydroxy methacrylate. Monomer concentration: 1.53 mol/L. Molar ratio: GMA:HEMA = 89.5: 10.5. Initiator: V65. Initiator concentration: 3.28×10^{-2} mol/L. Solvent: ethyl propionate. Polymerization temperature: 40°C.

ultrasonic waves at 5°C for a given time. The centrifugation, vigorous shaking, and ultrasonic vibration were repeated five times. As these processes were repeated, the liquid in which the microspheres were dispersed was gradually changed from polymerization solvent to ethanol and, finally, to water.

Characterization

The morphology of the microspheres was investigated using scanning electron microscopy (SEM) and optical microscopy. The densities of the microspheres were determined by the density gradient configuration method. The density gradient was formed using the static layers of an aqueous solution of potassium tartrate. The Rayleigh ratio $R(\theta)$ was measured by the dynamic light-scattering method with a light-scattering photometer, Model DLS-700 (Otsuka Electronics Co., Ltd.). The Rayleigh ratio measured in units of cm^{-1} is defined by eq. (1):

$$R(\theta) = r^2 I_\theta / V_\theta I_0 \qquad (1)$$

where θ is the light-scattering angle; V_θ, the light-scattering volume; r, the distance between a light-scattering center and the measuring point; I_0, the intensity of the incident light; and I_θ, the intensity of the scattering light.

Equation (1) is simplified to eq. (2) to take account of the light-scattering apparatus:

$$R(\theta) = \varphi n_s^2 (A/B) \sin\theta \qquad (2)$$

where φ is the constant of the apparatus; A, the light-scattering intensity measured with a photomultiplier; B, the laser light intensity measured with a photodiode; and n_s, the refractive index (n^D = 1.38394) of ethyl propionate at 20°C.

The Rayleigh ratio of the polymerization system at an angle of 90° was measured during the polymerization under ordinary conditions in the photocell of the light-scattering photometer, DLS-700 at 40°C. The Rayleigh ratio measurement is significant in terms of understanding the initiation reaction of precipitation polymerization in this type of system, i.e., a polymerization system which is homogeneous at the initial stage but which later becomes inhomogeneous accompanied by a turbidity increase and the appearance of insoluble oligomers.

RESULTS AND DISCUSSION

Polymerization

More platelike substances were produced using a glass tube than using a polytetrafluoroethylene tube. One of the reasons for this phenomenon is probably

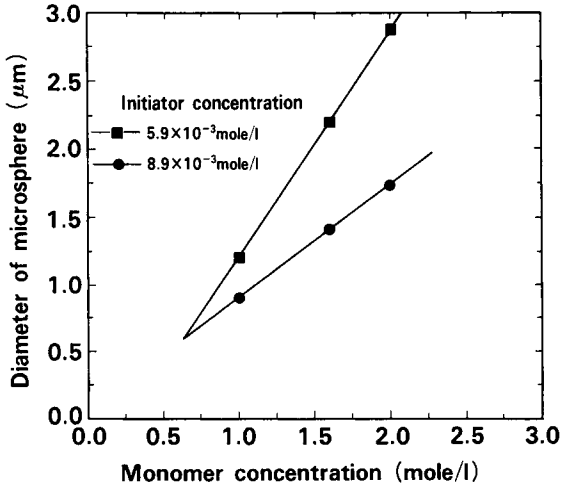

Figure 4 Relation between monomer concentration and microsphere diameter. Molar ratio: GMA:HEMA:3G = 85: 10:5. Polymerization temperature: 40°C. Polymerization time: 4.5 h. Initiator: V-65. Solvent: ethyl propionate.

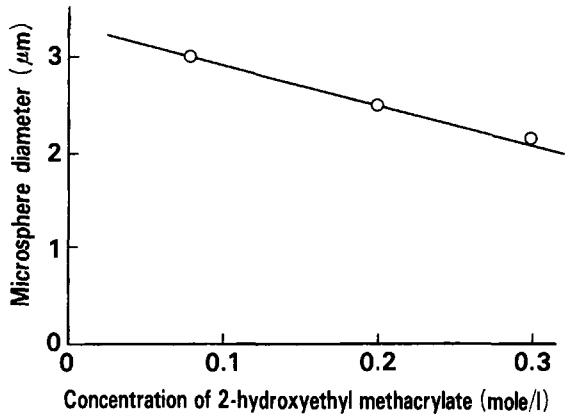

Figure 5 Relation between microsphere diameter and 2-hydroxyethyl methacrylate concentration. [GMA] + [HEMA] = 1.0 mol/L. Initiator: V-70. Initiator concentration: 10×10^{-3} mol/L. Polymerization temperature: 40°C. Polymerization time: 1.0 h. Crosslinking reagent concentration: 0.05 mol/L.

that more polymer microsphere nuclei adhere to the wall of the glass tube than to the wall of the fluorinated polymer tube. The relation between polymerization time and microsphere diameter is shown in Figure 1. This result shows that the polymer microsphere diameter increases with polymerization time until about 40 min. The relation between the Rayleigh ratio of the dynamic light scattering and polymerization time is shown in Figure 2. The system was homogeneous prior to the polymerization, so the Rayleigh ratio maintained a low constant value.[9] The ratio increased rapidly after a GMA polymerization time of about 27 min. In the case of GMA monomer only, the intensity of the turbidity reached a maximum at about 68 min and then slowly decreased. The aggregation and precipitation of the microspheres was negligible.

The relation between the Rayleigh ratio and the polymerization time of GMA and HEMA (molar ratio of GMA to HEMA is 89.5:10.5) is shown in Figure 3. It is shown that the turbidity of the solution without a crosslinking reagent increased rapidly after 70 min of polymerization. This shows that the polymerization initiation reactions occur simultaneously in the mixture. This is supported by the uniformity of the microsphere radii.

These phenomena reveal that the initiation reactions take place simultaneously in the solution and that the polymer chains grow until they reach a critical length where they exceed their solubility and precipitate from the solution.[10] The oligomers of GMA and HEMA at the initial stage are intrinsically insoluble in ethyl propionate. When a crosslinking reagent is used, linear polymers are transformed into crosslinking polymers and, finally, microspheres are formed. This is because crosslinking reagents, which are di- or polyfunctional compounds, may produce intra- and/or interchain bridges.[11] When the turbidity of the polymerization solution is increased, the microsphere radius increases, because there seems to be very little generation of new microspheres. The diameter of insoluble polymer particles may be around 20 nm.[12] Crosslinking reagents seem to shorten the time for which insoluble stable polymer from the solution is visible. As described above, the greater the concentration of the crosslinking reagent, the stronger the microspheres become and also they seem to have less tendency to aggregate.

Figure 4 shows the relation between monomer concentration and microsphere diameter. This result shows that the microsphere diameter is related linearly to the monomer concentration. The molar ratio of GMA : HEMA : 3G is 85 : 10 : 5. The effect of the concentration of catalyst V-65 was investigated between 5.9×10^{-3} and 8.9×10^{-3} mol/L. We found that as the catalyst concentration was increased the diameter of the microspheres became larger. This is because more propagating sites were generated in one microsphere at a higher catalyst concentration. The relation between the HEMA/GMA concentration ratio and microsphere diameter is shown in Figure 5. The microsphere diameter decreased linearly with HEMA concentration. This result shows that the polymerization rate of HEMA is a little slower than that of GMA. The chemical structure of the obtained polymer microspheres is shown in Figure 6. Because the two species of monomer and one crosslinking reagent were all in the homologous

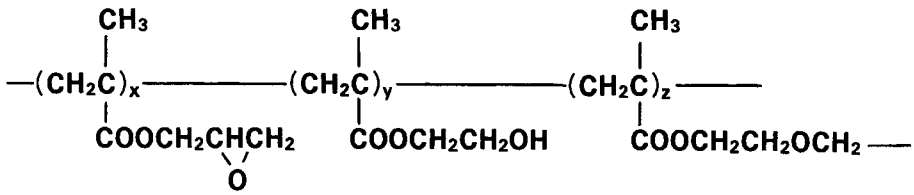

Figure 6 Chemical structure of polymer microsphere.

Table I Effect of Polymerization Solvent on Microsphere Diameter

No.	Solvent	Boiling Point (°C)	Diameter (μm)
1	Ethyl propionate	99	3.0
2	Isobutyl propionate	138	0.8
3	Phenyl propionate	211	No polymerization
4	Diethylhexyl phthalate	> 350	0.6

Monomer concentration: 1.05 mol/L. Molar ratio: GMA:HEMA:3G = 81:9.5:9.5. Initiator: V-70. Initiator concentration: 1.0×10^{-2} mol/L. Polymerization temperature: 40°C. Polymerization time: 1.0 hr.

series of the methyl methacrylate compound, the monomer reactivity ratios (mrr) of the three kinds of the monomer appear to be equal. The monomer distribution in the polymer chain was almost the same as the feed composition of the monomers. But we were not able to determine the ratio of monomer composition in the microspheres because the resulting microspheres were crosslinked. The glycidyl group and 2-hydroxyethyl group had almost the same chemical affinities as those of the polymerization solvent.

Solvent Effect

The effect of the kind of polymerization solvent on microsphere diameters is shown in Table I. The microsphere diameter decreased as the solvent boiling point increased. It appears that the velocity of the initiation radicals toward the microspheres strongly depended on solvent viscosity. It is shown that the rate-determining step of the increasing diameter of the microspheres is related to the velocity of primary radicals approaching the original microsphere. Figure 7 shows the relation between the volume ratio of isobutyl propionate/ethyl propionate and microsphere diameter. In the 5–30% volume range, the microsphere diameter decreased linearly with increases in the isobutyl propionate ratio. The total monomer concentration was sustained at 1 mol/L in all the polymerization reactions to avoid microsphere aggregation.

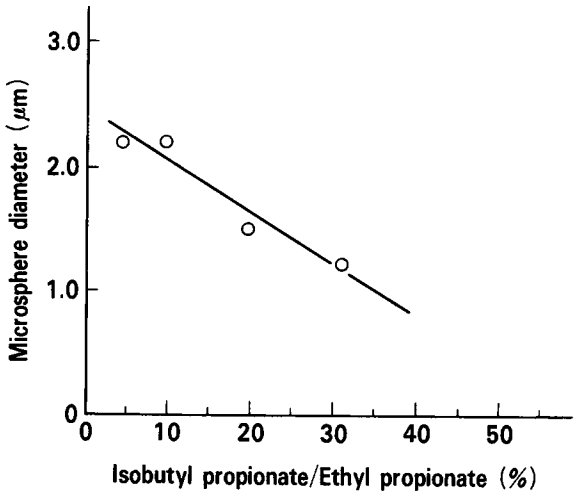

Figure 7 Relation between volume ratio of isobutyl propionate/ethyl propionate and microsphere diameter. Monomer concentration: 1.0 mol/L. Molar ratio: GMA: HEMA:3G = 85:10:5. Initiator: V-70. Initiator concentration: 10×10^{-3} mol/L. Polymerization temperature: 40°C. Polymerization time: 1.0 h.

Dispersion

After the polymerization reaction, the polymerization mixture was poured into more than five times its volume of vigorously stirred acetone at about 5°C. After several polymerization runs, we noticed that cooled acetone was very effective in realizing microsphere monodispersal. If the polymer yield was sufficient for aggregation to occur in the polymerization tube, the aggregated microspheres became impossible to separate completely. The procedure for microsphere monodispersion and purification consists of alternate centrifugation and redispersion of the

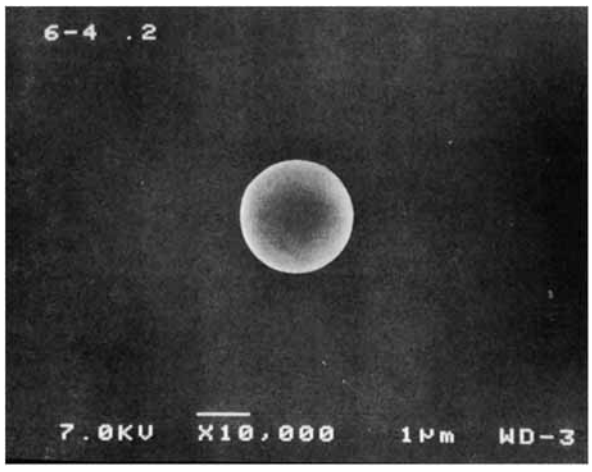

Figure 8 SEM photograph of microsphere.

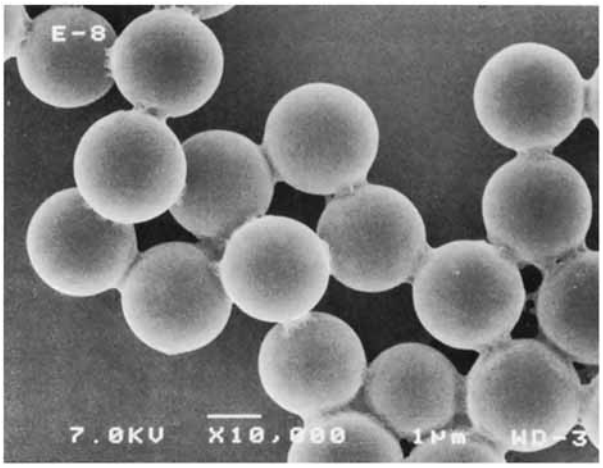

Figure 9 SEM photograph of melted microspheres.

polymerization mixture in five times its volume of cooled acetone under ultrasonic (power 400 W) dispersion. Finally, five times its volume of cooled ethanol was added under ultrasonic irradiation to the microsphere suspension and five centrifugation cycles were undertaken at 1500 rpm (227*g*) for 10 min at 4°C. These operations were performed alternately. Microsphere aggregation occurred when the solvent was changed. To prevent microsphere aggregation when replacing acetone with ethanol, it was necessary to allow the microspheres to stand for over 1 h in the new solvent under ultrasonic irradiation below 5°C.

The degree of dispersion was intermittently observed and checked with an optical microscope. If the monodispersibility of the microspheres was insufficient and there was aggregation, the microspheres were further treated by alternate cycles of ultrasonic dispersion and centrifugation at 5°C until microsphere monodispersion was attained. To obtain microspheres dispersed in water as the final product, cold distilled water was added in three cycles to the ethanol solution diluted with water in which microspheres were dispersed, under conditions of ultrasonic irradiation and centrifugation. About 10 mL of microspheres suspended in ethanol solution was poured into 40 mL of cold distilled water at 4°C under ultrasonic irradiation. The ethanol-to-water solvent-replacement procedure at 5°C was performed five times. By the third time, the microsphere dispersion solution was converted completely from organic solvent to water.

The size-distribution ratio between weight-average and number-average, dw/dn, was 1.09–1.16, measured with dynamic light scattering (DLS).

The monodispersion mechanism was considered to be as follows: The swollen microspheres adhered to each other when the solvents were changed. After about 1 h under ultrasonic irradiation, the microspheres became more rigid at low temperature, and as the microspheres became harder, there was a stronger tendency for them to separate from each other at low temperature. As the solvents were changed in succession, i.e., from ethyl propionate to acetone to ethanol with water, the tendency of the microspheres to adhere to each other weaken and the microsphere distribution proceeded monodispersibly. These phenomena occurred because the glycidyl groups in the microspheres were swollen easily in organic solvents and the molar ratio of the groups in glycidyl methacrylate monomer units on the microsphere surface was about 85 mol %. However, the glycidyl groups had weak affinity with water in water solvent and did not swell. In water, 2-hydroxyethyl groups in 2-hydroxyethyl methacrylate monomer units were solvated and swelled well, but, because their monomer feed was about 10 mol % in the copolymerization, the area ratio of the microsphere surface was very small compared with the surface area ratio of the glycidyl groups in the copolymer microspheres. This may be the main reason

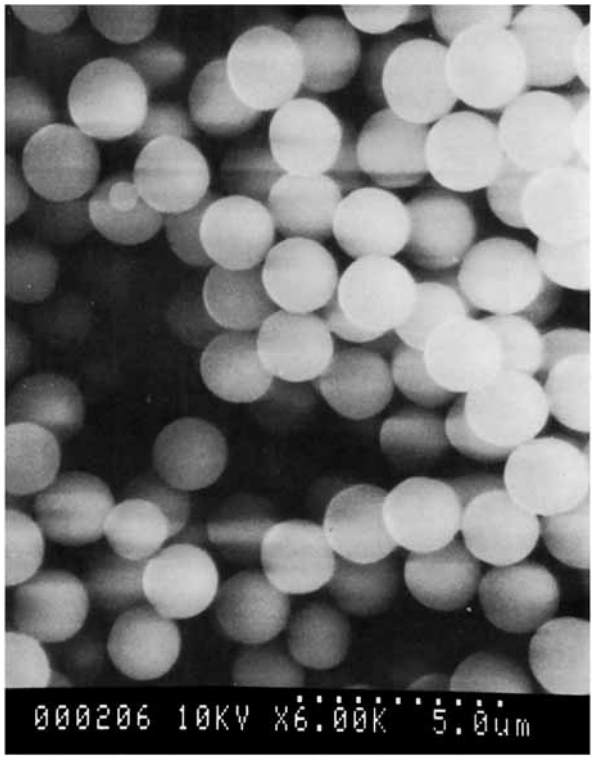

Figure 10 Aggregated microsphere.

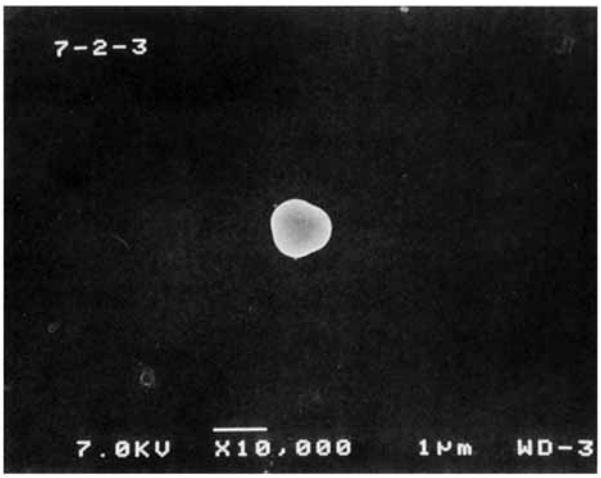

Figure 11 Triangular microsphere.

for the monodispersibility of the microspheres in water.

Microsphere Morphology

SEM photographs of microspheres are shown in Figures 8–11. Almost all the microspheres seem to be true spheres, as shown in Figure 8. The microspheres which were generated immediately before were very soft and sticky. If they touched one another, they stuck and aggregated immediately. Figure 9 shows that the skins of the microspheres melted and stuck to each other. Figure 10 shows aggregated microspheres. It can be seen that this microsphere aggregation contained a few smaller microspheres. Figure 11 shows a triangular microsphere. This may be have been formed by the triangular growing area of the oligomeric microspheres.

Microsphere Density

The apparent density of the microspheres in water was determined as 1.2383 g/mL in the polymerization condition of the molar ratio: GMA : HEMA : 3G = 81 : 9.5 : 9.5. Total monomer concentration was 1.05 mol/L. The polymerization time and temperature were 1 h and 40°C, respectively. The catalyst concentration was 5.9×10^{-3} mol/L of V-70. The obtained microspheres were porous and the amount of water that they contained depended on their porosity. Water was absorbed in the pores of the microspheres, which, in turn, were swollen by the water because the HEMA monomer was used, which is hydrophilic.

Microsphere density seemed to be greatly affected by the concentration of the crosslinking reagent. The apparent density should be in the 1.23 to 1.24 g/mL range for use in the LMIA method, because the microspheres must remain suspended in water without precipitation.

Thin-film Substances

Figure 12 shows thin-film substances in the microsphere dispersion solution. The mechanism of thin-film substance formation resembled that of microsphere formation. The difference between the two is thought to be in the nucleation steps and the anisotropy of the oligomer. The true spherical polymer particles seemed to be formed by the isotropic adsorption of monomers on the nucleus oligomer chain radicals. On the other hand, the thin-film substances seem to be formed when the nuclei adsorb

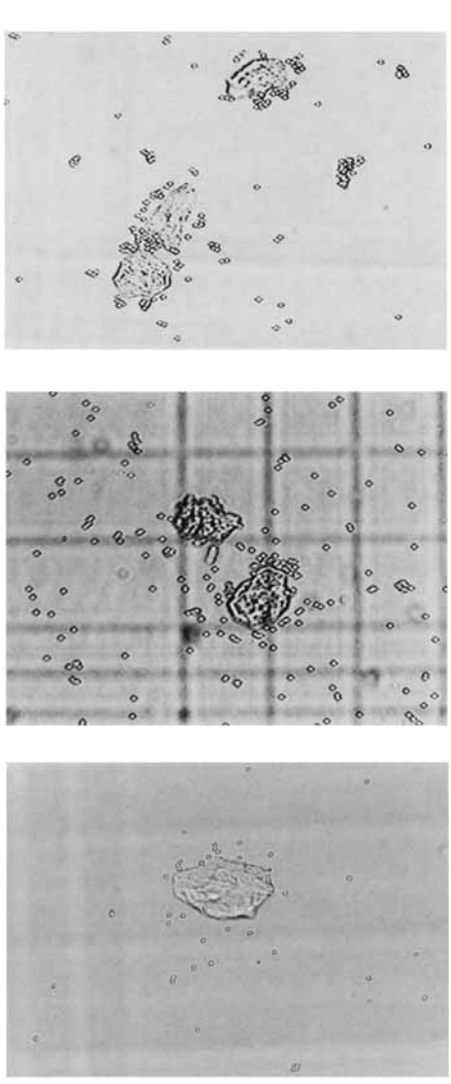

Figure 12 Photograph of thin film substances.

Table II No. Thin-film Substances

Sample No.	No. Thin-film substances (A)	No. Beads (B)	A/B	Average
1	22	17,170	129×10^{-5}	
	26	17,170	153×10^{-5}	125×10^{-5}
	16	17,170	94×10^{-5}	
2	14	22,220	63×10^{-5}	
	7	22,220	32×10^{-5}	44×10^{-5}
	8	22,220	36×10^{-5}	
3	10	29,694	34×10^{-5}	
	11	29,694	37×10^{-5}	30×10^{-5}
	6	29,694	20×10^{-5}	
4	7	26,462	27×10^{-5}	
	4	26,462	15×10^{-5}	19×10^{-5}
	4	26,462	15×10^{-5}	
5	5	24,846	20×10^{-5}	
	5	24,846	20×10^{-5}	17×10^{-5}
	3	24,846	12×10^{-5}	
6	7	30,906	23×10^{-5}	
	5	30,906	16×10^{-5}	16×10^{-5}
	3	30,906	10×10^{-5}	
7	6	32,522	18×10^{-5}	
	1	32,522	3×10^{-5}	13×10^{-5}
	6	32,522	18×10^{-5}	
8	2	17,776	11×10^{-5}	
	3	17,776	17×10^{-5}	11×10^{-5}
	1	17,776	6×10^{-5}	
9	3	24,644	12×10^{-5}	
	2	24,644	8×10^{-5}	11×10^{-5}
	3	24,644	12×10^{-5}	
10	2	24,240	8×10^{-5}	
	2	24,240	8×10^{-5}	6×10^{-5}
	0	24,240	0	
11	2	106,656	1.9×10^{-5}	
	0	106,656	0	0.9×10^{-5}
	1	106,656	0.9×10^{-5}	
12	1	177,760	0.6×10^{-5}	
	1	177,760	0.6×10^{-5}	0.8×10^{-5}
	2	177,760	1.1×10^{-5}	
13	0	48,076	0	
	0	48,076	0	0.7×10^{-5}
	1	48,076	2.1×10^{-5}	
14	0	99,990	0	
	1	99,990	1×10^{-5}	0.3×10^{-5}
	0	99,990	0	
15	0	147,258	0	
	1	147,258	0.7×10^{-5}	0.2×10^{-5}
	0	147,258	0	

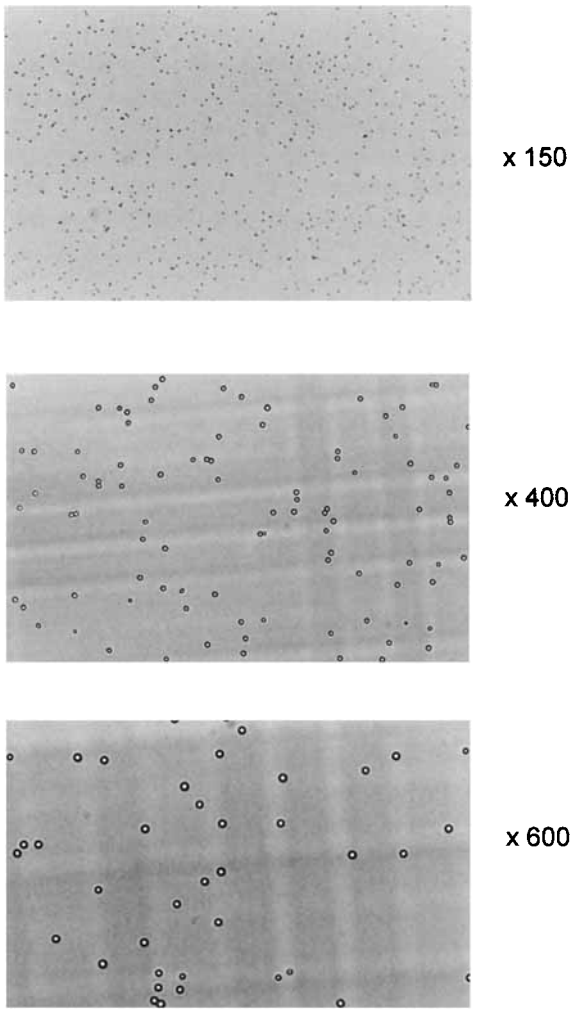

Figure 13 Photograph of microspheres taken with optical microscope.

three kinds of monomer anisotropically. The nuclei adsorbed monomers mainly from two directions, and, consequently, polymeric thin films were formed. The active polymer radical ends grew two-dimensionally in the nucleus polymer.

It is difficult to determine the number of thin-film substances in a microsphere dispersion solution. We adopted the method of counting the number of thin films among a given number (10^5) of microspheres which were put into the small gap of a Burker Turk red cell determination plate under a microscope.

The number of thin films in a given number of microspheres is shown in Table II. The number of thin films in commercial microspheres was about 58 $\times$ 10^{-5} per microsphere. In our experiment, the number of thin films ranged between 125 $\times$ 10^{-5} and 0.2 $\times$ 10^{-5} in our 15 polymerization reaction runs. In the initial polymerization runs, the number of thin films was between about 16 $\times$ 10^{-5} and 125 $\times$ 10^{-5}, but when the concentration of the crosslinking reagent was increased, the number of thin films in the microsphere dispersion solution decreased to the 13 $\times$ 10^{-5} to 6 $\times$ 10^{-5} range. In the later polymerization runs, the number of thin films was almost constant at a value of less than 0.9 $\times$ 10^{-5}. As a result of suppressing the thin films to these low values, it became possible to use the remaining microspheres for our new assay: the laser magnetic immunoassay.

Photographs of microspheres taken with an optical microscope at magnifications of 150, 500, and 600 are shown in Figure 13. These photographs show that the microspheres are well separated.

CONCLUSIONS

Mechanical vibration and centrifugation cycles, performed alternately below 5°C, were very effective in terms of microsphere monodispersion. It was found that in order to prevent microsphere aggregation when a new solvent was added to the polymerization mixture it was necessary to ultrasonically irradiate the microspheres dispersed in the solvent at below 5°C for over 1 h.

The dispersion mechanism of the microspheres was evaluated by the difference between the surface area ratio of the glycidyl groups which were hydrophobic and the 2-hydroxyethyl groups which were hydrophilic. SEM photographs showed that the microspheres were almost equal in diameter. This means that the initiation and propagation reactions of the polymerization occurred at almost the same time. It was possible to suppress the number of thin-film substances to below 10 particles per 10^5 microspheres by adopting appropriate polymerization conditions and a new dispersion procedure.

Monodispersed microspheres were produced by using the following conditions: (1) We adopted a polytetrafluoroethylene tube; (2) we used an appropriate monomer concentration of about 1.0–1.5 mol/L and a molar ratio GMA : HEMA : 3G of 81–85 : 9–10 : 5–10; (3) we increased the concentration of the crosslinking reagent, i.e., 1.5–2 times that of ordinary crosslinking reagent; (4) we completely changed the oxygen gas in the air; (5) we prevented a convection current occurring in the polymerization solvent media; (6) the polymerization mixtures were poured into more than five times their own volume of cooled acetone in order to prevent microsphere aggregation; (7) we terminated at a low polymer

yield in order to prevent microspheres contacting each other; (8) we employed centrifugation and redispersion in a cold solvent at below 5°C; and (9) we prevented solvent vaporization by using centrifugal tubes with caps.

The authors would like to thank Dr. K. Fujiwara, Dr. S. Shibata, Dr. H. Mizutani, and Miss. M. Suzuki for valuable discussions and Dr. S. Sugawara for help with the polymerization experiments. The authors also thank Professor K. Nagai of Yamagata University for helpful discussions.

REFERENCES

1. H. Mizutani, M. Suzuki, H. Mizutani, K. Fujiwara, S. Shibata, K. Arishima, M. Hoshino, H. Ushijima, H. Honma, and T. Kitamura, *Microbiol. Immunol.*, **35**(9), 717–727 (1991).
2. K. Fujiwara, H. Mizutani, and H. Mizutani, in *International Symposium: Basic and Clinical Approaches to Virus Chemotherapy,* Helsinki, June 19–22, 1988.
3. H. Mizutani, H. Mizutani, and K. Fujiwara, in *International Symposium: Basic and Clinical Approaches to Virus Chemotherapy,* Helsinki, June 19–22, 1988.
4. S. Hosaka, Y. Murao, S. Masuko, and K. Miura, *Immunol. Commun.*, **12**(5), 509–517 (1983); (b) *Tokkai Shou* 58-29962, 59-11602, 63-15551.
5. S. Hosaka, Y. Murao, H. Tamaki, S. Masuko, K. Miura, and Y. Kawabata, in *International Symposium on Polymeric Microspheres,* Fukui, N-52, Oct. 23–26, 1991, pp. 245–250.
6. T. Uchida, S. Hosaka, and Y. Murao, *Immunol. Commun.*, **11**(6), 467–475 (1982).
7. T. Uchida, S. Hosaka, and Y. Murao, *Biomaterials,* **5,** 281–283 (1984).
8. S. Hosaka, *Surface,* **23**(7), 421–431 (1985) (in Japanese).
9. S. Shen, E. D. Sudol, and M. S. El-Aasser, *J. Polym. Sci. Part A Polym. Chem.*, **32,** 1087–1100 (1994).
10. C. M. Tseng, Y. Y. Lu, M. S. El-Aasser, and J. W. Vanderhoff, *J. Polym. Sci. Part A,* **24,** 2995–3007 (1986).
11. R. Arshady, *Polym. Eng. Sci.*, **29**(24), 1746–1758 (1989); Part II: *Coacervation,* **30**(15), 905–914 (1990).
12. H. Hamaguchi, Y. Yamada, S. Kataoka, Y. Morita, and Y. Ohtsuka: *Polym. J.* **23**(8), 955–962 (1991).

Received December 15, 1994
Accepted February 5, 1995