Continuous Preparative Method of Relatively Large and Uniform Polymer Beads and Their Application to Immobilization of Urease

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Synopsis

An apparatus for continuous preparative method of polymer beads was investigated. Monomer droplets formed in glycerol were continuously introduced to a rotating glass tube which was filled with warmed glycerol and polymerized. Polymer beads of around 4 mm diameter were obtained. As an application of the polymer beads, urease was immobilized on the beads and their properties were evaluated. Macroreticular type of beads prepared with a mixture of maleic anhydride, styrene, and divinylbenzene showed the highest enzymatic activity among the beads tested, and the urease immobilized beads could be successfully applied for determination of blood urea nitrogen in human sera.

INTRODUCTION

In the preparation of polymer beads by suspension polymerization method, bead sizes are affected by various factors, such as mechanical agitation, volume ratio of monomer phase to suspending liquid, viscosity of suspending liquid, sizes of polymerizing apparatus, and so on.[1] Thus the polymer beads are easily prepared by the suspension polymerization, but the products have a wider size distribution. Consequently, classification is necessary to obtain narrow size distribution of the beads. The beads of 3 μm–1.0 mm are used for column packing materials of high performance liquid chromatography and general utilization of ion exchange resins.[2,3] On the other hand, the beads having relatively large sizes (several mm) are also expected to apply in wide ranges of studies owing to ease in handling. However, it seems difficult to obtain the polymer beads having relatively large and uniform size by the usual suspension polymerization.

In this study, an apparatus for continuous preparation of relatively large and uniform crosslinked polymer beads was investigated. Four kinds of macroreticular and gel types of polymer beads crosslinked with divinylbenzene or ethylene glycol dimethacrylate were prepared by the proposed apparatus. Urease was immobilized on the beads, and their enzymatic properties were also studied.

EXPERIMENTAL

Materials

Styrene (ST), ethylene glycol dimethacrylate (EGDMA), and divinylbenzene (DVB) solution (nominally containing about 50% of DVB) were washed with $2N$ sodium hydroxide and water to remove inhibitor. The monomers

Journal of Applied Polymer Science, Vol. 35, 1283–1288 (1988)
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were dried over anhydrous calcium chloride. Maleic anhydride (MA), benzoyl peroxide, and glycerol used were of reagent grade. Urease (E.C.3.5.15., 71 units/mg, from Jack Bean, Worthington Diagnostic System Corp.) was used without further purification. Buffer solution used in enzymatic studies was 0.1 M phosphate buffer (pH 7.0) containing 5 m*M* EDTA.

Preparation of Polymer Beads

The apparatus used for the preparation of polymer beads is shown in Figure 1. It can be divided into three main sections, namely, a monomer inlet section, a rotary glass tube, and a beads collector. The rotary glass tube (1) [1.35 cm (I.D.) × 300 cm (length)] has a conduit (2) [length 5 cm, reduced section 0.4 cm (I.D.), enlarged section 0.85 cm (I.D.) and 1.0 cm (O.D.)] in the upstream part. The conduit is fitted tightly to the inner part of the rotary glass tube with a silicone rubber tube (3), which is partially cut on its outer surface to permit the carrier fluid to flow across 3 to the downstream section of the rotary glass tube. Contrarily to the rotary glass tube, monomer droplet forming part (4), heat exchanger (5), and beads collecting vessel (6) are tightly fixed to a framework of steel. The carrier fluid, after filling the parts 6, 1, and 7, is air trapped in the upper part of 7 and purged from outlet (stop cock) 8 on 7. The rotary glass tube mounted with a worm wheel is rotated with worm (9) attached to motor (10), and its rotation speed is changed (alternatingly slow and fast) at definite intervals (about 1–2 s) with controller (11). Parts connected to the rotary glass tube are sealed with vacuum seal (12) to prevent flowing out the carrier fluid or the water from the heat exchanger. There are two pathways of the carrier fluid flow. One of them is a constant flow into the upstream with peristaltic pump (13), and this stream is necessary not to leak the monomer droplet between the walls of 1 and 2 with subsequent break down to small particles. The other is a stream to the left end (0.4 cm, I.D.) of a T-shaped tube, and the flow rate is controlled with peristaltic pump (14). Monomer solution from reservoir (15) is introduced to the lower end (0.15 cm,

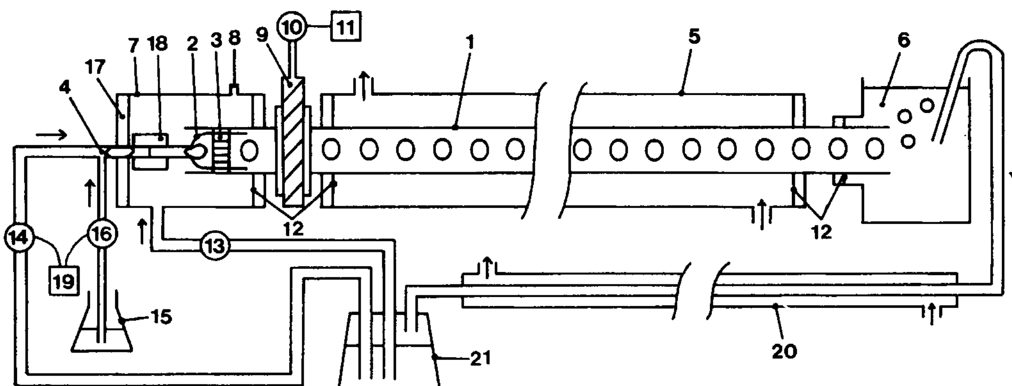

Fig. 1. Schematic diagram for preparation of beads: (1) rotary glass tube; (2) conduit; (3) silicone rubber tube; (4) monomer droplet forming part; (5, 20) heat exchanger; (6) collector; (7) upper part ware; (8) outlet for air; (9) worm; (10) motor; (11, 19) time controller; (12) seal; (13, 14, 16) peristaltic pump; (15) monomer reservoir; (17) silicone rubber stopper; (18) thick silicone rubber tube; (21) air trap.

I.D.) of the T-shaped tube with peristaltic pump (16). The right end (0.4 cm, I.D.) of the T-shaped tube is fixed to 7 with a silicone rubber stopper (17), and this end is connected to conduit (2) with thick silicone tube (18). The silicone tube is tightly fixed to the right end of the T-shaped tube and loosely fitted to the reduced end of conduit 2. The pumps (14) and (16) are controlled to work alternatively at appropriate intervals with time controller (19). Any desired size of droplets of the monomer solution can be made by controlling the frequency of pumps (14) and (16). The stream of carrier fluid controlled with pump (14) causes the occurrence of a droplet as well as the movement of this droplet to the downstream section of the rotary glass tube. The droplets are heated in the rotary glass tube and polymerized. The polymer beads thus prepared move to the downstream section with the stream of carrier fluid and are collected in vessel (6). The carrier fluid is heated again by means of heat exchanger (20) and passed through air trap (21) and then recirculated to the upstream part. Beads were washed with water and applied to a support for urease.

Immobilization of Urease on Beads. Twenty milliliters of urease solution (40 mg urease/20 mL phosphate buffer) was added to the beads [4 mm diameter, 40 particles (about 2 g)] and the mixture was incubated overnight at 8°C. Then the beads were filtered off and washed thoroughly with the buffer solution until the filtrate showed no enzymatic activity.

Enzymatic Activity of the Immobilized Urease. A piece of bead was added to 1 mL of the buffer solution, and then 5 mL of the buffer solution containing 5% of urea was added. The time course of the enzymatic reaction was studied at 38°C. The concentration of ammonia formed was determined by a urease–indophenol method.[4]

RESULTS AND DISCUSSION

Four kinds of spherical polymer beads of around 4 mm in diameter were prepared with the apparatus (Fig. 1). The proportions of chemicals of monomer solution for the preparation of beads are shown in Table I. In the preparation of beads, desirable sizes of droplets of monomer solution were formed in a monomer inlet section by controlling alternatingly the work of the peristaltic pumps for monomer and carrier fluid (glycerol). The droplets thus formed moved to the warmed rotary glass tube with the flow of carrier fluid

TABLE I
Proportions of Chemicals in Preparation of Beads[a]

	Monomer				Diluent (mL)			Benzoyl
Beads	MA (g)	ST (mL)	DVB (mL)	EGDMA (mL)	B	D	I	peroxide (g)
MA–ST–DVB (MR)	9.3	4	4	—	7	7	—	0.36
MA–ST–EGDMA (gel)	9.3	4	—	4	7	7	—	0.36
ST–DVB (gel)	—	5.1	0.9	—	—	—	—	0.09
DVB (MR)	—	—	10	—	—	—	10	0.15

[a] MA = maleic anhydride; ST = styrene; DVB = divinyl benzene; EGDMA = ethylene glycol dimethacrylate; B = benzene; D = dioxane; I = isooctane.

and then polymerized. The rotation speed of the tube was changed alternatingly slow and fast to obtain spherical beads, because ellipsoidal beads were liable to obtain when the glass tube was rotated at a constant speed. Polymerization was carried out at 70°C for the monomer solution containing MA and at 80°C for the other monomer solution. By adjusting the working intervals of the peristaltic pumps and the flow rate of the carrier fluid and the monomer solution, desirable sizes of droplets and distances of each droplet in the rotary tube and polymerization time (time to pass through the tube) can be selected ad libitum. When the monomer droplets move continuously at a distance of 2 cm to each other in the rotary glass tube, monomer droplets can be aligned about 150 droplets in the rotary glass tube (300 cm long), and the droplets are polymerized for 30 min. In these conditions, the polymer beads can be obtained 300 particles/h. In the case of the monomer solution containing MA, hard spherical beads of MA–ST–DVB (MR type) and MA–ST–EGDMA (gel type), could be obtained in 30 min of polymerization period (in a case of MA–ST–DVB, the monomer droplets changed from transparent to white in about 10 min). On the other hand, beads of ST–DVB (gel type) and DVB (MR type) were not so hard even after 30 min polymerization, so that these beads were transferred to a beaker with the carrier fluid and further polymerized at 80°C for 2 h. A photograph of the spherical beads prepared were shown in Figure 2. It has become clear that almost the same sizes of spherical beads (up to around 4 mm diameter) are able to prepare continuously with the apparatus proposed. It is expected that more larger sizes of beads can be prepared by changing the rotary glass tube into more large sizes (diameter) of rotary glass tube.

Small spherical polymer beads (0.1–0.7 mm) of MA–ST–DVB copolymer had already been successfully prepared with suspension polymerization using glycerol as suspension medium.[5] In the suspension polymerization, viscosity of the medium is relatively high, and hence large beads are not easily prepared. By using the proposed apparatus, however, relatively large polymer beads

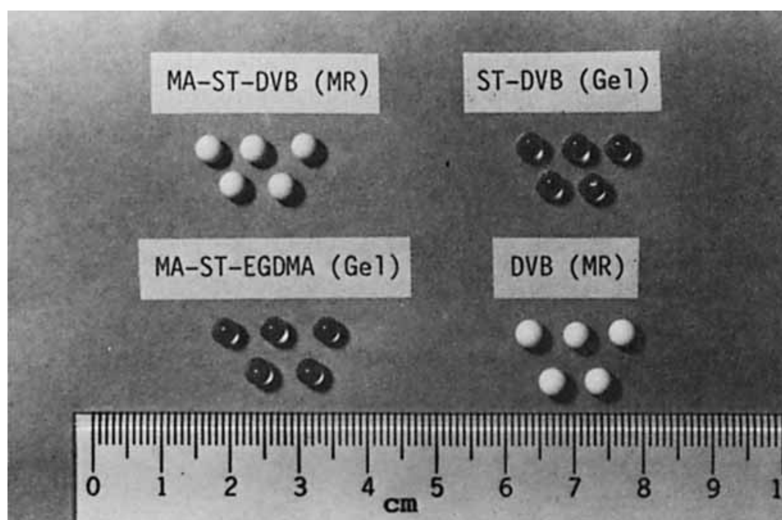

Fig. 2. Photograph of beads.

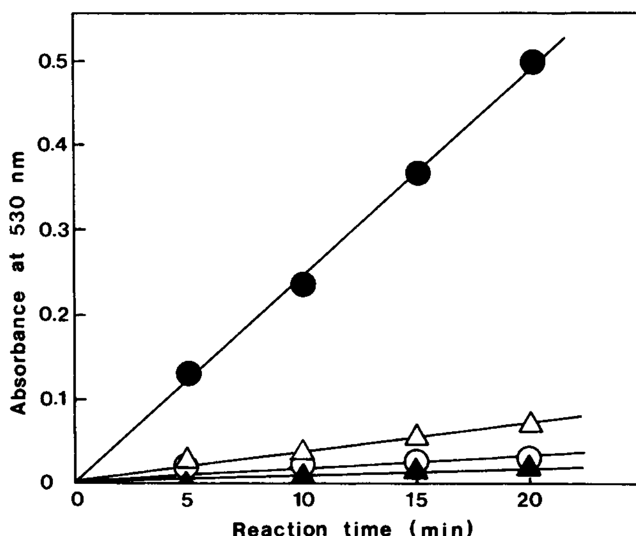

Fig. 3. Enzymatic reaction by using several kinds of urease immobilized beads: (●) MA–ST–DVB–urease; (△) DVB–urease; (○) MA–ST–EGDMA–urease; (▲) ST–DVB–urease; urease immobilized bead, one bead; substrate, 5% urea/buffer solution, 10 mL; buffer solution, 0.1*M* phosphate buffer containing 5 m*M* EDTA, pH 7.0; reaction temperature, 38°C.

could be obtained even in the high viscosity medium as glycerol. In the preparation of beads, stabilizer such as hydroxyethyl cellulose is unnecessary to add to carrier fluid, because monomer droplets do not collide with each other in the polymerization process. Of course, in the case of a monomer which is stable in water, glycerol can be replaced by aqueous solution.

As an application of the beads, urease, one of the well-known enzymes, was immobilized on these beads, and their enzymatic activities were studied (Fig. 3). In the beads tested, MA–ST–DVB beads showed the highest immobilization of urease. This is due to the MR structure and acid anhydride moiety on the MA–ST–DVB beads. A piece of MA–ST–DVB bead could immobilize about 5 μg of urease. The result of the determination of blood urea nitrogen (BUN) in normal human sera with the MA–ST–DVB–urease beads is shown in Table II. The data obtained by the immobilized urease showed almost

TABLE II
Determination of Blood Urea Nitrogen (BUN) by Urease-Indophenol Method[a]

	BUN (mg N/dL)	
Sample	Immobilized urease	Urease solution
A	7.1	8.3
B	9.1	9.2
C	11.2	11.3
D	9.3	9.4

[a]Sample: normal human sera, 100 μL; immobilized urease (MA–ST–DVB–urease), one bead; urease solution, 1 mg urease/10 mL buffer solution, 500 μL.

reasonable values (normal values for adults: 8–20 mg N/dL) compared with the standard method using urease solution.[6]

The authors are very grateful to Misses Tetsuko Fukuda and Syoko Hayashi for their technical assistance in the experimental work.

References

1. E. Trommsdorff and C. E. Schildknecht, in *Polymer Processes*, C. E. Schildnecht, Ed., Wiley-Interscience, New York, 1956, pp. 69–109.
2. H. Honma, *Handbook of High Performance Liquid Chromatography*, The Japan Society for Analytical Chemistry Ed., Maruzen, Tokyo, 1985, Chap. 4.
3. K. Dorfner, in *Ion Exchangers*, A. F. Coers, Ed., Ann Arbor Science, Michigan, 1972, pp. 28–31.
4. A. L. Cheng and E. P. Marbach, *Clin. Chem.*, **8**, 130 (1962).
5. N. Ogawa, K. Honmyo, K. Harada, and A. Sugii, *J. Appl. Polym. Sci.*, **29**, 2851 (1984).
6. I. Kanai and M. Kanai, *A Summary for Clinical Laboratory Test*, 28th ed., Kanahara Shuppan, Tokyo, 1978, Chaps. VII and XIV.

Received May 27, 1987
Accepted July 22, 1987