Preparation of Uniform-sized Functional Microsphere with Nano-porous Structure

Guanghui Ma[1], Ping Wang[2], Zhiguo Su[1]
[1]National Key Laboratory of Bio-chemical Engineering, Institute of Process of Engineering, Chinese Academy of Sciences, Beijing 100080, China
[2]Departmentt of Chemical Engineering, The University of Akron, 200 E. Buchtel Commons, Akron, OH 44325, USA

Introduction

Microspheres with nano-porous structure have important applications in immobilization of enzymes and construction of artificial cells. [1-5] However, the size distribution of microspheres is very broad by conventional suspension polymerization method, there are many disadvantages when polydispersed microspheres were applied in above applications. In this study, uniform-sized microspheres with functional group were prepared by combining a special glass membrane emulsification technique and a subsequent suspension polymerization process. That is, a mixture composed of functional monomer, crosslinker, diluent and initiator was pressed through the pores of the membrane into an aqueous phase to form uniform droplets under a controlled pressure, then the droplets were polymerized by elevating the temperature. The polarity of functional monomer is usually higher, it is difficult to obtain uniform droplet by a direct membrane emulsification technique because it wetted the membrane easily, a swelling technique of droplet was developed to attain to the purpose. In addition, when the monomer was polarity, it was difficult to obtain microspheres with porous structure on the surface, microspheres with porous structure inside and a skin on the surface usually were formed. In this study, the microspheres with homogeneous porous structure were prepared successfully by selecting an adequate porogen. The hollow microsphere with a porous wall was also prepared in order to construct an artificial cell.

Experimental

Preparation of PGMA microsphere with direct emulsification technique

An equipment for membrane emulsification technique process is shown in Fig.1. The monomer phase (oil phase) was stored in a oil tank, and the aqueous phase containing stabilizer was stored in an emulsion tank. A tubular glass membrane was installed in a stainless module. By increasing the nitrogen gas pressure to a critical pressure, the monomer phase was pressed through the pores of the membrane wall into the center of the tube to form droplets, which were brought away by circulated aqueous phase.

In the preparation of poly(glycidyl methacrylate) (PGMA) uniform microsphere, the oil phase composed of GMA monomer, divinyl benzene (DVB) crosslinker, porogen, and benzoyl peroxide (BPO) initiator (Table 1), and the aqueous phase was composed of water,

poly(vinyl alcohol) (PVA), sodium dodecyl sulfate (SDS), Na_2SO_4, and $NaNO_2$, which was used to inhibit the secondary nucleation in water phase. After the emulsification process was finished, the W/O emulsion was moved to a reactor. The temperature of the emulsion was raised to carry out polymerization after the nitrogen gas replacement. As the polymerization proceeded, uniform porous microsphere can be obtained due to the phase separation between crosslinked polymer and porogen when an adequate porogen was selected. The concentration of oil phase in water was always around 10 wt%.

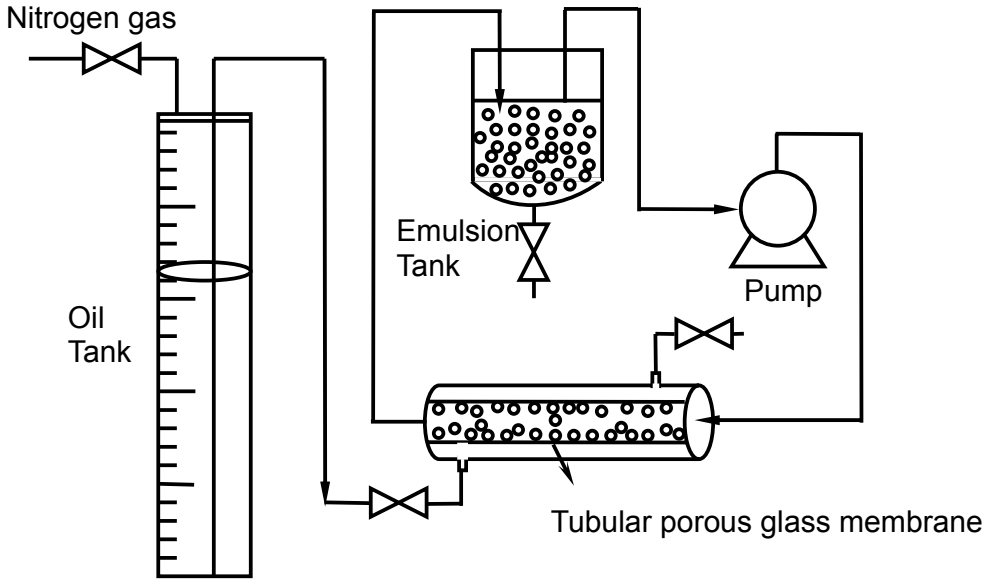


Fig.1 Schematic equipment for membrane emulsification technique

Preparation of PHEMA microsphere with swelling method of uniform droplet

Because the polarity of 2-hydroxyethyl methacrylate (HEMA) monomer is higher, the monomer will wet the pore of the membrane if the oil phase is pressed through the membrane directly. Therefore, a swelling method of the droplet was developed to prepare PHEMA porous microsphere. At the first, toluene dissolving hexadecane (HD) and BPO, was pressed through the pores of the membrane into the aqueous to form uniform seed droplet (primary emulsion), where the toluene is used as a porogen. A mixture composed of hydrophilic monomer HEMA and ethylene glycol dimethacrylate (EGDMA) crosslinker, was emulsified in water by a homogenizer to form the secondary emulsion with the diameter below 1μm. Then, two emulsions were mixed. Because the seed emulsion is very uniform and hydrophobic, it is stable, while the secondary emulsion is unstable due to its polarity and broad size distribution. As a result, the polar monomer in the secondary diffused into the aqueous phase, and then absorbed quickly by the seed droplet to form swollen droplet. After the secondary emulsion disappeared completely, the swollen emulsion was moved to a reactor to start polymerization.

Preparation of hollow PMMA microsphere with a porous wall

A double emulsion method was used to prepare hollow microsphere with porous wall. The internal water phase was dispersed in oil phase composed of MMA monomer, EGDMA crosslinker, porogen, initiator, and oil-soluble emulsifier, to form W/O primary emulsion by a homogenizer. Then, the primary emulsion was dispersed further into an external water phase containing PVA, SDS, $NaNO_2$, and Na_2SO_4 to form W/O/W double emulsion. The double emulsion was stirred gently for around 1 hour, allowing the multi-internal water phase to coalesce to a single large phase (Fig.2). Then, the double emulsion was moved to a reactor, the temperature was raised to start polymerization under nitrogen atmosphere. As the polymerization of the oil phase proceeded, the phase separation between polymer and porogen occurred, a porous wall can be obtained. And the internal water phase formed a hollow inside the microsphere.

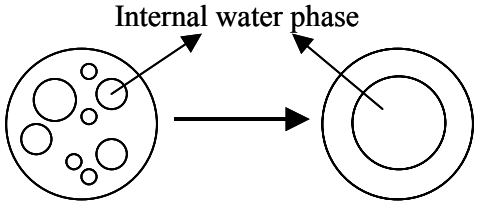


Fig.2 Coalesce of multi-internal water phase to a single large phase

Results and Discussion

Preparation results of porous PGMA microsphere

Effect of porogen on uniformity of emulsion droplets

PGMA is a convenient polymer. PGMA microsphere posses epoxy group, so the enzyme can be immobilized on it easily. We found that although GMA may wet the pore of the membrane, adding hydrophobic porogen in the monomer phase can avoid this phenomenon.

Table 1 Oil phase recipe to prepare PGMA microsphere

	Run No.				
	R100	R101	R102	R103	R104
GMA/DVB (wt/wt)	4/1	4/1	3/2	1/1	2/3
4-Methyl-2-pentonal content in porogen (wt%)	100	60	60	60	60
Isooctane content in porogen (wt%)	0	40	40	40	40
Monomer/porogen (wt/wt)	1	1	1	1	1
BPO/monomer (wt/wt)	1/100	1/100	1/100	1/100	1/100

Table 2 Standard recipe of aqueous phase for membrane emulsification

Deionized water	200 g
PVA GH20	2.0 g
SDS	0.04 g
$NaNO_2$	0.05 g
Na_2SO_4	0.05 g

The recipe of monomer phase was varied as Table 1. When 4-methyl-2-pentanol was used as the porogen (Run 100), the pore of membrane was wetted easily because the polarity of 4-Methyl-5-pentanol and GMA are higher, a jet-like stream was generated, uniform droplet was unable to form. When isooctane was used together with 4-methyl-2-pentanol (4-methyl-5-pentanol/isooctane=3/2 wt/wt, R101), relatively uniform emulsion droplets were obtained, the CV value which indicates the size distribution of the droplets was around 12.0%. The optical microscopic photographs (OM) of droplets for Run 100 and 101 are shown in Fig.3. Fig.4 is the SEM photo of Run 101 after polymerization, the microsphere was porous and uniform.

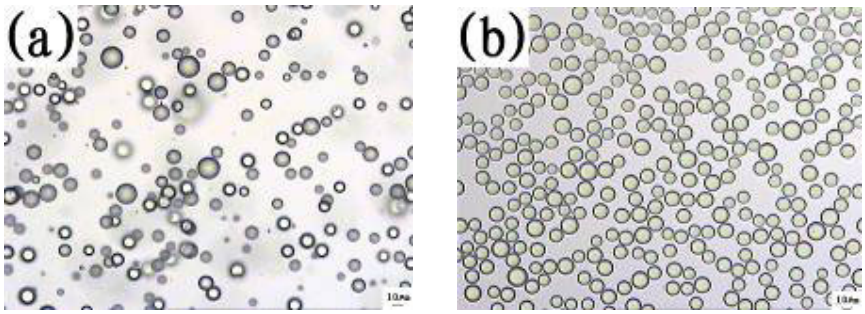


Fig.3 OM of emulsion droplets prepared by membrane emulsification technique. Pore Size of the membrane=5.2 μm. (a) Run 100; (b) Run 101

Effect of DVB content on morphology of microsphere

With the increase of DVB content in monomer, the size distribution of the droplet slightly decreased. This was because that DVB played a role of a hydrophobic additive in membrane emulsification process, as well as a crosslinker.

Effects of diluents and crosslinking degree on the morphology of microsphere

When 4-methyl-2-pentonal was used as the porogen, no pore was observed from SEM observation. On the other hand, when the mixture of 4-methyl-2-pentonal and isooctane was used as the porogen together, pores was observed on the microsphere (Fig.4). Isooctane was a precipitation agent of P(GMA-DVB) copolymer, which led to rapid phase separation between copolymer and porogen during the process of polymerization. After the porogen was removed, pores were formed. From above results, it was evident that

the addition of isooctane in oil phase not only improved the size distribution of microspheres, but also resulted in the formation of the pore in the PGMA microsphere.

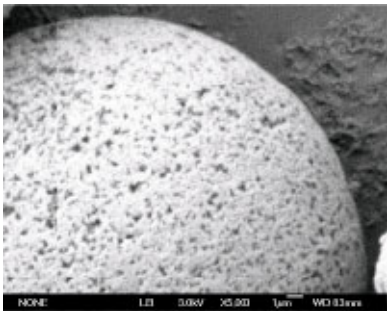


Fig.4 SEM photo of the porous feature of PGMA microsphere

With the increase of DVB content, the specific surface area of the microsphere increased and the average pore size decreased (Table 3). It is necessary to select an adequate pore size according to the molecular weight of enzyme which is intended to be immobilized.

Table 3 Effect of DVB content in monomer on pore size and specific surface area on PGMA microsphere

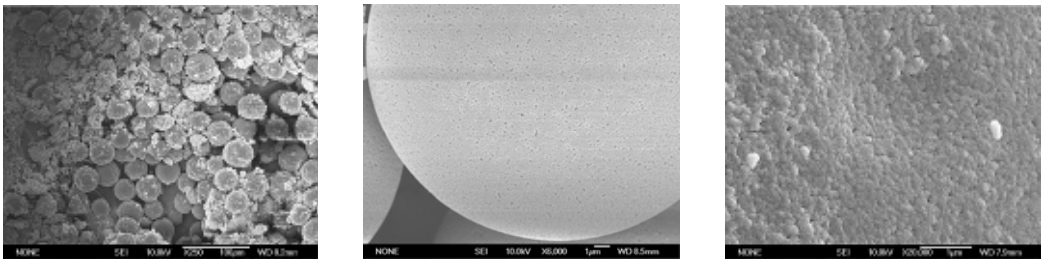
	Run101	Run102	Run103	Run104
Specific Surface area (m^2/g)	29.97	61.88	168.91	312.72
Average pore size (nm)	36.59	25.80	22.56	13.79

Preparation results of porous PHEMA microsphere

PHEMA uniform porous microsphere was prepared by a swelling technique of the droplets. Because the seed droplets are uniform, the rate of each droplet absorbing polar monomer through aqueous phase was similar, as a result, the obtained swollen droplets are also very uniform. In order to know how the crosslinking degree and toluene content affected the pore size and surface morphology of the P(HEMA-EGDMA) microspheres, toluene/monomer ratio and HEMA/EGDMA ratio were varied as shown in Table 4.

The typical SEM photographs showing the surface morphologies of the final microspheres are shown in Fig. 5. When the ratio of toluene/monomer was higher than 50% with a low cross-linking degree (EGDMA/HEMA = 4/8), collapsed microspheres were obtained as shown in Fig. 5(a) (Run A3). This was because the phase separation between toluene and polymer occurred before the surface of the swollen droplets began to solidify when the ratio of toluene/monomer exceeded an upper limit. When the cross-linking degree was higher (EGDMA/HEMA = 8/4), the microspheres were uniform and porous even by increasing the toluene/monomer ratio to 100%, as shown in Fig. 5(b) (Run C3). This was because the phase separation occurred timely and resulted in porous morphology during the polymerization process. However, the surface of the microsphere became compact,

and the pore was not apparent as shown in Fig. 5(c) (Run D1) when toluene content was relatively low. This was because the phase separation occurred late during the polymerization. It can be concluded that uniform porous microspheres can be successfully prepared only when the ratio of toluene/monomer was between a lower limit and an upper limit at a specified cross-linking degree.



(a) Run A3 (b) Run C3 (c) Run D1

Fig.5 SEM photographs of P(HEMA-EGDMA) microspheres.

Table 4 Morphological parameters of the P(HEMA-EGDMA) microspheres

Run No.	HEMA/EGDMA/Toluene (g/g/g)	Pore volume (ml/g)	Pore diameter (nm)	Specific surface area (m^2/g)
A1	8/4/3	0.1999	9.97	80.2
A2	8/4/4.5	0.3179	18.75	67.8
A3	8/4/6	—	—	—
A4	8/4/12	—	—	—
B1	4/4/3	0.2567	12.30	83.5
B2	4/4/6	0.4055	12.57	129.0
B3	4/4/8	—	—	—
C1	4/8/4	0.0882	7.45	47.4
C2	4/8/6	0.2084	16.85	49.5
C3	4/8/12	0.4234	44.97	37.7
D1	4/12/3	0.0020	0	0
D2	4/12/6	0.1584	6.80	93.5
D3	4/12/12	0.4534	20.60	88.0
D4	4/12/16	0.4536	26.00	69.9

"—": The microspheres in run A3, A4, and B3 collapsed, their pore properties were not measured.

The pore size and specific area of PHEMA microspheres are shown in Table 4. It can be seen that the average pore diameter of the microspheres increased with increase of toluene concentration. This phenomenon can be explained by assuming that higher toluene

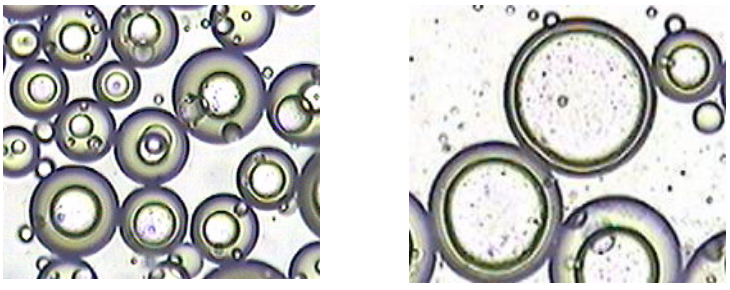
concentration in the swollen droplets resulted in higher porosity and larger total pore volume after removal of toluene. In this case, larger pores that contributed less to the specific surface area were formed and resulted in lower specific surface area. It was known from Table 4 that the highest total pore volume, largest average pore diameter, and highest specific surface area were 0.4536 ml/g, 44.97 nm, and 129.0 m^2/g, respectively, when the weight ratios of HEMA/EGDMA/toluene were 4/12/6, 4/8/12, and 4/4/6, respectively.

The advantage of the swelling method is that not only uniform polar microsphere was able to be obtained, but also very large microsphere with the diameter up to 100 μm can be obtained by increasing the swelling ratio. Further more, the pore size also can be varied according to the requirements, by adjusting the ratio of porogen and monomer.

Preparation results of hollow PMMA microsphere with a porous wall

It is difficult to prepare porous microspheres with a hollow inside. The recipe was optimized by mechanical method. Because it was prepared via a double emulsion process, it is necessary to obtain a stable double emulsion, otherwise, the internal water phase diffused out to the external water phase or the droplets coalesced together quickly, causing a large part of precipitates. This problem was overcome by adjusting density difference between internal water phase and oil phase to around 1.

The hollow size can be adjusted by increasing the salt concentration in the internal water phase, water in the external water diffused into the internal water aqueous to enlarge the internal water phase, and resulted in a thinner wall (Fig.6) After the polymerization of monomer oil phase, the oil phase became a wall, and the phase separation between polymer and porogen occurred, resulting a porous wall. The SEM photograph is shown in Fig.7, a hollow particle with a porous wall was successfully obtained. We are planned to construct an artificial cell by this kind of microsphere, the hollow can allow enzymes and their cofactors to be encapsulated inside, and substrate can pass through the wall freely. And, the high mechanical strength of synthesized microsphere can allow it to be used in chromatographic column, as well as in a reactor, compared with conventional gel microcapsules.



(a) Before swelling (b) After swelling

Fig.6 OM photos of swelling process when the salt concentration of internal water phase was higher

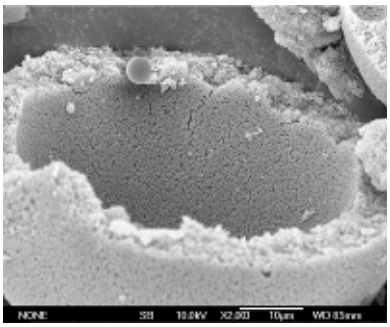


Fig.7 SEM photo of hollow microsphere with a porous wall

Reference

[1] Baughn, R. L., O. Adalsteinsson, and G. M. Whitesides, 1978. Large-Scale Enzyme-Catalyzed Synthesis of Atp from Adenosine and Acetyl Phosphate: Regeneration of Atp from Amp*. J. Am. Chem. Soc.* **100**(1): 304-306.

[2] Yamazaki, Y. and H. Maeda, 1982. The Co-Immobilization of Nad and Dehydrogenases and Its Application to Bioreactors for Synthesis and Analysis*. Agric. Biol. Chem.* **46**(6): 1571-1581.

[3] Chang, T. M. S. and S. Prakash, 2001. Procedures for Microencapsulation of Enzymes, Cells and Genetically Engineered Microorganisms*. Molecular Biotechnology*. **17**(3): 249-260.

[4] Buckmann, A. F., M.-R. Lula, R. Wichmann, and C. Wandrey, 1981. An Efficient Synthesis of High-Molecular-Weight Nad(H) Derivatives Suitable for Continuous Operation with Coenzyme-Dependent Enzyme Systems*. J. Appl. Biochem.* **3**: 301-315.

[5] West, J. L. and N. J. Halas, 2000. Applications of Nanotechnology to Biotechnology*. Current Opinion in Biotechnology*. **11**: 215-217.

Acknowledgement

We would like to thank the financial support of the National Nature Science Foundation of China under Contract 20125616 and 20221603. The financial supports from Chinese Academy of Sciences and Wang Kuan-Cheng scholarship for cooperation are gratefully acknowledged.