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막유화법에 의한 알지네이트 Microsphere의 제조

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Preparation of Alginate Microspheres Using Membrane Emulsification Method

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요 약: SPG (Shirasu porous glass) 관형 막이 설치된 회분식 막유화 장치를 사용하여 단분산 칼슘 알지네이트 미립자를 제조하기 위한 막유화 공정변수의 최적조건을 결정하였다. 막유화의 공정변수로는 연속상에 대한 분산상의 비율, 알지네이트 농도, 유화제의 종류와 농도, 안정제 농도, 가교제 농도, 교반속도, 막간 압력차 및 SPG 막의 세공크기로 설정하고, 이들 변수가 제조된 알지네이트 미립자의 입자 크기와 분포에 미치는 영향을 검토하였다. 막유화의 공정변수들 중에서 연속상에 대한 분산상의 비율, 막간 압력차 그리고 알지네이트 농도가 증가할수록 미립자의 크기가 증가하였다. 반면에 유화제의 농도, 교반속도 그리고 가교제의 농도가 증가할수록 미립자의 크기가 감소하였다. 세공 크기 2.9 μm인 SPG 막을 사용한 경우 막유화의 공정변수 조절을 통해 최종적으로 평균 입자 크기 6 μm, 크기 분산도 1.1인 단분산 알지네이트 미립자의 제조가 가능하였다.

Abstract: We prepared monodispersed calcium alginate microspheres by controlling various conditions of emulsification procedure using a lab-scale batch type membrane emulsification system equipped with SPG (Shirasu porous glass) tubular membranes. We determined the effects of process parameters of membrane emulsification (ratio of dispersed phase to continuous phase, alginate concentration, emulsifier concentration, type and concentration of stabilizer, transmembrane pressure, concentration of crosslinking agent, stirring speed and membrane pore size) on the mean size and size distribution of alginate microspheres. The increase of the ratio of dispersed phase to continuous phase, transmembrane pressure and alginate concentration led to the increase in the mean size of alginate microspheres. On the contrary, the increase in emulsifier concentration, stirring speed of the continuous phase and concentration of the crosslinking agent caused the reduction of the mean size of microspheres. Through controlling these parameters, monodisperse alginate microspheres with about 6 μm of the mean size and 1.1 of the size distribution value were finally prepared in case of the using SPG membrane with the pore size of 2.9 μm.

Keywords: *Membrane emulsification, SPG membrane, Microspheres, Calcium alginate*

1. Introduction

Emulsion manufacturing is a very important process in the food, chemical, mineral processing, cosmetics and pharmaceutical industries. Numerous studies have been reported on the preparation of both oil-in-water (O/W) and water-in-oil (W/O) emulsions. To produce emulsions, many emulsification systems such as a high-speed rotor system, colloid mill, homogenizer and ultra-sonicator are being used in various industrial fields. However, it is well known that a number of problems may be associated with such conventional methods.

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The droplet size and the size distribution cannot easily be controlled. The energy utilization for the large-scale production of emulsions using conventional methods is very poor and gets worse as vessel size increases. Reproducibility on a single piece of equipment is often poor and the quality of the products prepared from the same manufacture scale can even be different. Therefore, scale-up is a common and difficult problem: The transformation from laboratory to small-scale manufacture and/or to full-scale manufacture is difficult. This can lead to inflexibility of manufacturing systems[1-3].

Over the last 10 years or so, there has been an increasing interest in a new technique for making emulsions known as membrane emulsification. This method involves using low pressure to force a dispersed phase to permeate into a continuous phase through a microporous membrane having a uniform pore size distribution. This technique is highly attractive because of its simplicity, low energy consumption and monodisperse products[4]. Since the pioneering work on the preparation of monodisperse emulsions using Shirasu porous glass (SPG) membrane by Nakasima and Shimizu *et al*[5], various kinds of monodisperse emulsions and microspheres with a narrow size distribution have been developed[6].

Microspheres composed of natural polymers and biodegradable polymers such as gelatin[7], albumin[8], poly (lactide)[9,10], alginate[11,12], and other poly(saccharides)[13] can be used as the drug delivery systems (DDS) and carriers of proteins, DNAs and cells, because of their good biocompatibility. When they are used as DDS, adjusting the degradation rate of the polymers can control the release rate of drugs because the polymers can be degraded by hydrolysis or the action of enzymes *in vivo*. Furthermore, the polymers do not accumulate in a living body so that they will not inflict any harm on the body. On the other hand, the microspheres with a narrow size distribution are necessary in the DDS applications in order to decrease side effects of the drugs, especially anti-cancer agents, because the accumulated locations of the particles containing anti-cancer agents also depend on the size of the particles.

In this paper, the uniform microspheres of a natural polymer, calcium alginate, were prepared by controlling various conditions of membrane emulsification procedure using a lab-scale system. To prepare uniform microspheres, we considered various parameters of membrane emulsification procedure such as the ratio of dispersed phase to continuous phase, the type and concentration of emulsifiers, concentration of alginate, concentration of stabilizer, stirring speed of a continuous phase, pore size of membranes, transmembrane pressure (ΔP_{TM}) and concentration of a crosslinking agent.

2. Experimental

2.1. Materials

Sodium alginate (low viscosity grade) used as a dispersed phase was purchased from Sigma Co. (USA). Isooctane used as the oil (continuous) phase was obtained from APS Co. (Australia). Emulsifiers were provided from Aldrich Co. (USA): Sorbitan monooleate (Span 80, hydrophile-lipophile balance value; HLB = 4.2); sorbitan sesquioleate (Span 83, HLB = 3.7); and sorbitan trioleate (Span 85, HLB = 1.8). Arabic gum used as a stabilizer was purchased from Fluka Co. (Switzerland). Calcium chloride as a crosslinking agent was obtained from Ajax Chemicals Co. (USA). Ethanol and acetone were provided from Merck Co. (Germany). All chemicals were used without further purification. SPG membranes of average pore sizes, 1.45 and 2.9 μm, were purchased from Ise Chemical Co. (Japan). Distilled and deionized water used throughout the process was prepared from Milli-Q$^+$ Ultrapure Water System (USA).

2.2. Experimental System

A schematic diagram of the membrane emulsification system is shown in Fig. 1. The continuous phase containing an emulsifier was stirred in the vessel and the dispersed phase in a reservoir was permeated through the SPG membrane into the continuous phase under N_2

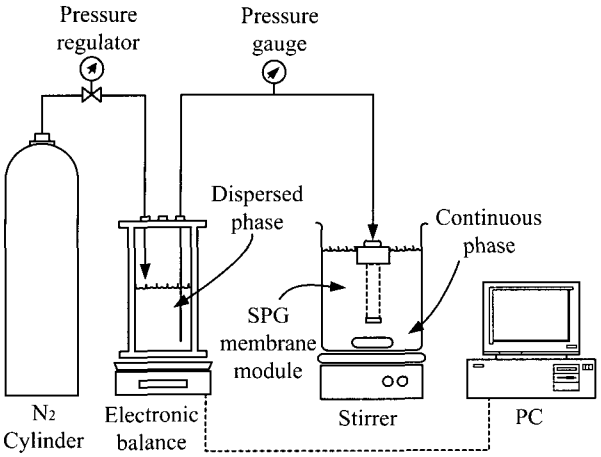


Fig. 1. Schematic diagram of membrane emulsification system.

gas pressure. Pressure was monitored by a pressure gauge connected on the tube between the dispersed phase reservoir and the membrane module. The dispersed phase flux was calculated on the basis of data obtained from the weight change of the dispersed phase reservoir with an electronic balance (PB8001-S, Mettler Toledo Co., Switzerland) and a computer. Used SPG membrane tubes were 10 mm outer diameter, 1 mm thickness, and 30 mm length, and treated with octadecyltrichlorosilane (ODS) and trimethylchlorosilane (TMS) to render SPG membranes hydrophobic[14].

2.3. Preparation of Alginate Microspheres

A typical procedure for the preparation of alginate microspheres is shown in Fig. 2. Before emulsification, the membrane module was immersed into the oil phase and treated with an ultrasonicator for 10 min prior to use so that its surface was entirely wetted by the continuous phase. This module wetted by the oil phase was installed in the membrane system. The dispersed phase, a mixture of deionized water, sodium alginate and stabilizer, was prepared and stored in the dispersed phase reservoir. The continuous phase, a mixture of isooctane and an emulsifier, was stirred in a vessel. The dispersed phase is permeated through the membrane module under nitrogen gas pressure into the continuous phase and dispersed. Calcium chloride solu-

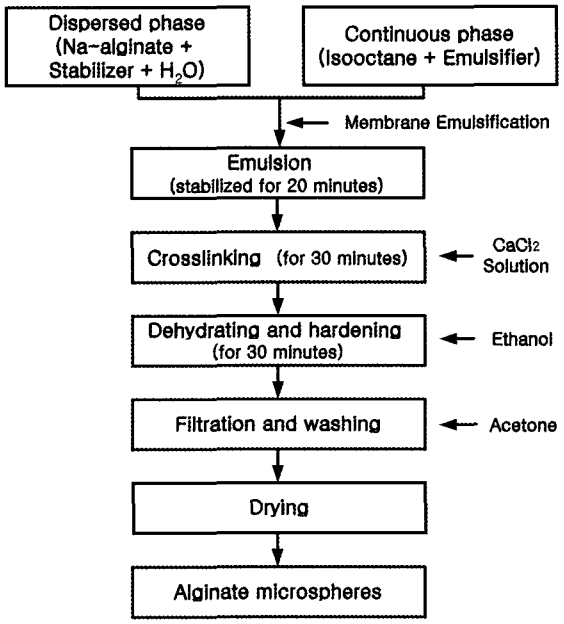


Fig. 2. Procedure for preparation of alginate microspheres using membrane emulsification method.

tions with different concentrations were added and the dispersion was mixed for 30 min. Ethanol was then used to dehydrate and further harden the formed microspheres. The microspheres were collected by filtration, washed three times with acetone and finally dried at 37°C for 24 h. Experimental conditions for the preparation of alginate microspheres by membrane emulsification are shown in Table 1 and Table 2.

2.4. Determination of Size and Size Distribution of Microspheres

The volume-averaged diameters of the microspheres and their size distributions were measured with a light scattering particle size analyzer (Master Sizer 2000, Malvern Instruments Ltd., UK). The size distribution was evaluated with the value α defined as follows [1, 2]:

$$\alpha(-) = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \quad (1)$$

Here, $D_{N\%}$, (N = 10, 50, 90) means the diameter with volume percentage of microspheres up to N%.

Table 1. Preparation Conditions for Manufacturing of Alginate Microspheres[a)]

Ratio of D/C[b)] [%(w/v)]	Emulsifier concentration [%(w/v)]	Alginate concentration [%(w/v)]	Stabilizer concentration [%(w/w)]	Crosslinking agent concentration [%(w/v)]
2.0 **4.0** **8.0** **16.0**	2.0	3	11	5
4.0	**0.5** **1.0** **2.0** **4.0**	3	11	5
4.0	2.0	**1.0** **2.0** **3.0** **4.0**	5	5
4.0	2.0	3	**0** **5** **11** **18**	5
4.0	2.0	3	5	**2.5** **5.0** **10.0** **20.0**

a) Process conditions: stirring speed = 700 rpm, transmembrane pressure = 40 kPa, pore size = 2.9 μm, b) means the ratio of dispersed phase to continuous phase

Table 2. Process Conditions for Manufacturing of Alginate Microspheres[a)]

Stirring speed (rpm)	Transmembrane Pressure (kPa)	pore size of SPG membrane (μm)
300 **500** **700** **900**	40	2.9
700	**20** **30** **40** **60**	**2.9**
700	**100** **120** **140** **160**	**1.45**

a) Preparation conditions; ratio of D/C = 4%(w/v), alginate concentration = 3%(w/v), emulsifier concentration = 2%(w/v), stabilizer concentration = 5%(w/w), crosslinking agent concentraion = 5%(w/v)

The smaller the value α, the narrower the size distribution.

2.5. Morphology Analysis of Microspheres

To observe the surface feature of alginate microspheres, they were dried in a freeze dryer (FD5510-01, Ilshin Lab Co., Korea) for 24 h. Then the shape and surface morphology of the microspheres were observed with a field emission scanning electron microscope (LEO-1530FE, LEO Instrument Co., Germany).

3. Results and Discussion

3.1. Effect of the Ratio of Dispersed Phase to Continuous Phase

The effect of the ratio of dispersed phase to continuous phase (ratio of D/C) on the mean size and the size distribution of the microspheres prepared by membrane emulsification method was investigated. The ratio of D/C was adjusted with 2, 4, 8, and 16%(w/v). The concentration of emulsifier (Span 80) in the continuous phase was fixed to 2%(w/v). The mean size and the size distribution of prepared microspheres are shown in

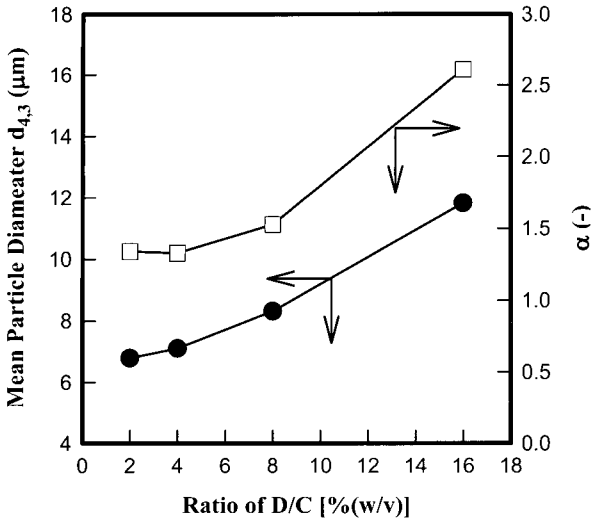


Fig. 3. Effect of ratio of dispersed phase to continuous phase on mean size and size distribution of microspheres. (alginate concentration = 3%(w/v); stabilizer concentration = 11%(w/w); emulsifier (Span 80) concentration = 2%(w/v); transmembrane pressure (ΔP_{TM}) = 40 kPa; stirring speed = 700 rpm; membrane pore size = 2.9 μm).

Fig. 3. When the ratio of D/C was 2%(w/v), the most uniform microspheres were prepared. As the ratio of D/C increased from 2 to 16%(w/v), the mean size of prepared microspheres increased gradually and the size distribution became broader. It is demonstrated that the increase in the ratio of D/C makes emulsion unstable because of the gradual decrease in the emulsifier amount to disperse the droplet formed at the membrane surface.

3.2. Effect of Type and Concentration of Emulsifier

The effects of the type of emulsifiers on the mean size and the size distribution of microspheres were studied with three kinds of emulsifiers that are sorbitan monooleate (Span 80, HLB = 4.2), sorbitan sesquioleate (Span 83, HLB = 3.7) and sorbitan trioleate (Span 85, HLB = 1.8) as shown in Fig 4. It was observed that the most uniform and smallest microphores were prepared with Span 80 at HLB 4.2. As HLB value decreased, the mean size and size distribution of the microspheres were significantly worse. The mean size of microspheres prepared with Span 85 was approximately twice as large as those prepared with Span 80. This result indicated that low HLB was unsuitable to prepare uniform microspheres because of inducing rapid coalescing between emulsion droplets.

The effect of emulsifier concentration in the continuous phase on the mean size and the size distribution of prepared microspheres is shown in Fig. 5. Sorbitan

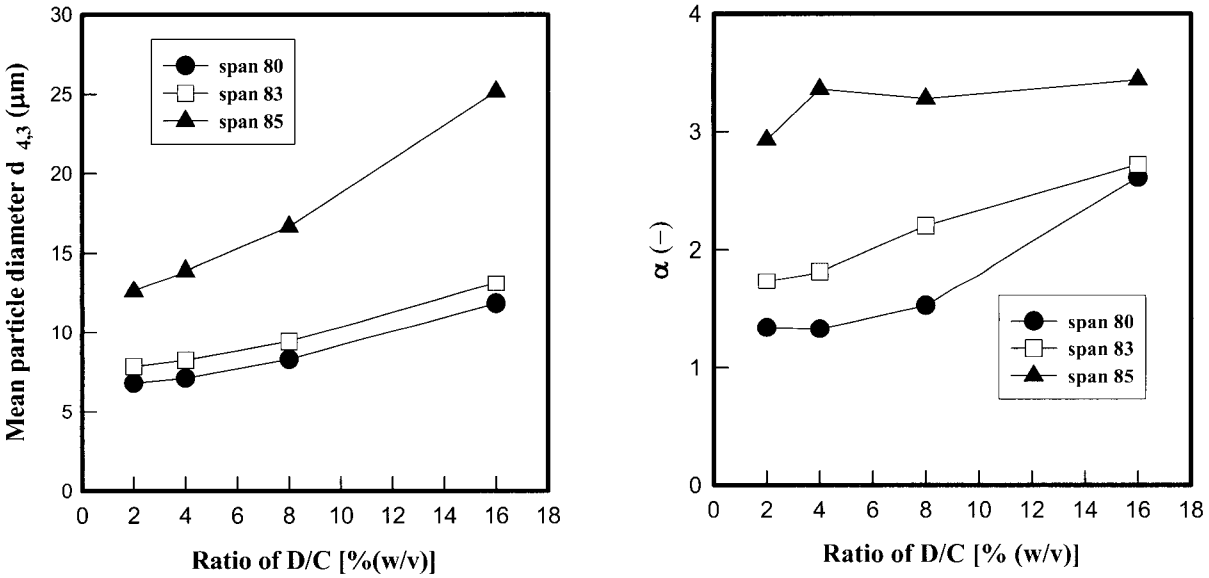


Fig. 4. Effect of types of emulsifier on mean size and size distribution of microspheres (alginate concentration = 3%(w/v); stabilizer concentration = 11%(w/w); emulsifier concentration = 2%(w/v); ΔP_{TM} = 40 kPa; stirring speed = 700 rpm; membrane pore size 2.9 μm).

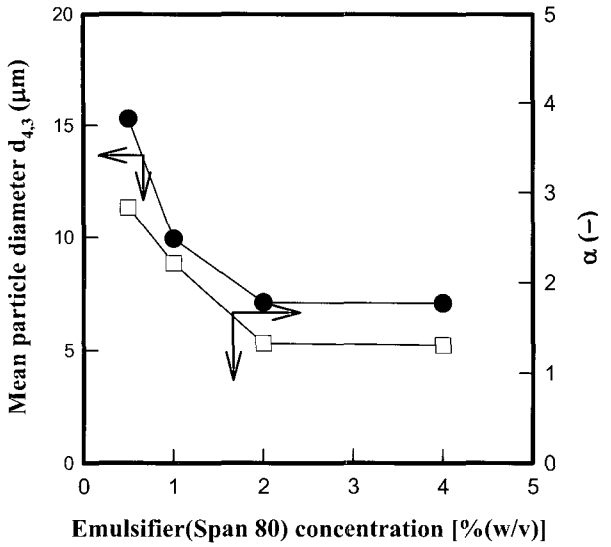


Fig. 5. Effect of emulsifier concentration on mean size and size distribution of microspheres (ratio of D/C = 4%(w/v); stabilizer concentration = 11%(w/w); ΔP_{TM} = 40 kPa; alginate concentration = 3%(w/v); stirring speed = 700 rpm; membrane pore size = 2.9 μm).

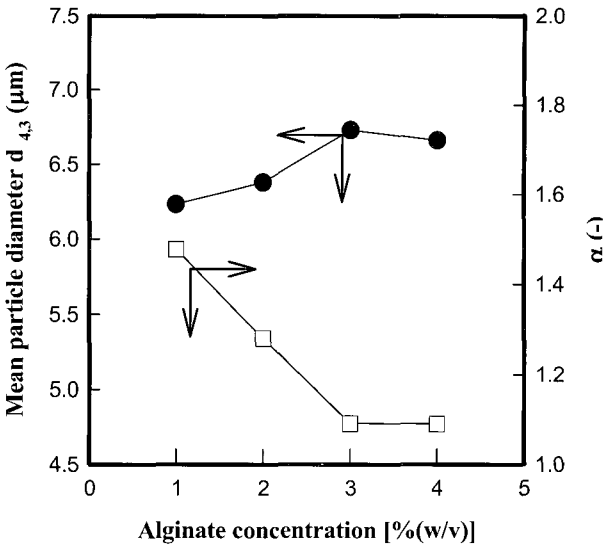


Fig. 6. Effect of alginate concentration on mean size and size distribution of microspheres (ratio of D/C = 4%(w/v); emulsifier (Span 80) concentration = 2%(w/v); stabilizer concentration = 5%(w/w); ΔP_{TM} = 30 kPa; stirring speed = 700 rpm; membrane pore size = 2.9 μm).

monooleate was used as an emulsifier and the ratio of D/C was fixed at 4%(w/v). A Span 80 concentration was changed at 0.5, 1, 2, and 4%(w/v).

Emulsifiers have two main roles to play in the formation of an emulsion. Firstly, they lower the interfacial tension between oil and water. Schröder and Schubert [2] have suggested that the interfacial tension force is one of the essential forces holding a droplet at a pore of a membrane. They found that the higher the equilibrium interfacial tension the larger droplets are produced. Secondly, emulsifiers stabilize the droplets against coalescence and aggregation. This will depend on both the type of emulsifier and the concentration[15].

As shown in Fig. 5, when the concentration of Span 80 decreased, the mean size of microspheres increased dramatically and the size distribution became broad. It is explained that droplets detach at large diameters from pores of a membrane and the coalescence probability at the membrane surface is high because Span 80 with concentration of 0.5%(w/v) reduces the interfacial tension of droplets formed in a pore more slowly than Span 80 with concentration of 4%(w/v).

3.3. Effect of Alginate Concentration

The effect of alginate concentration in the dispersed phase on the mean size and the size distribution of prepared microspheres is shown in Fig. 6. Also, Fig. 7 shows SEM photographs of alginate microspheres prepared with various alginate concentrations. The concentration was varied from 1 to 4%(w/v). As shown in Fig 6, it was observed that the mean size of microspheres increased gradually with the increase in alginate concentration. The matrix structure of microspheres prepared at high concentration was much denser. When the concentration was 1%(w/v), the mean size of microspheres was the smallest but the size distribution was broad owing to low viscosity of alginate solution. Large pores were found at the surface of prepared microspheres due to their poor solidity. On the other hand, the mean size of microspheres at high concentration of alginate solution was larger than that at low concentration because of the increase in viscosity. Although uniform microspheres were prepared at high concentration (4%(w/v)), the dispersed phase flux decreased considerably and the permeation time was significantly increased because the flow rate of the dis-

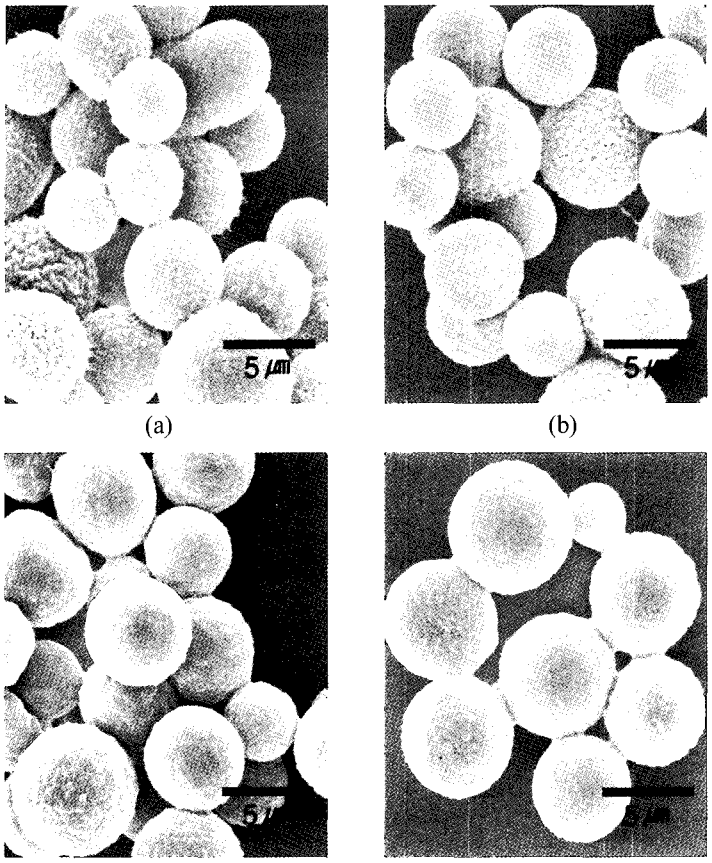


Fig. 7. SEM photographs of alginate microspheres prepared with various alginate concentrations (the same conditions as in Fig. 6) (a) 1%(w/v) solution (b) 2%(w/v) solution, (c) 3%(w/v) solution, and (d) 4%(w/v) solution.

persed phase through the pores of the membrane became slow owing to high viscosity.

3.4. Effect of Stabilizer Concentration

The effect of stabilizer concentration in the dispersed phase on the mean size and size distribution of prepared microspheres is shown in Fig. 8. The gum arabic was used as a stabilizer and emulsifier concentration was fixed at 2%(w/v). The stabilizer amount per the Na-alginate amount was changed at 0, 5, 11, and 18%(w/w).

As the gum arabic amount increased, it was observed that the mean size of alginate microspheres decreased gradually from 7.6 to 7.1 μm because dispersion stability of formed emulsion was improved by the gum arabic controlling coalescence of droplets in emulsion. However, the effect of stabilizer concentration on emulsion stability was not as dramatic as

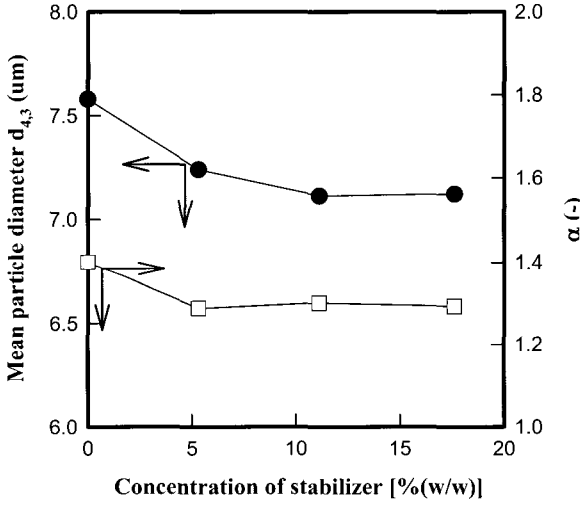


Fig. 8. Effect of the amount of stabilizer on mean size and size distribution of microspheres (ratio of D/C = 4%(w/v); emulsifier (Span 80) concentration = 2%(w/v); ΔP_{TM} = 40 kPa; alginate concentration = 3%(w/v); stirring speed = 700 rpm; membrane pore size = 2.9 μm).

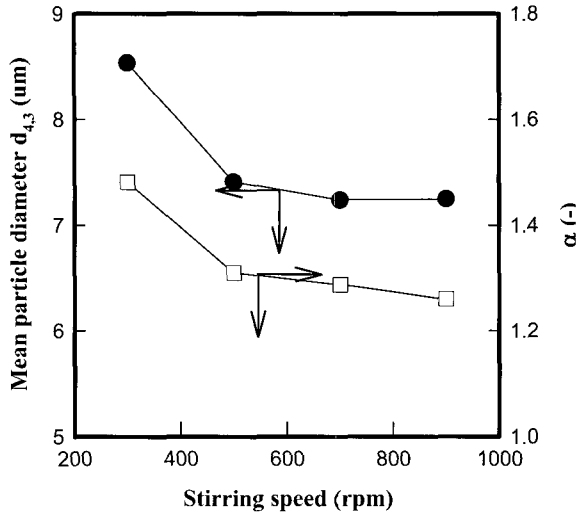


Fig. 9. Effect of stirring speed of continuous phase on mean size and size distribution of microspheres (ratio of D/C = 4%(w/v); alginate concentration = 3%(w/v); stabilizer concentration = 5%(w/w); ΔP_{TM} = 40 kPa; emulsifier (Span 80) concentration = 2%(w/v); membrane pore size = 2.9 μm).

that of emulsifier concentration.

3.5. Effect of Stirring Speed

The stirring speed of the continuous phase is considered as one of the main parameters affecting the membrane emulsification method because droplets formed on the surface of the membrane detach under the influence of the flowing continuous phase. The effect of stirring speed of the continuous phase on the mean size and the size distribution of prepared microspheres is shown in Fig. 9. The stirring speed was adjusted at 300, 500, 700, and 900 rpm. As shown in Fig. 9, the largest change in the mean size of microspheres occurred at lower stirring speed range. These results can be explained as follows: At lower stirring speed the formed droplet size increases rapidly and the size distribution becomes much broader because the droplets grow and coalesce at the membrane surface before finally being detached. However, there is no significant influence on the mean size and the size distribution of microspheres at high stirring speed. The similar results were reported by Joscelyne *et al* [16] and Schröder *et al*[2].

3.6. Effect of Transmembrane Pressure and Membrane Pore Size

The effect of transmembrane pressure, ΔP_{TM}, on the mean size and the size distribution of prepared microspheres is shown in Fig. 10. Microspheres were prepared with changing of transmembrane pressure using 1.45 and 2.9 μm membranes. For 1.45 and 2.9 μm

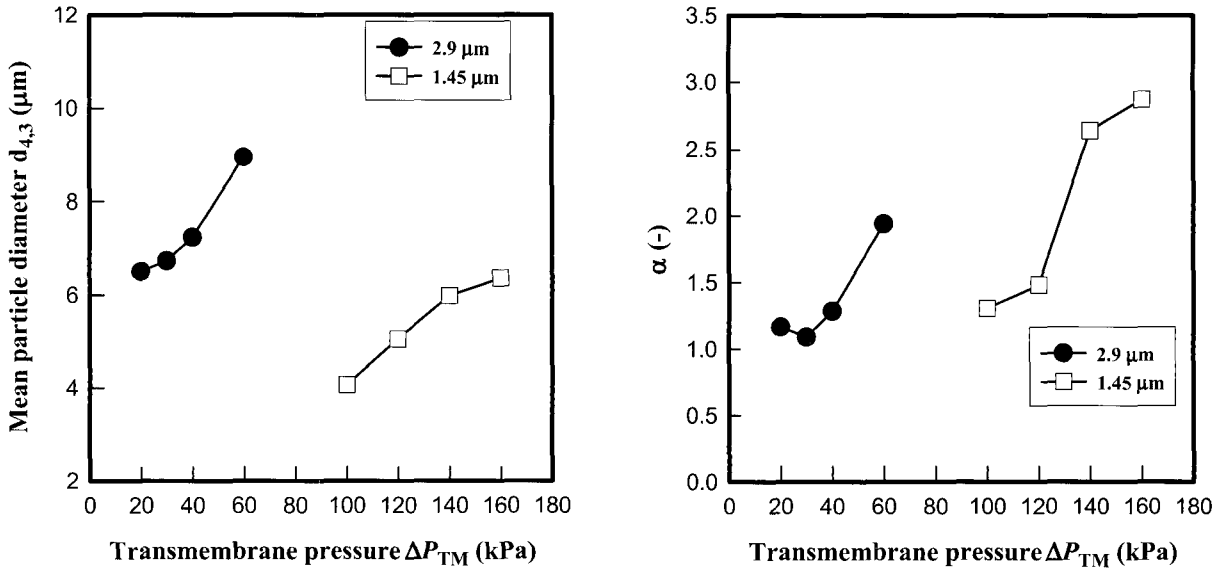
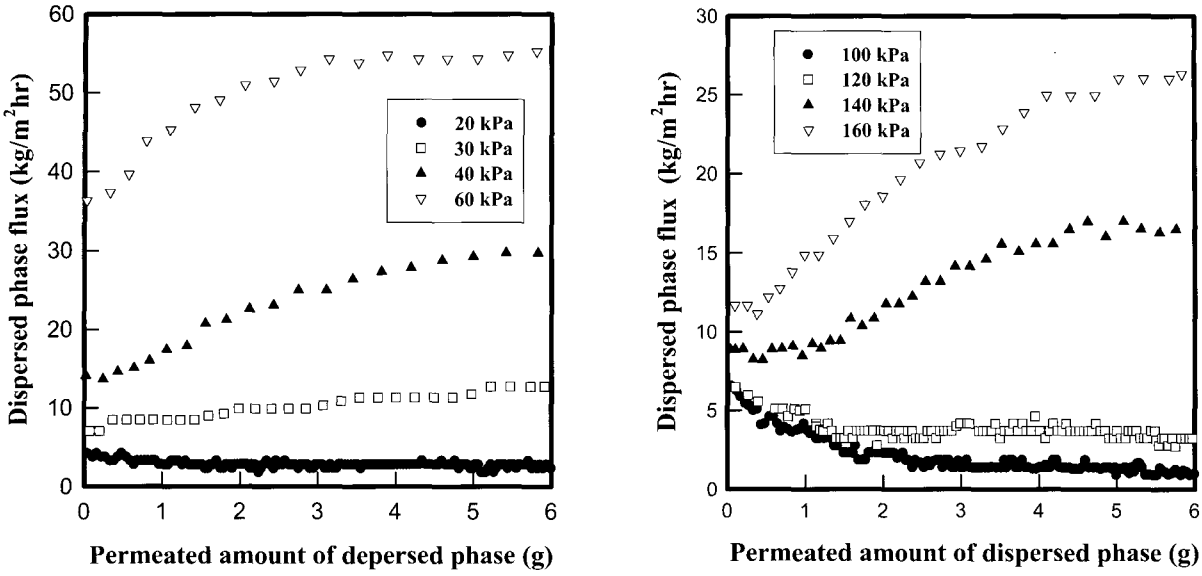


Fig. 10. Effect of transmembrane pressure and membrane pore size on mean size and size distribution of microspheres (ratio of D/C = 4%(w/v); alginate concentration = 3%(w/v); stabilizer concentration = 5%(w/w); emulsifier (Span 80) concentration = 2%(w/v); stirring speed = 700 rpm; pore size = 2.9 μm, 1.45 μm).



(a) pore size : 2.9 ㎛ (b) pore size : 1.45 ㎛

Fig. 11. Effect of transmembrane pressure on dispersed phase flux (ratio of D/C = 4%(w/v); alginate solution = 3%(w/v); stabilizer concentration = 5%(w/w); emulsifier (Span 80) concentration = 2%(w/v); stirring speed = 700 rpm; pore size = 2.9 μm, 1.45 μm).

membranes, transmembrane pressure was adjusted by a pressure gauge with 20~60 kPa and 100~160 kPa, respectively. Higher pressure was needed to force the dispersed phase into the continuous phase through a membrane with a smaller pore size. It was observed that the mean size and the size distribution of microspheres increased sharply with the increase in transmembrane pressure because the droplets grew and coalesced on the membrane surface at high pressure before finally being detached.

Fig. 11 shows the effect of transmembrane pressure on the dispersed phase fluxes through the different pore size membranes. When the membrane with larger pores was used, the increase in the flux occurred apparently with the increase in transmembrane pressure. The increase in the flux was the fastest for the membrane with a pore size of 2.9 μm at 60 kPa. It was observed, however, that as the change in transmembrane pressure, the flux was not constant but increased or decreased with permeated amount of dispersed phase. When transmembrane pressure decreased, the flux was constantly maintained or decreased gently with time. And then the flux was finally reached a steady state

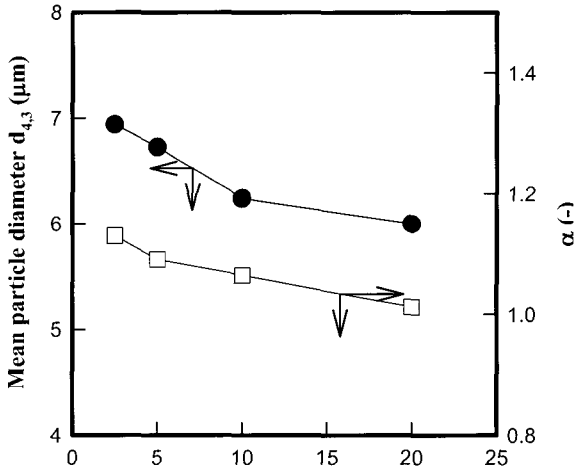


Fig. 12. Effect of concentration of crosslinking agent on mean size and size distribution of microspheres (ratio of D/C = 4%(w/v); alginate concentration = 3%(w/v); emulsifier (Span 80) concentration = 2%(w/v); stabilizer concentration = 5%(w/w); ΔP_{TM} = 30 kPa; stirring speed = 700 rpm; pore size = 2.9 μm).

value. It is explained that the decrease in the flux results from the fact that not all pore paths through the membrane are used to permeated at the same time, given differences in path lengths and pore diameters.

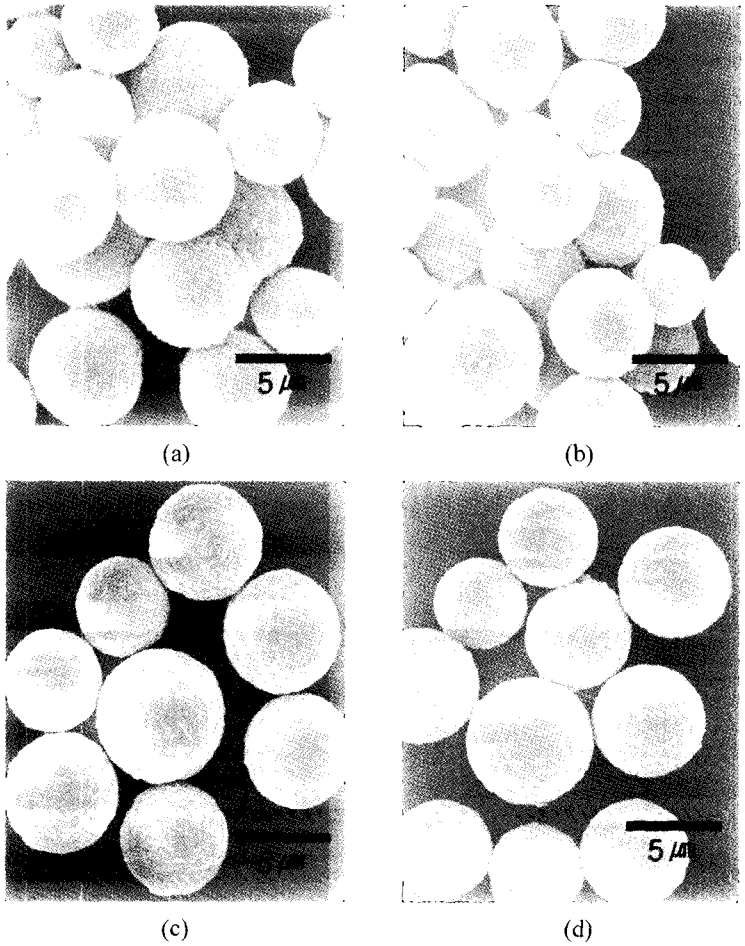


Fig. 13. SEM photographs of alginate microspheres prepared with various concentrations of crosslinking agent (the same conditions as in Fig. 12). (a) 2.5%(w/v), (b) 5%(w/v), (c) 10%(w/v), and (d) 20%(w/v).

3.7. Effect of Concentration of Crosslinking Agent

Fig. 12 shows the mean size and the size distribution of microspheres with the concentration of crosslinking agent. Their concentration varied with 2.5, 5, 10, and 20%(w/v). When the concentration of the crosslinking agent increased, the mean size of microspheres decreased because of the high degree of crosslinking. Also, the size distribution was slightly improved. As shown in Fig. 13, when the concentration was 2.5%(w/v), large pores were found at the surface of prepared microspheres due to their poor solidity by the low degree of crosslinking. On the contrary, the matrix structure of microspheres became gradually dense with the increase in the concentration of the crosslinking agent.

Through the experiments of membrane emulsification above, uniform microspheres with the mean diameter of about 6 μm were finally prepared and the optimal conditions for manufacturing of these microspheres with about 6 μm diameter using membrane emulsification were obtained as follows; 1) ratio of D/C = 4%(w/v), 2) alginate concentration = 3%(w/v), 3) emulsifier (Span 80) concentration = 2%(w/v), 4) stabilizer concentration = 5%(w/w), 6) ΔP_{TM} = 30 kPa, 7) stirring speed = 700 rpm, 8) membrane pore size = 2.9 μm and 9) concentration of crosslinking agent = 10%(w/v).

4. Conclusions

Alginate microspheres were prepared by the membrane emulsification method using SPG membrane

tubes. Effects of experimental conditions of membrane emulsification on the size and size distribution of alginate microspheres were investigated. Results are as follows:

(1) Uniform microspheres with the mean diameter of about 6 μm were finally prepared using membrane emulsification and the optimal conditions for manufacturing of these microspheres were as follows; ratio of D/C = 4%(w/v), alginate concentration = 3%(w/v), emulsifier concentration = 2%(w/v), stabilizer concentration = 5%(w/w), ΔP_{TM} = 30 kPa, stirring speed = 700 rpm, membrane pore size = 2.9 μm and concentration of crosslinking agent = 10%(w/v).

(2) The mean size of alginate microspheres increased with the increase in the ratio of dispersed phase to continuous phase, transmembrane pressure and alginate concentration.

(3) The increase of emulsifier concentration, stabilizer concentration, stirring speed of the continuous phase and concentration of the crosslinking agent decreased the mean size of alginate microspheres.

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