Preparation of Uniform Microspheres and Microcapsules by Modified Emulsification Process

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Summary: Uniform microspheres and microcapsules have been prepared by developing a direct membrane emulsification technique from O/W, W/O and W/O/W emulsions in previous studies, and have been applied in bio-separation and drug delivery systems. The diameter can be controlled from several microns to above 100 microns. However, smaller microsphere with submicron size, especially from W/O/W emulsion was difficult to be prepared. In this article, a modified emulsification technique was developed to overcome the problem. That is, a pre-emulsion (W/O or W/O/W) with broad size distribution of droplets was prepared firstly by homogenization, sonification or mechanical stirring method, then the pre-emulsion was pressed through the uniform pores of a Shirasu Porous Glass (SPG) membrane to obtain relatively uniform smaller droplets, finally the droplets were solidified to form uniform microsphere or microcapsule. Uniform chitosan microsphere and poly(lactic-glycolic acid) (PLGA) microcapsule with submicron size were prepared from W/O and W/O/W emulsions, respectively. Further more, uniform polymer-magnetite microcapsule was prepared by combining this technique and a new post-precipitation process of magnetite.

Keywords: magnetite; microcapsule; microsphere; modified membrane emulsification technique; uniform,

Introduction

Polymer microspheres and microcapsules have important applications in bio-separation and drug delivery systems.[1–3] In these applications, the control of size and size distribution of microspheres and microcapsules is necessary. If the size distribution is broad, for example, the separation resolution will be lower when the microspheres are used as chromatographic media[4,5]; the bio-availability of drug will be lower because the bio-distribution of drug-carrier in vivo depends apparently on the size of microcapsules.[6] In conventional technique, however, mechanical stirring method and spray drying method were often used, by which the size is difficult to control and the size distribution of microsphere is usually broad. This also result in poor reproducibility of the product, which is undesirable in user's applications, especially is difficult to get FDA permission for application in drug delivery systems.

In previous studies, we have prepared uniform microspheres and microcapsules by developing direct membrane emulsification technique (''direct'' is used here to distinguish it from ''modified'' technique in this study), including polystyrene porous microsphere from O/W emulsion,[7,8] chitosan microsphere from W/O emulsion,[9,10] and poly(lactide) microcapsule from W/O/W emulsion.[11,12] And, we found that the direct membrane emulsification technique had many advantages: (1) the size was

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uniform, the CV (coefficient variation) value which is an indicator of size distribution was around 10%; (2) the size was easy to control, could be controlled between 1 to above 100 microns just by selecting the membrane with different pore size; (3) the coalescence and break-up of the droplets less occurred, and the Oswald degradation was retarded, because of the uniform size, as a result, the encapsulation efficiency of drug or other functional compounds was usually much higher than conventional mechanical stirring method; (4) the emulsification condition was mild, it is suitable for encapsulating bio-active drug such like protein drug, which is easy to deactivate under higher shear force; (5) reproducibility was excellent, because the size of droplets is determined mainly by pore size of the membrane.

By utilizing uniform-sized microspheres and microcapsules, we obtained excellent application results in bio-separation and drug delivery systems. For example, we used polystyrene (PST) porous microspheres in separation of Icariin (Traditional Chinese Medicine) by reversed-phase chromatography,[4] it was found the Icariin can be separated completely from its impurities; much higher separation resolution was obtained, compared with non-uniform porous media. Another example was that, we found chitosan microsphere showed autofluoresce when it was crosslinked by aldehyde-type crosslinker.[13] By utilizing the uniform size and autofluoresce of chitosan microsphere, the effect of size and structure of chitosan microsphere on bioavailability can be investigated quantitatively when it was used as oral administration carrier.[6,14,15] It was found the biodistribution of chitosan microsphere depended apparently on the diameter and the structure of microspheres, one should choose the best diameter to obtain the highest bio-availability of the encapsulated drug,[6] and hollow-porous chitosan microsphere containing Insulin showed higher bio-availability, the blood glucose level was lowered closely to a normal value.[15]

On the other hand, it was also found that the direct membrane emulsification technique had its limitation, the microsphere smaller than 1 μm was difficult to prepare. In this technique, because the droplet is formed by utilizing the high interfacial tension between the dispersed phase and the membrane, the droplet size is usually 3–6 times as large as the membrane pore size. Therefore, the emulsification rate will be extremely low even under very high transmembrane pressure when we use the membrane with submicron-pores. It is also relatively difficult to prepare microcapsules from W/O/W emulsion by the direct membrane emulsification technique, because the primary W/O emulsion must be very stable during the membrane emulsification process, the density balance of the oil phase and water phase had to be adjusted, and the oil-soluble emulsifier should be optimized. In this report, we developed a modified membrane emulsification technique to overcome the above difficulties. The preparation of chitosan microsphere and PLGA microcapsule with submicron size, and polymer microcapsule including magnetite by combining a post-precipitation process of magnetite, were introduced.

Experimental Part

Preparation of Chitosan Microsphere with Submicron Size[16]

Chitosan ($M_W = 50000$, deacetylation degree = 89%) was dissolved in 1 wt% acetic acid aqueous phase. Then, it was mixed with the oil phase (mixture of liquid paraffin and petroleum ether) by a homogenizer (14000 r/min, 60 s) to form W/O pre-emulsion. Subsequently, the pre-emulsion was pressed through the uniform pores of SPG membrane (pore size: 1.4 μm) under a high nitrogen pressure (0.95 MPa) and this process was repeated 3 times to obtain uniform chitosan droplets with submicron size. Finally, GST (Glutaraldehyde Saturated Toluene) was slowly

dropped into the emulsion to solidify the chitosan droplets into microspheres.

Preparation of PLGA Microcapsule with Submicron Size[17]

PLGA (200 mg, Mw = 15000) was dissolved in ethyl acetate (EA, 10 mL), then 1 mL GLP-1 (Glucagon-like Peptide-1) water phase (5 mg/mL) was mixed with PLGA oil phase by homogenization (6000 r/min for 15 s) to form W_1/O primary emulsion, which was further mixed with poly(vinyl alcohol) (PVA, 1.9 wt%) aqueous phase under mechanical stirring (300 r/min, 60 s) to obtain $W_1/O/W_2$ pre-emulsion. The $W_1/O/W_2$ pre-emulsion was pressed through the uniform pores of SPG membrane (pore size: 1.4 μm) for three times to obtain uniform $W_1/O/W_2$ droplet. Then, it was poured in a large amount of water (800 mL) and was stirred for 30 min under 500 r/min to remove EA.

Preparation of Polymer-Magnetite Microcapsule by Combining a Post-precipitation Process of Magnetite

A new process combining modified membrane emulsification technique and post-precipitation process of magnetite was developed to prepare polymer-magnetite microcapsule, as shown in Figure 1.

Firstly, $FeCl_2$ (0.31 g) and $FeCl_3$ (0.13 g) were dissolved in deionized water (1.8 g), then it was emulsified in oil phase composed of poly(styrene-2-hydroxyethyl methacrylate) [P(ST-HEMA)] (0.05 g, $Mw = 1.70 \times 10^5$, HEMA = 5 mol%), dichloromethane (4.5 ml) and emulsifier Span 85 (0.033 g) by sonification (Branson Sonifier) for 15 s in a ice bath to form W_1/O primary emulsion. Then, it was emulsified into an external water phase (150 ml) containing PVA (0.75 g) to form pre-double emulsion ($W_1/O/W_2$) by a magnetic stirrer for 40 s at 300 rpm, and then the pre-double emulsion was pressed through the pores of SPG membrane (pore size: 19.9 μm) to form relatively uniform $W_1/O/W_2$ emulsion with the decreased size. The above double emulsion was transferred into a three-neck glass flask under an agitation rate of 150 r/min by a four-moon shaped impellor, and ammonia solution (25%, 30 ml) was added to the double emulsion slowly (10 ml/min) under nitrogen atmosphere. Ammonia diffused from external water phase to oil phase, finally to internal aqueous phase, and then magnetic nanoparticles were formed by a co-precipitation reaction with $FeCl_2$, $FeCl_3$ and ammonia in the internal water phase (post-precipitation). The reaction was kept at room temperature for 2 h under nitrogen atmosphere; as a result, the $W_1/O/W_2$ emulsions was changed to an $S/O/W_2$ emulsion. Finally, the organic solvent of above $S/O/W_2$ emulsion was evaporated at room temperature for 24 h under a gentle stirring at a rate of 180 rpm. After the solvent was evaporated, the solid polymer-magnetite microcapsules was obtained.

Results and Discussion

Preparation Results of Chitosan Microsphere with Submicron Size

The preparation condition was optimized by investigating the effects of the amounts of emulsifier in oil phase, composition of oil phase, concentration of chitosan, ratio of water to oil phase, transmembrane pressure, and pass number of transmembrane on the size distribution of chitosan microsphere. As a result, the uniform-sized chitosan microsphere with submicron size was successfully obtained by the modified membrane emulsification technique, as shown in Figure 2, where the membrane with pore size of 1.4 μm was used, and optimum condition to obtain the narrowest size distribution was as follows: the transmembrane pressure was 0.95 MPa, the concentration of chitosan solution was 0.5 wt%, oil phase was the mixture of liquid paraffin and petroleum ether [1:2(v/v)] containing 4 wt% PO-500 (hexaglycerin penta ester) emulsifier, the volume ratio of water to oil phase was 1:30, the amount of solidification agent was set as that the molar ratio between amino group and

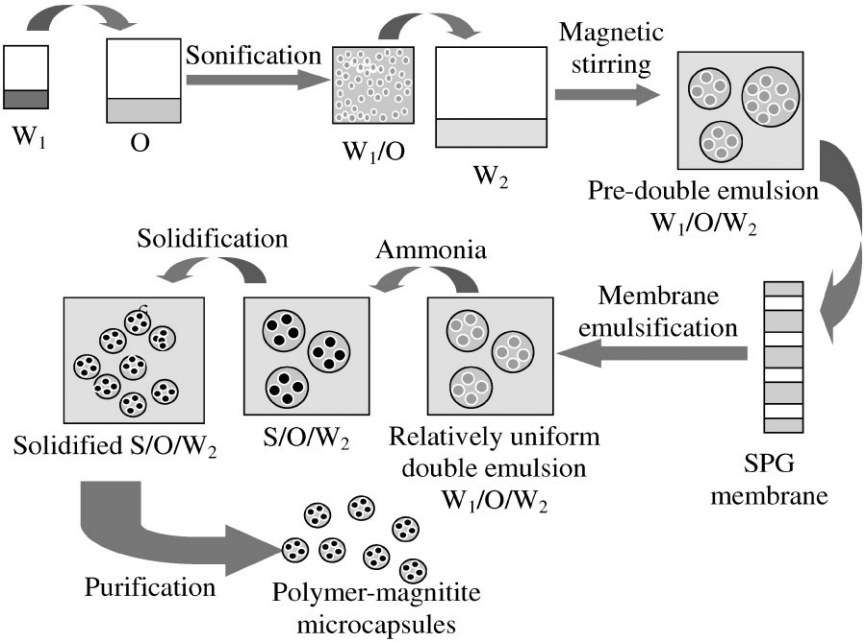

Figure 1.
Schematic illustration for the preparation of polymer-magnetite microcapsule by the modified membrane emulsification.

aldehyde group was 1:1, and the solidification reaction time was 10 h. The diameter of the obtained chitosan microspheres was 200–300 nm, the CV value was as low as 7.4%. Further more, the microspheres with the diameters from 300 nm to 1.85 μm were obtained just by changing the pore size of the membrane from 1.4 μm to 9.0 μm.

Preparation Results of PLGA Microcapsule with Submicron Size

The uniform PLGA microcapsule containing GLP-1 (Glucagon-like peptide-1) was successfully obtained as shown in Figure 3, after investigating the various factors on size distribution of PLGA microcapsules. The optimized preparation condition was

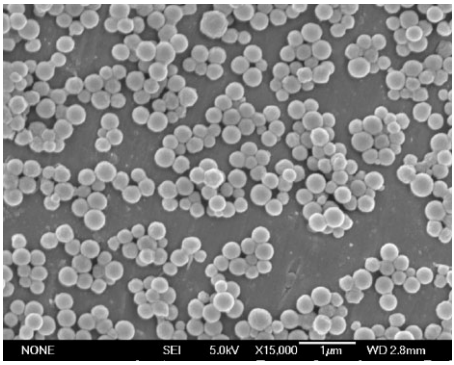

Figure 2.
SEM photograph of chitosan microsphere prepared by modified membrane emulsification technique.

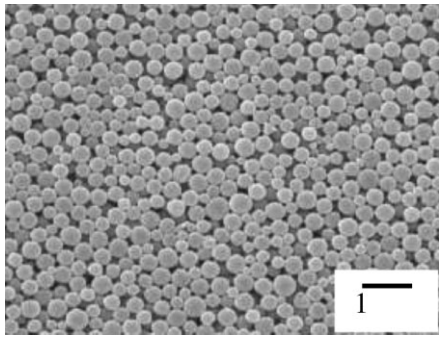

Figure 3.
SEM photograph of PLGA microcapsule prepared by modified membrane emulsification technique.

as follows: transmembrane pressure was 1 MPa, transmembrane number 3 times, PVA concentration in water phase 1.9 wt%, and oil/water ratio 1/5 (v/v). The CV value of the obtained PLGA microcapsule under optimum was 11.4%; the encapsulation efficiency attained to 65%, the bioactivity of GLP-1 was maintained at 85%.

We have ever prepared uniform PLA microcapsules from $W_1/O/W_2$ emulsion by direct membrane emulsification technique,[11] where the primary W_1/O emulsion was pressed through the pores of the membrane into the external water phase to form $W_1/O/W_2$ emulsion. In this case, W_1/O emulsion should be very stable because the direct emulsification process took a relatively longer time. The density of the oil phase and the type and amount emulsifier should be optimized to avoid phase separation. Further more, it was difficult to prepare $W_1/O/W_2$ emulsion with submicron size by direct membrane emulsification. The modified membrane emulsification process can be finished with several minutes, it overcame above problems.

Preparation Result of Polymer-Magnetite Microcapsules by a New Process

Based on above modified membrane emulsification technique, we developed a new process to prepare relatively uniform polymer-magnetite microcapsules. We used this modified emulsification technique to prepare $W_1/O/W_2$ emulsion firstly, the internal water phase contained $FeCl_2$ and $FeCl_3$, oil phase was composed of P(ST-HEMA) copolymer and dichloromethane. After uniform $W_1/O/W_2$ emulsion was prepared by this modified membrane emulsification technique, ammonia was added in external water phase. Ammonia diffused into the internal water phase through oil phase to react with $FeCl_2$ and $FeCl_3$, forming nanoparticles of magnetite. Here, we investigated the effects of process parameters in details, to help to understand this new process.

1) Effect of Transmembrane Pressure

The transmembrane pressure was an important factor for preparation of uniform-sized polymer-magnetite microcapsules. When the higher transmembrane pressure was used, the pre-double emulsion passed through the SPG membrane quickly, which led to vigorous collisions between pre-emulsion and tortuous wall in the pore of SPG membrane. As shown in Figure 4, with the increase of pressure, polymer-magnetite microcapsules with smaller size and narrower size distribution were obtained.

2) Effect of Number of Transmembrane

The effect of transmembrane number of emulsification on size distribution is shown in Figures 5 and 6.

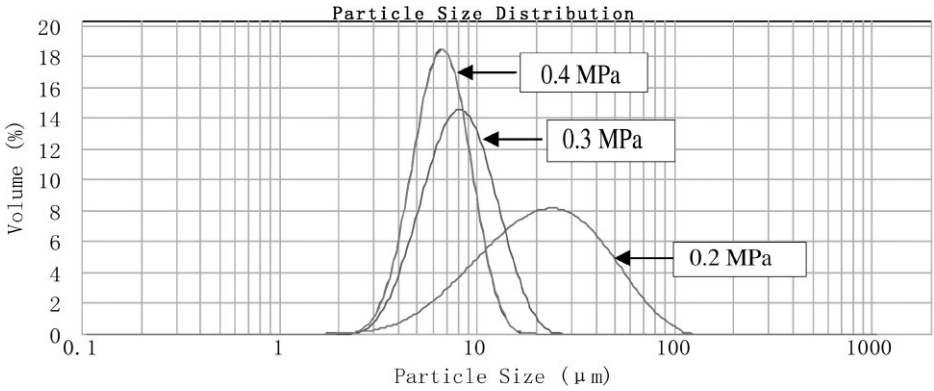

Figure 4.
Size distribution of microcapsules prepared under different transmembrane pressure.

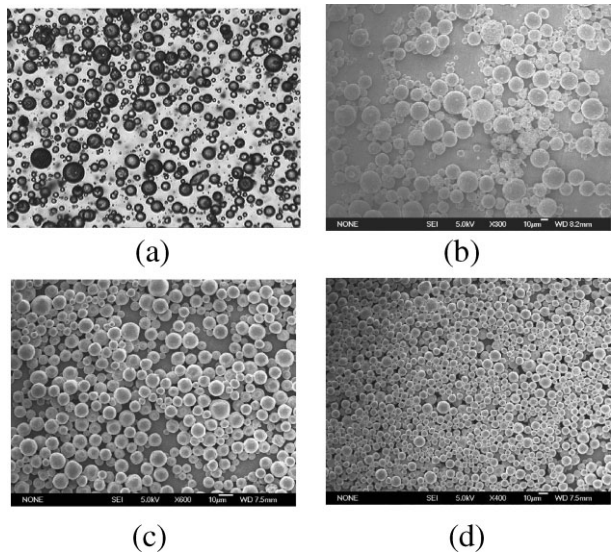

Figure 5.
Optical micrograph (a) of pre-double emulsion and SEM photographs of polymer-magnetite microcapsules with one time (b), two times (c), and three times (d) of transmembrane.

When the pre-double emulsion was pressed through the membrane for more times, larger droplets were broken into smaller ones, and the size distribution became narrower. The effect of transmembrane number on magnetic properties of microcapsules is shown in Figure 7. It can be seen that the saturation magnetization of polymer-magnetite microcapsules decreased with the increase of the transmembrane number. This was because the internal water phase would escape to external water phase during the transmembrane process, especially when the droplets were decreased to submicron size.

3) Effect of Size of Pre-double Emulsion

In order to investigate the size effect of pre-double emulsion, the stirring rate of magnetic stirrer during preparation of pre-double emulsion was varied from 300 to 3000 r/min; the size of pre-double emulsions was adjusted from 14.8 μm to 42.3 μm. As shown in Table 1, the size of the

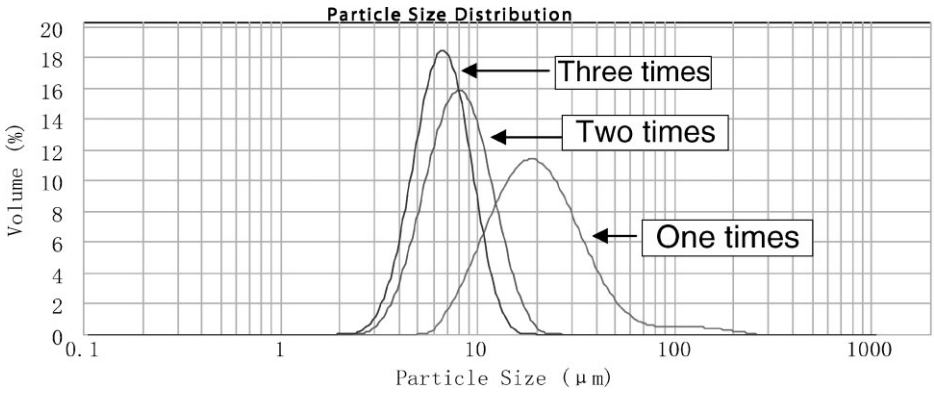

Figure 6.
Size distribution of polymer-magnetite microcapsules with different transmembrane number.

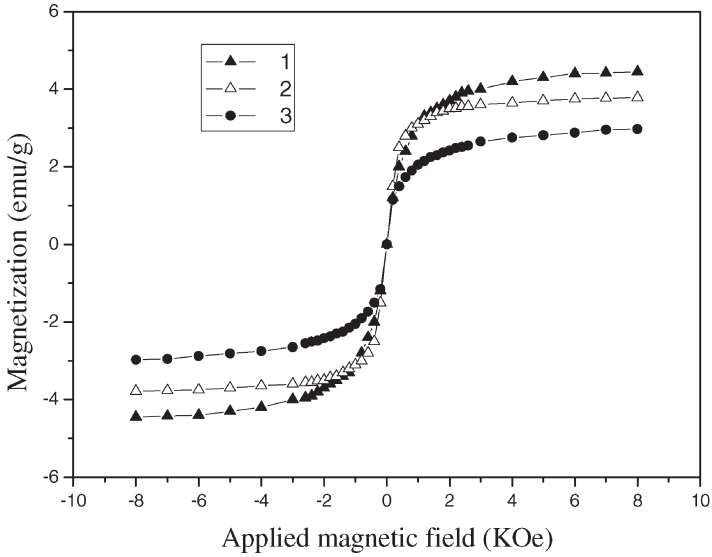

Figure 7.
Magnetization curves of polymer-magnetite microcapsules. Transmembrane number: 1 represents one time; 2 two times; 3 three times.

Table 1.
Size distribution of microcapsules with different sizes of pre-double emulsion.

Volume-mean diameter of pre-double emulsion (μm)	CV of pre-double emulsion (%)	Volume-mean diameter of microcapsules (μm)	CV of microcapsules (%)
42.31	33.7	6.93	27.7
37.15	32.2	7.01	28.4
27.91	35.2	6.68	26.3
14.83	34.5	6.45	27.1

pre-double emulsion played little role in the size and size distribution of the final polymer-magnetite microcapsules. This result implied that the modified emulsification technique was a robust process, can provide the products with excellent reproducibility.

The polymer-magnetite microcapsules with CV value below 26% was obtained by modified SPG membrane emulsification, which was lower than pre-double emulsion. However, the size distribution of polymer-magnetite microcapsules was broader compared with the results of preparation of chitosan microsphere and PLGA microcapsule. This was probably because that the double emulsion in this case was unstable during solidification and co-precipitation of magnetite due to the higher contents of Fe^{2+} and Fe^{3+}. One hand, diffusion of water from external water phase to internal water phase would occur; other hand, the density of internal water phase became much higher than oil phase, resulting in instability of double-emulsion.

Conclusion

In order to overcome the problems in direct emulsification technique, a modified membrane emulsification technique was developed to prepare microsphere with submicron size or microcapsules. Uniform chitosan microsphere and PLGA microcapsule with submicron size were successfully

prepared, respectively from W/O pre-emulsion and W/O/W pre-emulsion. And, polymer-magnetite microcapsule with relatively narrow size distribution was obtained by utilizing this technique and a new post-precipitation process of magnetite. It was found the transmembrane pressure and the number of passes showed apparent effect on size distribution of microsphere and microcapsule.

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