Microencapsulation of Proteins by Rapid Expansion of Supercritical Solution with a Nonsolvent

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A new method—rapid expansion from supercritical solution with a nonsolvent (RESS-N)—is reported for forming polymer microparticles containing proteins such as lysozyme (from chicken egg white) and lipase (from Pseudomonas cepacia). A suspension of protein in CO_2 containing a cosolvent and dissolved polymer is sprayed through a nozzle to atmospheric pressure. The polymers are poly(ethylene glycol) (PEG4000; MW = 3,000, PEG6000; MW = 7,500, PEG20000; MW = 20,000), poly(methyl methacrylate) (PMMA; MW = 15,000), poly(L-lactic acid) (PLA; MW = 5,000), poly(DL-lactide-co-glycolide) (PGLA; MW = 5,000) and PEG − poly(propylene glycol) (PPG) − PEG triblock copolymer (MW = 13,000). The solubilities of these polymers in CO_2 increase significantly with low-molecular-weight alcohols as cosolvents. The particles do not tend to agglomerate after expansion, since the pure cosolvent is a nonsolvent for the polymer. The structure and morphology of the microcapsules were investigated by TEM, SEM, and optical microscopy. The thickness of the polymer coating about the protein, as well as the mean particle diameter and particle-size distribution, could be controlled by changing the feed composition of the polymer.

Introduction

The encapsulation of pharmaceuticals, including proteins into polymers, has been attracting a significant amount of attention for controlled-release applications (Gutcho, 1976; Martin, 1978; Harris, 1992; Benita, 1996). For drug delivery, it is often desirable to produce globular polymer particles on the order of 10 microns containing hydrophilic pharmaceuticals (Deasy, 1984). Despite a considerable number of studies of the preparation of pharmaceuticals coated with polymers for controlled release applications (Martin, 1978; Deasy, 1984; Whateley, 1992), the development of a microencapsulation method with environmentally benign solvents still remains a challenge. Solvent impurities are often toxic and also may degrade pharmaceuticals within a polymer matrix.

Fluidized-bed coating and spray coating have been suitable only for particles greater than about 100 microns in diameter because of particle coalescence and adhesion (Gutcho, 1976; Nixon, 1976; Kondo, 1979; Deasy, 1984; Benita, 1996). For microparticles smaller than 100 microns, coating methods such as coacervation and *in situ* polymerization may be used (Gutcho, 1976; Nixon, 1976; Kondo, 1979; Deasy, 1984). However, these methods often require toxic organic solvents and/or surfactants. Our goal is to develop a new microencapsulation method in which the particle size can be controlled while avoiding the use of toxic organic solvents and/or surfactants. In addition, the method must minimize emissions to the environment.

Carbon dioxide (CO_2), which is inexpensive, essentially nontoxic, and nonflammable, has easily accessible critical conditions, such as, $T_c = 31°C$ and $P_c = 7.37$ MPa. In some cases, it can be utilized as an environmentally benign solvent substitute for hydrocarbons, chlorofluorocarbons, and other organics (McHugh and Krukonis, 1988; Hoefling et al., 1991; Goel and Beckman, 1994a,b; DeSimone et al., 1992; Johnston, 1994; Harrison et al., 1994; McFann et al., 1994). Supercritical (SC) fluids, including CO_2, have been used in a process called rapid expansion from supercritical solution (RESS) to produce a variety of organic and inorganic powders and fibers by many investigators (Matson et al., 1987a,b;

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Chang and Randolph, 1989; Mohamed et al., 1989; Ohgaki et al., 1990; Lele and Shine, 1992, 1994; Tom and Debenedetti, 1991; Tom et al., 1994; Mawson et al., 1995). In RESS, a supercritical fluid solution is expanded across a fine throttling device, such as a capillary or orifice nozzle, leading to rapid supersaturation and often the production of small particles. The type of phase behavior and the proximity to the phase boundaries during RESS may be expected to have a profound effect on the morphology, as has been shown in mechanistic studies (Lele and Shine, 1994; Mawson et al., 1995).

Polymers in general have very limited solubility in SC-CO_2 at temperatures below 80°C (Lele and Shine, 1992, 1994; Tom et al., 1994; O'Neill et al., 1998). However, solubilities can increase significantly at higher temperatures and very high pressures, often above 300 bar (Lele and Shine, 1994; Tom et al., 1991; Mawson et al., 1995). In previous studies, it has been shown that a useful property to describe the solvent strength of CO_2 (with regard to solubility behavior) is the polarizability per volume (McFann et al., 1994; Harrison et al., 1994). The molecular polarizability, a constant for a molecule, divided by the molar volume is a measure of the number and strength of van der Waals interactions in a given volume. This function has been compared for several fluids vs. pressure (McFann and Johnston, 1991). The van der Waals forces for CO_2 are considerably weaker than those of ethane and ethylene. Recently, it was found that polymer solubility in CO_2 at temperatures below 80°C increases with a decrease in the surface tension of the polymer, a pure component property that reflects its cohesive energy density (O'Neill et al., 1998). The trend in decreasing solubility with surface tension is observed in the following order for the series: poly(fluorooctyl acrylate), poly(dimethylsiloxane) > poly(propylene oxide) > poly(acrylates) > poly(ethylene oxide). Because CO_2 has a lower cohesive energy density than any of these polymers, polymer solubility tends to increase as the cohesive energy density of the polymer decreases.

RESS of polymer solutions in CO_2 has been limited by low polymer solubility. Low-molecular-weight oligomers of the semicrystalline polymers, for example, poly(1-butene), poly(ethylene succinate), poly(caprolactone), and poly(hexamethylene sebacate), have been sprayed to form particles (Lele and Shine, 1992). Tom et al. (1994) used CO_2 to produce submicron biodegradable polymer particles for encapsulated drug delivery systems, including poly(*L*-lactic acid) (*L*-PLA), poly(*DL*-lactic acid), and poly(glycolic acid). Initial solubility of the *L*-PLA in CO_2 was 0.14 wt. %, but rapidly dropped off to less than 0.05 wt. % once the lowest molecular-weight oligomers had been extracted. With the addition of 27 wt. % chlorodifluoromethane as a cosolvent, the solubility increased by only a modest amount (Tom et al., 1994).

The crystalline fluoropolymer, poly(1,1,2,2-tetrahydroperfluorodecylacrylate) is one of most soluble polymers in CO_2 (Mawson et al., 1995). Solutions from 0.5 to 2.0 wt. % of this polymer were sprayed by RESS to produce submicron- to several-micron-sized particles and fibers without the need for any cosolvent. Another spray technique is the UNICARB coating process to form films. A formulation, consisting of a polymeric resin, an oxygenated solvent, and CO_2 as a dissolved solute to serve as a diluent, is mixed (Nielson et al., 1990). Here, CO_2 is used to replace a hydrocarbon diluent,

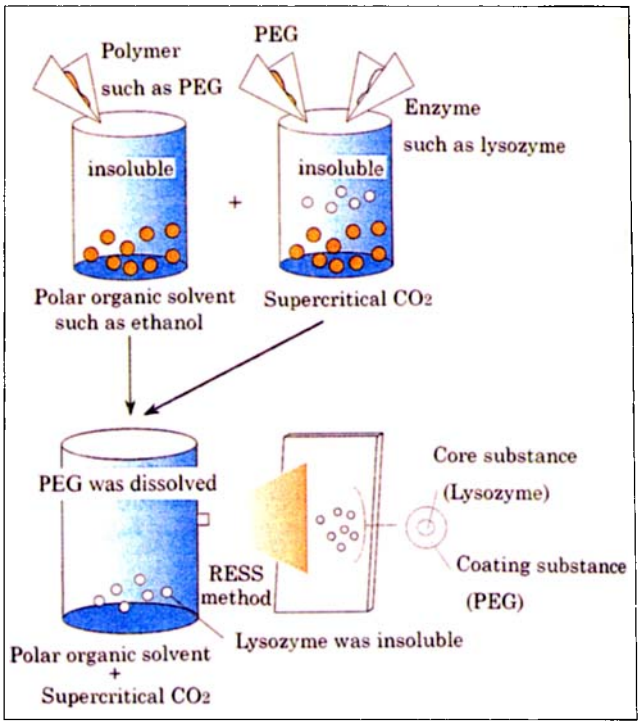

Figure 1. Principle of forming polymeric microcapsules using the rapid expansion of supercritical fluid.

and volatile organic carbon emissions may be reduced up to two-thirds. In our case we are interested in producing polymer microparticles containing protein rather than films.

The objective of this study is to utilize a new process—rapid expansion from supercritical solution with a nonsolvent (RESS-N) for the formation of nonagglomerated microparticles to produce micron-sized polymer particles containing proteins for controlled release applications. Figure 1 provides a conceptual framework of the process. Because the polymers used here are insoluble in CO_2 at our operating temperatures and pressures, several cosolvents were used, including ethanol, methanol, and 1-propanol. In pure form, these cosolvents are nonsolvents for the polymers; thus, they are only sparingly soluble in the polymer particles produced during expansion. Since the cosolvent does not swell the polymer product, it is not expected to cause agglomeration (Dixon et al., 1993). For low-molecular-weight solutes (Nakanishi and Asakura, 1977) and polymers (Mishima et al., 1996), it has been demonstrated that cosolvents that cause large increases of solubilities in CO_2 need not be good solvents for the solute. In this study, we wish to take advantage of the difference in solubilities of the protein and polymer in the CO_2–cosolvent mixture. The insoluble protein is suspended, whereas the polymer is soluble. During RESS, the polymer will precipitate and coat the protein microparticles.

The first part of this study explores the solubility behavior for polymer weight percent approaching 25 wt. % as a function of cosolvent concentration. The second part examines the performance of the RESS-N microcoating process in terms of particle morphology, particle-size distribution, and other properties.

Mishima et al. (1996) used a nonsolvent, such as ethanol, as a cosolvent to raise polymer solubility and avoid polymer agglomeration during RESS-N in order to make polymeric microparticles. This method did not require toxic organic solvents and/or surfactants. The produced powder did not agglomerate and could be used for powder coating applications without the need for undesirable organic solvents such as toluene or any surfactants.

In this method, it is not necessary to use complex spray equipment for the actual coating, as can be the case in the UNICARB process. The present study extends this method to examine microencapsulation of pharmaceuticals. The new RESS-N process is very different from the UNICARB process for several reasons. The CO_2 concentration is much higher, such that atomization leads to particles instead of a film. Another reason for particle rather than film formation is that the pure cosolvent is a nonsolvent for polymer. The organic solvent is a good solvent in the UNICARB process, such that expansion produces highly swollen particles that coalesce to produce a film. The use of a nonsolvent as a cosolvent to raise polymer solubility in the supercritical fluid phase and to avoid particle agglomeration is the key novel feature of this work.

Experimental Studies

Materials

In order to check the applicability of the RESS-N process, eight polymers were used as coating materials. The polymers were poly(ethylene glycol) (PEG) fractions (PEG4000; MW = 3,000, PEG6000; MW = 7,500, PEG20000; MW = 20,000), poly(methyl methacrylate) (PMMA; MW = 15,000), poly(Lactic acid) (PLA; MW = 5,000), poly(styrene) (PS; MW = 13,000), poly(*DL*-lactide-*co*-glycolide) (PGLA; MW = 5,000) and PEG–poly(propylene glycol) (PPG)–PEG triblock copolymer (MW = 13,000). PEG and PLA were purchased from Wako Pure Chemical Industries Ltd., PMMA was purchased from the Aldrich Company Ltd., and PGLA was purchased from Birmingham Polymer, Inc. The approximate weight fraction of glycolide in PGLA was 50%. PEG–PPG–PEG copolymer samples were donated by Daiichi Seiyaku Company Ltd. The approximate weight fraction of PEG in the PEG–PPG–PEG copolymer was 85%.

Lipase from *Pseudomonas Cepacia* (commercial name: lipase PS, specific activity of 30 olive oil units/mg) was kindly supplied by the Amano Pharmaceutical Company, Ltd. Lysozyme from chicken egg white was purchased from the Aldrich Company Ltd. Methanol, ethanol, 1-propanol, acetone, and toluene (Wako Pure Chemical Industries Ltd.) had purities of more than 99.9%. All the alcohols were kept over activated molecular sieves (3A) and used without further purification. The purity of carbon dioxide (CO_2) (Fukuoka Sanso Company) was greater than 99.9%. A 0.5 wt. % aqueous solution of ruthenium tetroxide (RuO_4) was used to stain lysozyme and lipase (Electron Microscopy Sciences Company).

Procedure

The solubilities of the polymers in mixtures of SC-CO_2 and cosolvent were determined by observing the cloud point visually with the experimental apparatus as described previously (Mishima et al., 1998). In this work, the cloud point is defined as the point where the solution turns slightly translucent. A 500-cm^3-high pressure cell (AKICO Company, model SCE-500), equipped with sapphire windows (10×140 mm) for observation of the phase behavior, was placed in a thermostated air chamber. Known amounts of CO_2 and cosolvent were charged into the cell by using a CO_2 pump (AKICO Company, NRXM-90-G5M) and a sample feed pump (AKICO Company, MDP-300-2). CO_2 was charged by actual weight using a CO_2 pump equipped by measuring the total flow at a controlled temperature. The actual weight of CO_2 was calibrated by a wet-gas meter. The mixture of CO_2, cosolvent, and polymer was stirred by an agitator rotating at 150 rpm for 2 h and then kept for more than 48 h without agitation at 308.15 K. A certain amount of cosolvent was added into the cell until a cloud point was detected. Near the cloud point, cosolvent was carefully charged at more than 30-min intervals. After observing the cloud point, the mixture was stirred for more than 1 h and then kept for more than 12 h without agitation to confirm that two phases remained. The dispersed phase coalesces into two distinct phase in this 12-h period.

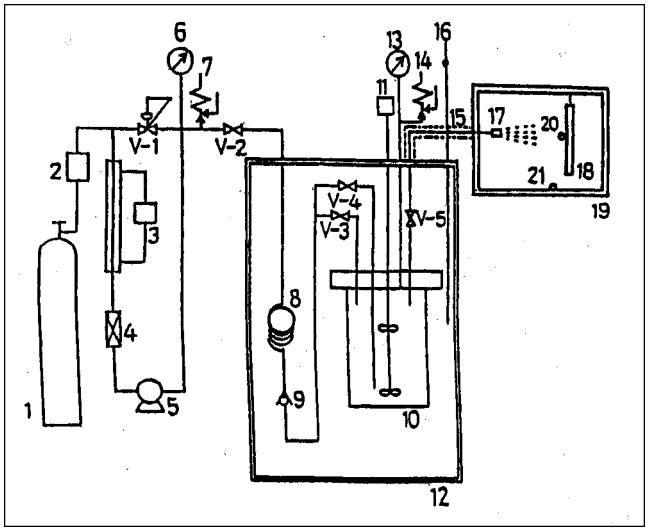

Figure 2. Experimental apparatus for making the polymeric microcapsules using the rapid expansion of supercritical fluid.

1. Gas cylinder, 2. dryer, 3. cooling unit, 4, filter, 5. pump, 6. pressure gauge, 7. safety valve, 8. preheater, 9. stopper, 10. extraction cell, 11. motor, 12. water bath, 13. pressure gauge, 14. safety valve, 15. heating unit, 16. thermometer, 17. expansion nozzle, 18. plate, 19. air bath, 20, 21. samping locations, V-1. back pressure regulator, V-2–V-5. shut-off valve.

Figure 2 presents the experimental apparatus for making polymeric microcapsules. In this apparatus, the CO_2 and cosolvent charging parts were similar to the apparatus for polymer solubility measurements. The high-pressure cell (AKICO Company, SCV500A), about 500 cm^3 in volume, was equipped with a sapphire window (10 mm in diameter). The system pressure was controlled by a back pressure regulator (V-1) (Tescom Company, model 26-1721-24, accurate to 0.1 MPa) and monitored by a digital pressure gauge (Shinwa electron-

ics Company, model DD-501, accuracy ±0.3%). Temperature was kept at the desired value within ±0.1 K with a water bath. Known amounts of the polymer and cosolvent were placed in the high-pressure cell. SC-CO_2 was pumped through a preheater, 1/8-in. stainless-steel tube, 3 m long, and then to the high-pressure cell. This mixture was stirred by an agitator rotating at 200 rpm for about 4 h and then kept for more than 1 h without agitation. Prior to the expansion of the polymer solution, it was confirmed that all of the feed polymer was dissolved and the proteins were dispersed homogeneously by observation through the sapphire window. The dissolved polymer/dispersed protein mixture was sprayed through a capillary nozzle (Tungsten Carbide Company, model 500017-TC) 0.28 mm in diameter, toward a target plate (30 cm × 60 cm × 1 mm, aluminum plate) for a short time (less than 3 s) by opening a valve (V-5) placed before the nozzle. The nozzle was maintained at 313.15 K with an electric heater. The target plate was placed in a chamber (70 cm × 80 cm × 80 cm) under atmospheric pressure. The distance from the tip of the nozzle to the plate varied from 10 to 50 cm. After expansion, precipitation of the polymer led to microspheres. The collection chamber was completely closed, and the gas was vented after the sedimentation of particles through a filter. Yields of collected polymeric microcapsules depend on the solubility of the polymer in the mixtures of SC-CO_2 and cosolvent. When the target polymer, such as PEG6000, was completely dissolved in the mixtures of SC-CO_2 and ethanol, about 3.5 g of microcapsules were formed in approximately 3 s of spraying at our experimental condition.

Characterization

The microparticles were analyzed by an optical microscope (Olympus, BH-2), transmission electron microscopy (TEM, JEOL 200CX), and scanning electron microscopy (SEM, Hitachi S-2100B). For sample preparation of SEM, collected polymeric particles were mounted on a small glass plate and sputter-coated with silver palladium. For preparing samples for TEM, the microparticles were placed onto a collodion film. The particles on the film were exposed to ruthenium tetraoxide (RuO_4) vapor at room temperature for 30 min in the presence of 0.5 wt. % aqueous RuO_4 solution. The TEM was operated at 120 kV. For preparing samples for the optical microscope, RuO_4 solution was used to stain the protein. The particle-size and particle-size distribution and coating thickness of the microcapsules were determined by a laser-diffraction particle-size analyzer (SALD, Shimadzu Company Ltd., SALD-2000) and an optical microscope (Olympus, BH-2). To analyze the particle size and distribution, the product microcapsules were dispersed in ethanol or water with surfactant. Physical properties of the polymeric microparticles were investigated by high performance liquid chromatography (HPLC, Tosoh Company Ltd., HPLC-8000) and differential scanning calorimetry (DSC, Seiko Instruments Inc., DSC-120).

Results and Discussion

Phase behavior

The solubilities of PEG6000 in a mixture of SC-CO_2 and ethanol at 308 K and 16 MPa are shown in Figure 3. Without

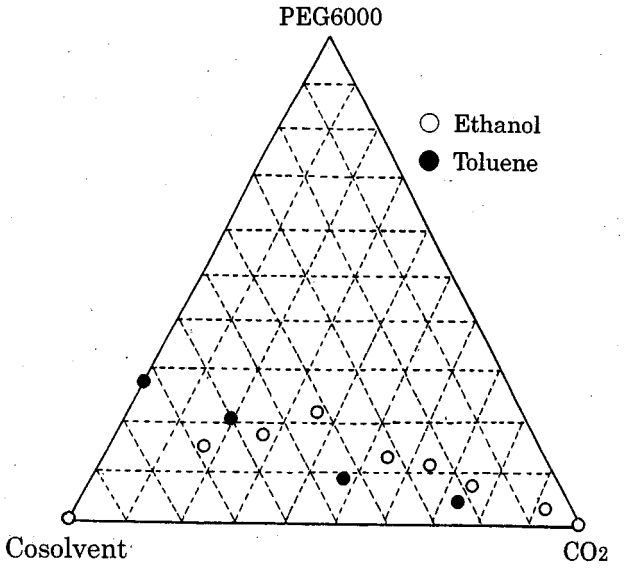

Figure 3. Solubility of PEG6000 in a mixture of CO_2 and cosolvent at 16 MPa and 308 K.

polymer, the mixtures of CO_2 and ethanol form a single supercritical fluid phase at this temperature and pressure according to vapor–liquid equilibrium data in the literature (Jennings et al., 1991). The dissolution of the polymer is strongly dependent upon the concentration of the cosolvent. For the binary system without cosolvent, Daneshvar et al. (1990) reported that the solubility of PEG1000 (MW = 1,000) in SC-CO_2 is about 0.1 wt. % at 323 K. Our solubility of PEG6000 at 308 K and 16 MPa is in general agreement. However, with the addition of about 25 wt. % ethanol (polymer-free basis), the solubility of PEG reaches about 8.0 wt. %. Even higher solubilities may be achieved with toluene as a cosolvent, which is not surprising since pure toluene is such a good solvent for PEG, as shown in Figure 3.

It is interesting that ethanol is such a good cosolvent despite the fact it is a nonsolvent for the polymer. Solubilities of polymer in the pure cosolvent are shown in Table 1. Solubilities of PEG6000 are very low. High-molecular-weight PEG is insoluble in ethanol. Even with the addition of 25 wt. % CO_2 to ethanol, the solubility is not so high. The maximum solubility is achieved at about 50 wt. % ethanol (polymer free). Similar effects were observed for other small alcohols, such as methanol and 1-propanol. Similar results were also obtained for other polymers including PMMA, PEG, PEG–PPG–PEG, as shown in Table 1. The solubilities of PEGs in a mixture of SC-CO_2 and ethanol increase with a decrease in the molecular weight of PEG. The solubility of PEG6000 actually decreased with the addition of CO_2 to acetone or toluene. For some other polymers—PS and PLA—the addition of ethanol as a cosolvent did not raise the solubility significantly. Compared with a conventional ternary system containing polymer solution such as PS in liquid mixtures of acetone and *n*-alkanes, the phase separation of a polymer–CO_2–cosolvent system is extremely fast. Phase-separation rate by pressure quench is faster than that by temperature quench or compositional quenching with a nonsolvent.

Table 1. Solubilities of Polymers in Ethanol and Mixtures of Supercritical Carbon Dioxide and Ethanol at 308 K and 16 MPa

Polymer	Solubilities of Polymer [wt. %] Measured: In Pure Solvent (Pure Ethanol)	Measured: In 35 wt. % Cosolvent Solution of SC-CO_2, w_p^{exp} (Polymer-Free Basis)	Calculated*: Mean Value of Solubilities in 35 wt. % Cosolvent Solution of SC-CO_2, w_p^{cal}	Cosolvency Effect** Δw_p [wt. %]
PEG4000	2.50	12.9	0.88	12.0
PEG6000	0.42	13.2	0.15	13.1
	0.50†	10.8†	0.18†	10.6
	0.60††	0.54††	0.22††	0.32
	5.21‡	1.65‡	0.82‡	−0.17
	26.8‡‡	6.67‡‡	9.39‡‡	−2.7
PEG20000	0.23	3.13	0.09	3.0
PEG−PPG−PEG	32.6	14.9	11.4	3.5
PMMA	0.0	1.20	0.0	1.2
PS	0.0	0.0	0.0	0.0
	62.8‡‡	15.8‡‡	21.9‡‡	−6.1
PLA	16.8	3.5	5.89	−2.4

*Calculated as $0.35 \times$ solubility (in solvent) $+ 0.65 \times$ solubility (in SC-CO_2).
**Cosolvency effect, $\Delta w_p = w_p^{exp} - w_p^{cal}$.
†Solvent or cosolvent is methanol.
††Solvent or cosolvent is 1-propanol.
‡Solvbent or cosolvent is acetone.
‡‡Solvent or cosolvent is toluene.

Lysozyme and lipase used in this work are insoluble in mixtures of SC-CO_2 and ethanol for ethanol weight percent from 0 to 100. The low-molecular-weight alcohols are nonsolvents for these substances.

Evaluation of microencapsulation

A SEM, TEM, and optical microphotograph of a polymeric microcapsule containing lipase or lysozyme produced by RESS and collected on the surface of a target plate 30 cm from the nozzle is shown in Figure 4. The preexpansion pressure was 20 MPa and the temperature was 308 K. Ethanol was used as a cosolvent at a concentration of 27.1 wt. %. The composition ratio of lipase to PEG6000 in the feed was 1.4.

In the SEM photograph, the particles did not adhere to each other, because the ethanol was volatile and a nonsolvent for the polymer. The globular particles had a fairly monodisperse particle-size distribution. On the other hand, microcapsules formed with toluene or acetone as cosolvents agglomerated as these solvents dissolved in the polymer after RESS.

In the TEM photograph and optical microphotograph, the lipase phase, stained by the 0.5 wt. % aqueous solution of RuO_4, is shown by the dark area. It is completely covered by the PEG phase, shown as the light nonstained area. In the microcapsule, the volume ratio of the lipase phase to the PEG phase is almost identical to the feed composition ratio of lipase to PEG. The lipase is coated thoroughly by PEG. The collodion film under the microcapsule was also slightly light, and the boundary between the PEG phase in the microcapsule and the collodion film is clearly observed. It is likely that the insoluble lipase particles in suspension in the expanding jet served as a nucleating agent for the precipitating polymer, aiding the very thorough encapsulation. When the composition ratio of lipase to PEG6000 in the feed was 1.4, the volume ratio of the dispersed lipase particles to the volume of PEG continuous phase in the microcapsules approximately agreed with the feed composition, as shown in Figure 4. The volume ratio was determined by visual observation such as TEM and optical microphotograph.

Similar results were obtained for lysozyme microencapsulated with PEG6000, as shown in Figure 4. The protein stained by aqueous RuO_4 solution is slightly darker than the unstained PEG. Again, the protein is coated thoroughly by PEG6000. The sizes of the microcapsules of lipase are smaller than those of lysozyme. The surface of the nearly spherical microcapsules of lipase coated with PEGs is smooth. The surface of the microcapsules of lysozyme coated with PEGs is rougher than that of lipase. This tendency for less smooth surfaces of microcapsules is even more evident for copolymers, such as PEG−PPG−PEG, relative to PEG. The smoother surface for lipase-containing microcapsules may be due to the more hydrophilic surface of lipase relative to lysozyme. To obtain information about hydrophobic and hydrophilic properties of protein surface, partition coefficients of them in the PEG6000−dipotassium hydrogenphosphate (K_2HPO_4)−water aqueous two-phase system (Mishima et al., 1995) were determined at 298 K. An aqueous two-phase system is expected to provide a powerful method for the characterization of biomolecules (Haynes et al., 1993; Zaslavsky, 1995), because the surface properties of target substance such as hydrophobicity can be analyzed under the mild condition. Based on the studies of biomolecule partitioning in an aqueous two-phase system by Zaslavsky (1995), it can be concluded that the hydrophobic effect, such as partition coefficients, is the determining factor. In general, partition coefficients increase with an increase in the surface hydrophobicity of a partitioning substance in the aqueous two-phase system.

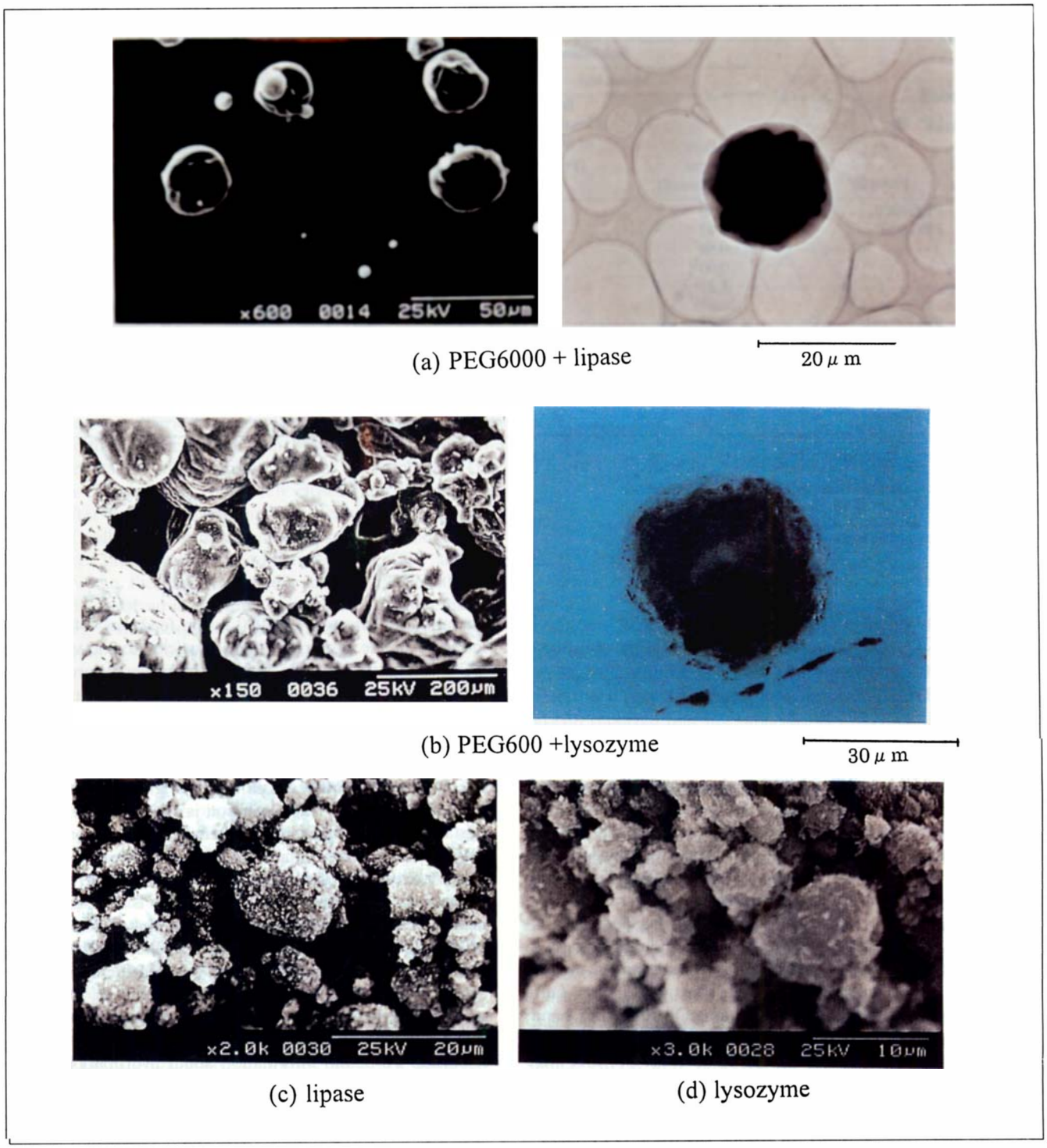

Figure 4. TEM, SEM, and optical microphotograph of a polymeric microcapsule of lipase and lysozyme by polymer produced by RESS under the preexpansion conditions.

Temperature—308 K; pressure—20 MPa; core substance—(3.0 wt. %); cosolvent–ethanol (27.1 wt. %); coating material—PEG6000 (2.2 wt. %).

For partitioning of proteins, 14.9 wt.% PEG6000/9.2 wt. % K_2HPO_4 systems were selected (Mishima et al., 1995). Partition coefficients of lysozyme and lipase in the PEG6000(1)–K_2HPO_4(2)–water(3) system are 30.5 and 5.3, respectively. As the results of partitioning experiments, partition coefficients of lysozyme are higher than that of lipase. It may be considered that hydrophobicity of the lysozyme surface is larger than that of the lipase surface.

Table 2. Observed Polymer Morphology Produced by Rapid Expansion of Supercritical Carbon Dioxide Solution Containing Cosolvent at 308 K and 20 MPa

No.	Core Material	Coating Material	Cosolvent	Core Substance [wt. %]	Cosolvent [wt. %]	Polymer [wt. %]	CO_2 [wt. %]	Primary Particle Dia. [μm]	Geometric Standard Deviation†
1	Lipase	PEG6000	Ethanol	0.3	27.1	2.2	70.4	13	1.52
2	Lipase	PEG6000	Methanol	0.3	27.1	2.2	70.4	17	1.61
3	Lipase	PEG6000	Propanol	0.3	27.1	2.2	70.4	18	1.63
4	Lipase	PEG6000	Acetone	0.3	27.1	2.2	70.4	—	—
5	Lipase	PEG6000	Toluene	0.3	27.1	2.2	70.4	—	—
6	Lipase	PEG6000	Ethanol	0.1	27.1	2.2	70.6	9	1.52
7	Lipase	PEG6000	Ethanol	2.0	27.1	2.2	68.7	8	1.52
8	Lipase	PEG6000	Ethanol	3.0	27.1	2.2	67.7	11	1.52
9	Lipase	PEG6000	Ethanol	0.3	8.9	20.4	70.4	62	1.75
10	Lipase	PEG6000	Ethanol	0.3	17.8	11.5	70.4	33	1.65
11	Lipase	PEG6000	Ethanol	0.3	20.7	8.6	70.4	22	1.61
12	Lipase	PEG6000	Ethanol	0.3	28.1	1.2	70.4	11	1.60
13	Lipase	PEG6000	Ethanol	0.3	13.6	2.2	83.9	15	1.52
14	Lipase	PEG6000	Ethanol	0.3	40.7	2.2	56.8	18	1.63
15	Lipase	PEG6000	Ethanol	0.3	54.2	2.2	43.3	20	1.69
16	Lipase	PEG 4000	Ethanol	0.3	27.1	2.2	70.4	9	1.52
17	Lipase	PEG 20000	Ethahol	0.3	27.1	2.2	70.4	18	1.58
18	Lipase	PLA	Ethanol	0.3	27.1	2.2	70.4	11	1.67
19	Lipase	PMMA	Ethanol	0.3	27.1	2.2	70.4	12	1.75
20	Lipase	PEG–PPG–PEG	Ethanol	0.3	27.1	2.2	70.4	18	1.54
21	Lipase	PGLA	Ethanol	0.3	27.1	2.2	70.4	12	1.64
22*	Lipase	PS	Ethanol	0.3	27.1	2.2	70.4	—	—
23**	Lipase	PS	Toluene	0.3	27.1	2.2	70.4	—	—
24**	Lipase	PS	Acetone	0.3	27.1	2.2	70.4	—	—
25	Lysozyme	PEG 6000	Ethanol	0.3	27.1	2.2	70.4	30	1.69
26	Lysozyme	PLA	Ethanol	0.3	27.1	2.2	70.4	45	1.63
27	Lysozyme	PMMA	Ethanol	0.3	27.1	2.2	70.4	30	1.70
28	Lysozyme	PEG–PPG–PEG	Ethanol	0.3	27.1	2.2	70.4	37	1.71
29	Lysozyme	PGLA	Ethanol	0.3	27.1	2.2	70.4	12	1.65
30*	Lysozyme	PS	Ethanol	0.3	27.1	2.2	70.4	—	—
31**	Lysozyme	PS	Toluene	0.3	27.1	2.2	70.4	—	—
32	Lipase	—	Ethanol	0.3	27.1	0.0	72.6	6	1.51
33	Lysozyme	—	Ethanol	0.3	27.1	0.0	72.6	7	1.52

*Samples are not obtained.
**Film is obtained.
†Standard deviation, $\ln \sigma = \sqrt{(1/\Sigma\, n)\left(\Sigma\, n(\ln D_p - \ln \tilde{D})^2\right)}$, where n is the number of particle, D_p is the particle diameter, $\tilde{D}$ is the primary particle diameter.

Effect of various factors on particle size

The effects of various factors, the preexpansion pressure, temperature, feed compositions, injection distance and polymer molecular weight on the mean particle diameter, and the standard deviation of particle diameter are discussed as shown in Table 2. The particles were analyzed by a laser-diffraction particle-size analyzer. The particle-size distribution of PEG6000 microparticles obtained from a preexpansion condition of 20 MPa and 308 K is relatively narrow, as shown in Figure 5. In this case, mean particle diameter and standard deviation are 22 micron and 1.61, respectively.

The effect of PEG6000 feed concentration on the mean particle diameter of microcapsules containing lipase is shown in Figure 6. The mean particle diameter of the microcapsules, or likewise the coating thickness, increases with an increase in PEG6000 concentration. In the case of lipase, one lipase particle is coated with PEG6000, as shown in Figure 4. The size of the lipase particle in the core is almost the same as that of the lipase particle fed to the system in this experimental condition. The method for the determination of particle sizes of lipase and lysozyme without polymer are given as follows. Lipase or lysozyme is dispersed in mixtures of SC-CO_2 and ethanol, and the dispersed proteins were sprayed. Particle sizes of the resulting protein particles were determined by a laser-differential particle-size analyzer. Photomicrographs and particle sizes of pure lysozyme/ethanol/CO_2 and lipase/ethanol/CO_2 particles produced by RESS without any polymer are shown in Figure 4 and Table 2. The protein particles did not adhere to each other. The thickness of the coating increases with an increase in the polymer concentration for both lipase and lysozyme, as shown Figure 7. Furthermore, the standard deviation in the particle diameter increases with an increase with the feed composition of polymer. A key result of this study is the ability to control the thickness and particle-size distribution of the microcapsules with the feed concentration of the polymer.

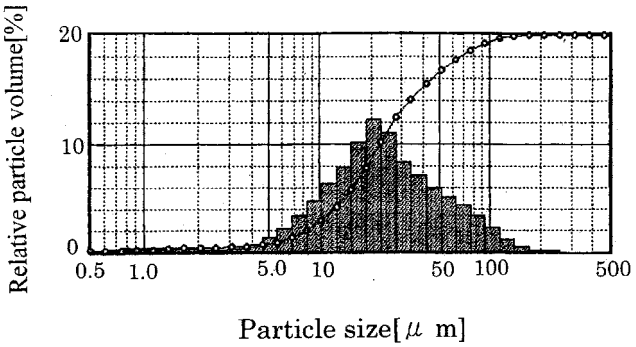

Figure 5. Particle size distribution of microcapsules with lipase.

Temparature: 308 K, pressure: 20 MPa, core substance: lipase (0.3 wt. %), cosolvent: ethanol (20.7 wt. %), coating material: PEG6000 (8.6 wt. %).

The mean particle diameter of PEG particles is almost constant for several conditions, including the preexpansion pressure, temperature, molecular weight of polymer, and injection distance. The standard deviation of the particle diameter decreases slightly with an increase in the preexpansion pressure and injection distance. A linear relationship is observed between the standard deviation and these factors. For each of these variables, except the polymer feed composition, the particle size of the polymeric microcapsules is almost constant in these experimental conditions. Therefore, the polymer feed composition is a key variable for controlling the particle size of the microcapsules.

Similar results were obtained for the other polymers in this study. Various physical properties of the microspheres were compared with those of the source polymers. Glass transition temperatures of the feed polymer and polymeric microspheres were measured by DSC and found to be the same. Consequently, little solvent remained in the polymer or it would have depressed the glass transition temperature. To determine the amount of residual solvent in the microcapsules, the latter were dried at about 373 K and the change in mass was measured. The amount of residual ethanol in the microcapsules was less than 1 wt. %. The HPLC retention times of the feed and product polymer were identical, indicating that the molecular weight did not change. This result suggests that the CO_2–cosolvent mixture dissolved all of the polymer rather than a fraction of the molecular weight distribution. For reasons given earlier, it is clear that physical properties such as molecular weight and glass transition temperature of the polymer materials have not been varied through the RESS processing.

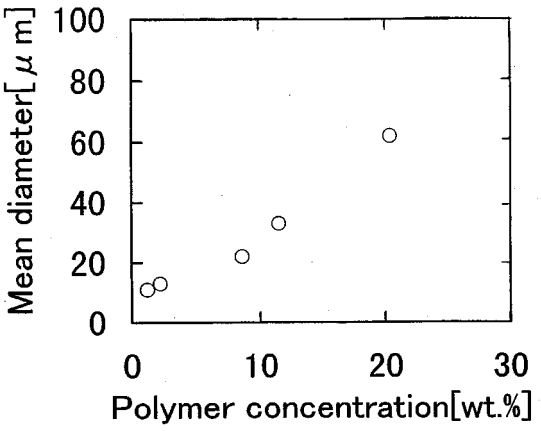

Figure 6. Influence of polymer concentration on particle-size distribution of PEG6000 microcapsules with lipase.

Temperature—308 K; pressure—20 MPa; cosolvent—ethanol; coating material—PEG6000; core substance—lipase.

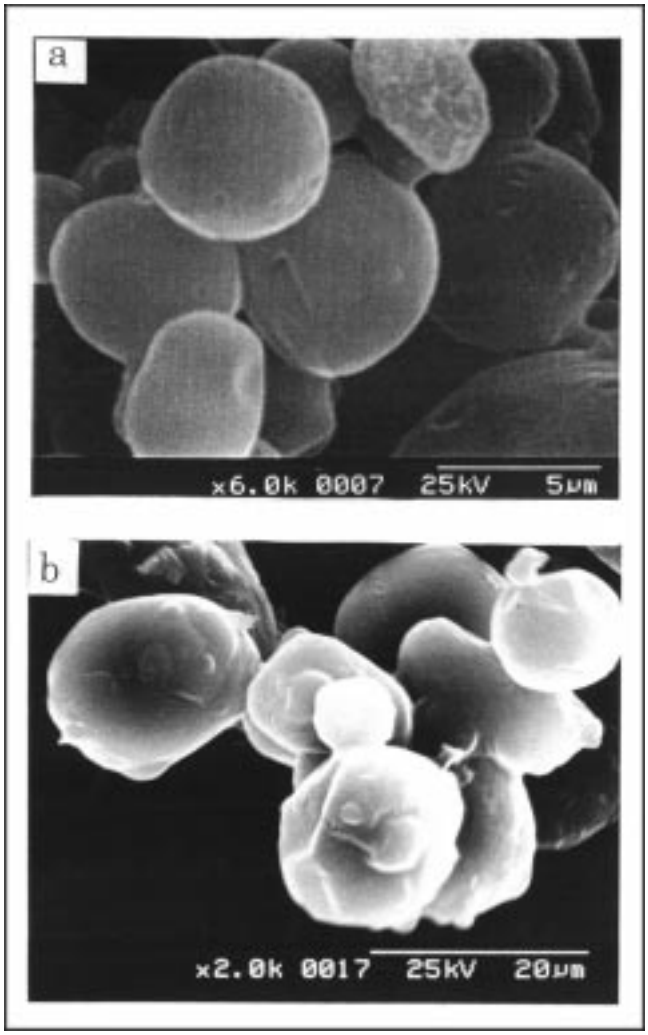

Figure 7. SEM photograph of PEG6000 microcapsules of lipase by polymer produced by RESS under the preexpansion conditions.

Temperature—308 K; pressure—20 MPa; core substance: lipase; cosolvent: ethanol; (a) polymer feed compositions 2.2 wt. %, (b) polymer feed compositions 11.5 wt. %.

Conclusion

RESS with a cosolvent has been utilized to produce polymeric microcapsules of lysozyme and lipase without agglomeration. The cosolvent, ethanol, is far less toxic than most

organic solvents, and no surfactant is required. It was found that the solubilities of the polymers—PEGs (PEG4000, PEG6000, and PEG20,000), PMMA, and PEG–PPG–PEG triblock copolymer—increase considerably by adding a small amount of methanol, ethanol, or propanol. An interesting feature of this process is that these alcohols, which in pure form are nonsolvents for PEG, can become good cosolvents when mixed with CO_2. Because ethanol is a nonsolvent for the polymers, the polymeric microparticles produced by rapid expansion from supercritical solution did not agglomerate. The globular microparticles were reasonably monodisperse in size.

The particle-size distribution of microcapsules could be controlled by changing the polymer feed composition. It changed very little with the preexpansion pressure, temperature, molecular weight of polymer, and injection distance.

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