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A new microencapsulation method using an ultrasonic atomizer based on interfacial solvent exchange

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Abstract

Reservoir-type microcapsules were produced in a mild and highly efficient manner using a coaxial ultrasonic atomizer. To understand the microencapsulation mechanism, the atomizer was operated in different modes. The results suggested the reservoir-type microcapsules were formed by midair collision of the microdrops of the two component liquids consisting of a polymer solution and an aqueous solution. The encapsulation efficiency and the distribution of the microdrops captured in midair were dependent on the ratio of the flow rates of the two liquids, which suggested that the collision involved multiple microdrops. This method allowed lysozyme to be encapsulated without loss of functional integrity and to be released with near zero-order kinetics for over 50 days. The ultrasonic atomizer provided a new method of preparing reservoir-type microcapsules.
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Keywords: Solvent exchange; Microencapsulation; Ultrasonic atomizer; Protein delivery; Reservoir-type microcapsule

1. Introduction

Proteins and peptides are excellent therapeutic compounds which have highly specific and potent biological functions. Advances in biotechnology have produced a number of recombinant protein drugs in large quantities, and more new protein drugs will be developed as a result of the information obtained from the genome project. The delivery of protein drugs, however, has been limited to parenteral administration, and frequent injection is required due to their short half lives in the blood. In this regard, long-term protein delivery ranging from weeks to months is highly attractive for patient compliance and convenience.

Microencapsulation technologies have advanced significantly during the past few decades, leading to many successful commercial products. Since the early promise of sustained protein delivery [1], research on protein microencapsulation has been increased exponentially. Microencapsulation of protein drugs, how-

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ever, still remains one of the most challenging subjects in the controlled drug delivery area. Due to the high sensitivity of proteins to harsh conditions that can occur during the microencapsulation process, maintaining the functional integrity of the encapsulated protein drugs is not easy [2–6].

Microparticles loaded with therapeutic proteins have been typically produced by the double emulsion–solvent extraction/evaporation method [7,8]. Proteins microencapsulated by those methods frequently become unstable and easily denatured upon prolonged exposure to stressful conditions, such as a large water–organic solvent interfacial area, high shear stress, acidic and hydrophobic microenvironments, or elevated temperature [3–6]. It is not surprising to see only a limited number of formulations on the market despite more than three decades of research on the protein microencapsulation. A number of strategies have been developed for improving protein microencapsulation, and they include variations in formulation parameters, development of different polymeric systems, and modifications of the existing encapsulation methods.

For example, acidification of the microenvironment by degrading polymers was counteracted by coencapsulating basic excipients along with active ingredients [9,10]. Surface-eroding polymers, such as polyanhydride and poly(ortho esters), were suggested as alternatives to typical poly(lactic-co-glycolic acid), which accumulates acidic degradation products within the microparticles [11]. Recently, a new way of microencapsulation that utilized multiple concentric nozzles to generate double-walled microspheres was developed adding flexibility in combined use of the different polymers [12]. In an attempt to reduce the exposure of encapsulated drugs to the deleterious environments, such as water–organic solvent interfacial area, anhydrous microencapsulation processes were demonstrated with promising results [5,13]. The hydrophobic interaction between the protein and polymers has been minimized by encapsulating proteins within hydrophilic excipients prior to polymeric encapsulation [14,15], including adsorption competitors within the microcapsules [16], and encapsulating the protein in hollow microcapsules [17]. Although advances have been made in preserving protein stability and achieving desired release profiles, the problems associated with high shear

stress and complexity of the emulsion-based encapsulation methods are virtually untouched and remain to be improved. There has been a great need for developing new microencapsulation techniques that can encapsulate proteins under simple and mild conditions.

A new microencapsulation method based on interfacial solvent exchange was previously reported by our group [18,19]. In this method, reservoir-type microcapsules were generated using a dual micro-dispenser system that involves two ink-jet nozzles. Series of drops of polymer solution and aqueous drug solution are separately produced using ink-jet nozzles, and then they are induced to collide in the air. Following the collision, the two liquid phases are separated as a core and a membrane within the merged microdrops due to the surface tension difference of the two liquids. Recently, it was found that a coaxial ultrasonic atomizer can also be utilized to generate reservoir-type microcapsules under the similar principle, yet, in a simple, mild, and highly efficient manner [20]. The objective of this study is to demonstrate the new microencapsulation method using lysozyme as a model protein and to understand the microencapsulation mechanism.

2. Materials and methods

2.1. Materials

Poly(lactic-co-glycolic acid) [PLGA; 50/50 lactic acid/glycolic acid; nominal inherent viscosity=0.59 dl/g, weight average molar mass (Mw)=44 kD] were purchased from Birmingham Polymers (Lot: D02022). PLGA (50/50 lactic acid/glycolic acid; nominal inherent viscosity=0.15 dl/g, Mw=13 kD) was obtained from Alkermes (Lot: 1257–532). Polyvinyl alcohol (PVA; 98.0–98.8% hydrolyzed, Mw ~195,000) was obtained from Fluka. Sodium dodecyl sulfate (SDS) and ethyl acetate (EA) were obtained from Mallinckrodt Baker. Coomassie brilliant blue R-250 was purchased from Bio-Rad. DiO (3,3′-dioctadecyloxacarbocyanine perchlorate), fluorescein isothiocyanate labeled dextran (FITC-dextran, Mw ~42,000), Nile Red, and lysozyme were purchased from Sigma. The bicinchoninic acid (BCA) and microBCA assay agents were obtained from Pierce.

2.2. Preparation of microcapsules

Fig. 1 describes a coaxial ultrasonic atomizer system. PLGA solution (2–5%) in ethyl acetate (PLGA-EA) and an aqueous solution containing optional solutes were separately fed into an ultrasonic atomizer through coaxial cables (Sono-Tek, Milton, NY). Lysozyme (3%) was included in the aqueous solution for the release study. Coomassie brilliant blue R-250 was added to the aqueous solution to visualize aqueous cores. For confocal microscopy, FITC-dextran and Nile Red were added to the aqueous solution and the PLGA-EA solution, respectively. The two solutions were delivered using syringe pumps at controlled flow rates. Microcapsules, which were produced upon the onset of atomizer vibration, were collected in a water bath containing 200 ml of 0.5% PVA. Alternatively, two separate single-nozzle atomizers were used to deliver the two component liquids and were aligned to induce collision of two groups of liquid microdrops. After 2 h in the PVA bath, the microcapsules were collected using a centrifuge, washed with distilled water.

2.3. Confocal Laser Scanning Microscopy (CLSM)

Distribution of the PLGA solution and the encapsulated solution in the microcapsules was examined using a Bio-Rad MRC-1024 laser scanning confocal imaging system equipped with a krypton/argon laser and a Nikon Diaphot 300 inverted microscope. All confocal fluorescence pictures were taken with a 20× objective lens and excitation at 488 and 568 nm.
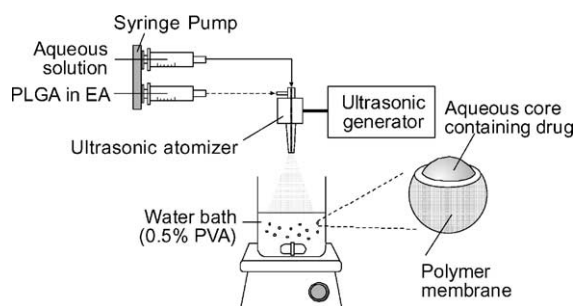

Fig. 1. Schematic description of the microencapsulation system using a coaxial ultrasonic atomizer. In the present example, Liquid 1 is an aqueous solution, and Liquid 2 is a PLGA-EA solution.

2.4. Determination of loading and encapsulation efficiency

Protein content in the microcapsules was determined using the dimethyl sulfoxide (DMSO)/sodium hydroxide (NaOH)/sodium dodecyl sulfate (SDS) method modified from the literature [21]. Less than 10 mg of microcapsules was precisely weighed and put into a microcentrifuge tube. DMSO (0.2 ml) was added into the tube to dissolve the polymer portion of the microcapsules. Then, 0.8 ml of a 0.05N NaOH solution containing 0.5% SDS was added to the tube and gently mixed. Following sonication for 90 min at 25 °C, samples were centrifuged at 10,000 rpm for 5 min. Supernatants were analyzed using the BCA assay method.

2.5. In vitro release profile

Collected microcapsules were further washed with 10 mM HEPES buffer (pH 7.4, ionic strength=150 mM) containing 0.02% sodium azide and then suspended in 3 ml of fresh HEPES buffer for a release study. The tubes were then stored in a 37 °C incubator. At selected time intervals, 1 ml of the release buffer was withdrawn and replaced by fresh HEPES buffer. The protein content in the sampled release medium was determined using the BCA or microBCA protein assay method. The amount of protein released in each interval was divided by the actual amount encapsulated in the microcapsules. The percentage of cumulative released protein was defined as the sum of the percentage of released protein by the specified time point.

2.6. Polymer degradation profile

Weight average molar mass (Mw) of the original PLGA polymers, as well as those in the degrading microcapsules, was determined by gel permeation chromatography. Blank microcapsules were incubated in the same release condition as that of in vitro release test described above, sampled at timed intervals, freeze-dried, and dissolved in tetrahydrofuran. Each sample of 20 µl was injected into a PLgel 5 µm mixed-D column (Polymer Laboratories, Amherst, MA). Tetrahydrofuran was used as a mobile phase at 1 ml/min at 35 °C. The weight average molar mass was

calculated from a calibration curve using a series of polystyrene standards.

2.7. Lysozyme biological activity assay

The biological activity of lysozyme in the release medium was determined by measuring turbidity change of a *Microcuccus lysodeikticus* bacterial cell suspension, following a reported method with slight modification [22]. A 0.1 ml aliquot of standard lysozyme solutions with known concentrations and the release samples were added to a cuvette containing 2.9 ml of 0.25 mg/ml *M. lysodeikticus* suspension in HEPES buffer (pH 7.4). Changes in the turbidity of the cell suspensions were monitored using a Beckman spectrophotometer. Lysozyme solutions in the range of 25 to 500 µg/ml induced a zero-order decrease in the absorbance of the substrate suspensions at 450 nm over at least 1 min. Linear changes in the absorbances were recorded over the initial 1–2.5 min to calculate the rate constant (k, min^{-1}). The percentage of retained biological activity (RBA) of lysozyme was calculated by percent RBA=$k_{apparent}$/$k_{theoretical}$. Here, $k_{apparent}$ is the observed rate constant, and $k_{theoretical}$ is the expected value for the release sample concentrations measured by the BCA or microBCA assay.

2.8. Examination of encapsulation pattern

Dependence of the encapsulation pattern on the flow rate ratio of the two liquids was examined as follows. A 2% PLGA-EA solution labeled with Nile Red and 0.2% sodium alginate solution containing FITC-dextran were fed into the coaxial ultrasonic atomizer using syringe pumps at controlled flow rates. The Q_{Pol} was varied from 0.25 to 1.5 ml/min, while the Q_{Aq} was fixed at 0.25 ml/min. Microdrops emerging from the ultrasonic atomizer were captured midair on a glass plate and observed using a Nikon Eclipse E1000 fluorescence microscope.

2.9. Microencapsulation using an underwater system

The coaxial ultrasonic atomizer was operated as submerged under water. A 2% PLGA-EA solution was allowed to flow through the outer nozzle at 1.5 ml/min. Through the inner nozzle, either an aqueous solution containing 0.2% sodium alginate or a non-

aqueous liquid, *n*-decane flowed at 0.25 ml/min. For confocal microscopy, Nile Red was added to the PLGA solution, while FITC-dextran and DiO were added to the alginate solution and *n*-decane, respectively. With the front horn of the atomizer immersed in a 0.5% PVA bath, the atomizer was vibrated at 60 kHz. A part of the water bath was sampled immediately and observed using a confocal microscope.

3. Results and discussion

An ultrasonic atomizer generates microdrops of a liquid by vibrating at ultrasonic frequencies [23]. Consisting of a front and rear horn and piezoelectric transducers which are located between the two horns, the ultrasonic atomizer vibrates in a direction parallel to the central axis of the transducers and breaks up the liquid delivered to the surface of the front horn. In this study, the ultrasonic atomizer was modified to have coaxial tubes, through which the two liquids were separately delivered. Reservoir-type microcapsules were obtained by spraying two component liquids over a water bath containing 0.5% PVA using an ultrasonic atomizer described in Fig. 1. Here, the component liquids were a PLGA-EA and an aqueous dye solution. Fig. 2A shows a bright-field microscopic image of the reservoir-type microcapsules. The polymer membrane covering the aqueous core was clearly shown in the CLSM image (Fig. 2B).

In elucidating the microencapsulation mechanism, two possibilities were considered. The first scenario was that the microcapsules would be formed by midair collision between the liquid drops of individual liquids. Previously, it was shown that collision of two liquid drops that were generated, respectively, by two ink-jet nozzles produced reservoir-type microcapsules [18,19]. Here, the aqueous drops were encapsulated by the polymer drops due to the surface tension gradient between the two liquid drops. In a similar manner, the coaxial ultrasonic atomizer might generate microdrops of component liquids, and their midair collision would result in microcapsules. Another possibility was that the two liquids would form an emulsion-type mixture prior to atomization. A liquid that is subjected to ultrasonic atomization forms a transitional liquid film on the atomizing surface by absorbing the underlying vibration energy [23]. In a
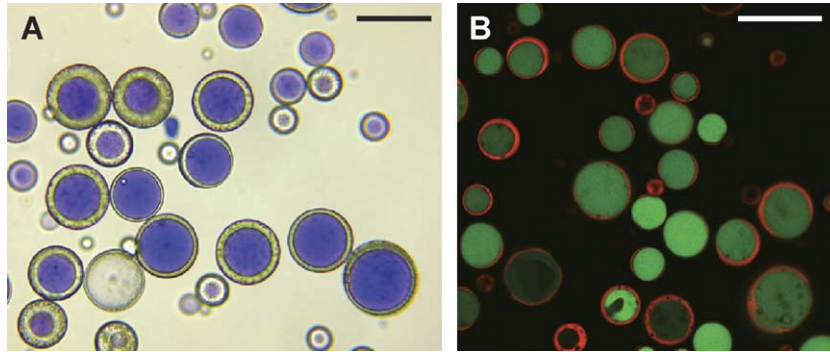
Fig. 2. (A) Bright-field microscopic image of the microcapsules produced by the coaxial ultrasonic atomizer. The aqueous cores look blue due to the presence of Coomassie brilliant blue R-250. (B) CLSM cross-sectional image of the microcapsules. The cores were labelled with FITC-dextran (green), and the polymer layer with Nile Red (red). Scale bars=100 μm.

coaxial atomizer, the liquid film might function as an intermediate stage in which the two liquids mix intimately to make a "transient emulsion," which then breaks up into microcapsules.

The fact that the encapsulation efficiency decreased upon increase of the ratio (Q_{Aq}/Q_{Pol}) of flow rates of aqueous solution (Q_{Aq}) to polymer solution (Q_{Pol}) could support both hypotheses. Encapsulation efficiency decreased from $53.9\pm5.8\%$ to $26.8\pm1.2\%$ (n=2) as Q_{Aq}/Q_{Pol} increased from (0.125 ml/min)/(3 ml/min) to (0.5 ml/min)/(3 ml/min). For the transient emulsion hypothesis, this result might mean that the relatively high Q_{Aq}/Q_{Pol} led to formation of oil-in-water (o/w)-type transient emulsion instead of water-in-oil (w/o) emulsion, preventing internalization of the aqueous phase within the polymeric phase. An alternative interpretation of this result is also possible.

The other observations strongly suggest that the microcapsules are formed by midair collision of the drops of two liquids. First, when a pair of ultrasonic atomizers (that separately delivered the two liquids) were arranged to bring the two groups of microdrops into collision, they produced the same kind of microcapsules as a coaxial atomizer. In this situation, the two liquids did not have an opportunity to share a common atomizing surface on which to form a transient emulsion, and the collision was the only chance for the two groups of liquid drops to contact each other. Second, the coaxial ultrasonic atomizer operating as submerged in the water bath also generated microcapsules; however, it was possible only when both liquids were nonaqueous solutions,

such as n-decane and PLGA solution (Fig. 3). When the usual combination of aqueous and PLGA solutions was used in the underwater system, the two liquids were separated immediately, and only the polymer phase was recovered as monolithic polymer particles. If the transient emulsion had occurred before the atomization, at least part of the aqueous phase would have been protected by the surrounding polymer solution and survived as parts of the microcapsules. In other words, the aqueous phase was put in direct contact with the water bath and immediately
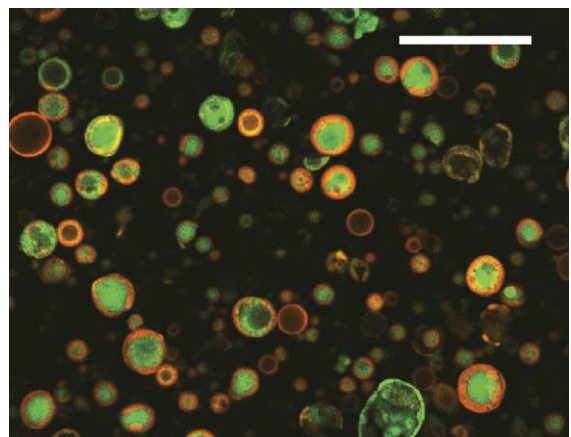

Fig. 3. CLSM cross-sectional image of the microcapsules produced by the coaxial ultrasonic atomizer operating underwater. N-decane appears green due to the presence of DiO. The PLGA phase containing Nile Red should fluoresce in red; however, the polymer layer appears rather orange because of partitioning of a part of DiO into the polymer phase. The microcapsules were relatively smaller than those produced in air and then collected in the water bath. Scale bar=100 μm.
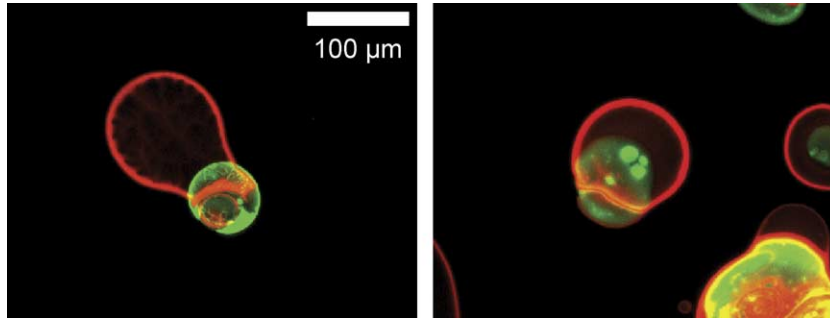
Fig. 4. Fluorescence microscopic images of microdrops captured on the glass plate. PLGA microdrops labelled with Nile Red (red) are encapsulating aqueous drops labelled with FITC-dextran (green). Q_{Aq}/Q_{Pol}=0.25 (ml/min)/1.5 (ml/min). The drops were flattened on the glass plate, and thus, they are not real representations of the three-dimensional liquid drops.

connected to the surrounding water before it could be encapsulated. In contrast, n-decane drops were still able to collide with the PLGA drops to make microcapsules without being diluted into the surrounding water. Third, when the microdrops emerging from the atomizer were collected on a glass plate and observed using a microscope, drops of an individual liquid were readily seen. A few polymer drops that were encapsulating the aqueous drops were also observed (Fig. 4). Based on these observations, it is concluded that the two component liquids are atomized into drops of individual liquids and then collide with each other to make microcapsules. However, because the two liquids come in brief contact with each other on the atomizing surface, it cannot completely be ignored that a minor degree of mixing between the two liquids may exist prior to the atomization.

In light of the above conclusion, the dependence of the encapsulation efficiency on the flow rate ratio (Q_{Aq}/Q_{Pol}) was reexamined. Microscopic examination of the microdrops that were captured midair on a glass plate revealed that the two component microdrops combined with each other immediately after their formation. On the other hand, it was interesting to find that the location of the two solutions in a combined drop was dramatically different depending on the Q_{Aq}/Q_{Pol} ratio. Fig. 5 shows that at a relatively low Q_{Aq}/Q_{Pol}, most aqueous drops were surrounded by the polymer drops, but the number of aqueous drops surrounded by polymer drops decreased with increasing Q_{Aq}/Q_{Pol} ratio. If only two drops of an equal size were involved in the collision, as in the two ink-jet nozzle system [18], the polymer solution

should encapsulate the aqueous drop according to the difference in surface tension, regardless of the Q_{Aq}/Q_{Pol} ratio. On the other hand, our previous study using the microdispenser system showed that the encapsulation of one drop by the other was affected not only by the surface tension gradient but also by the size ratio of the participating drops: when one of the contacting microdrops dominated the other in size, the larger one encapsulated the other due to the higher inertial force regardless of the surface tension gradient. Thus, the present observation suggests that the relative location of drops change according to the flow rate ratio, because the variation of the flow rates results in difference in the size of the two colliding drops. At a relatively high Q_{Aq}/Q_{Pol}, more water drops would be created than polymer drops and have better chances to make bigger drops prior to collision. At a relatively low Q_{Aq}/Q_{Pol}, the polymer drops would coalesce into bigger drops before they collide with the aqueous drops. The ãnormalã capsules (aqueous core surrounded by the polymer layer) would preferably occur in this condition; that is, when the size of the polymer drop is larger than that of the aqueous drop. For this reason, the encapsulation efficiency was relatively high at low Q_{Aq}/Q_{Pol} ratios. In the current observation, the normal capsules begin to outnumber the ãreversedã capsules when Q_{Aq}/Q_{Pol} is (0.25 ml/min)/(1.0 ml/min) (=1/4) or lower, which suggests that this is the condition that the polymer drops begin to dominate the aqueous drops in size sufficiently.

According to the theoretical prediction of the size distribution of drops produced by the ultrasonic atomizer [23], $d_{N,0.5}$=0.34 $(8\pi s/\rho f^2)^{1/3}$, where s and
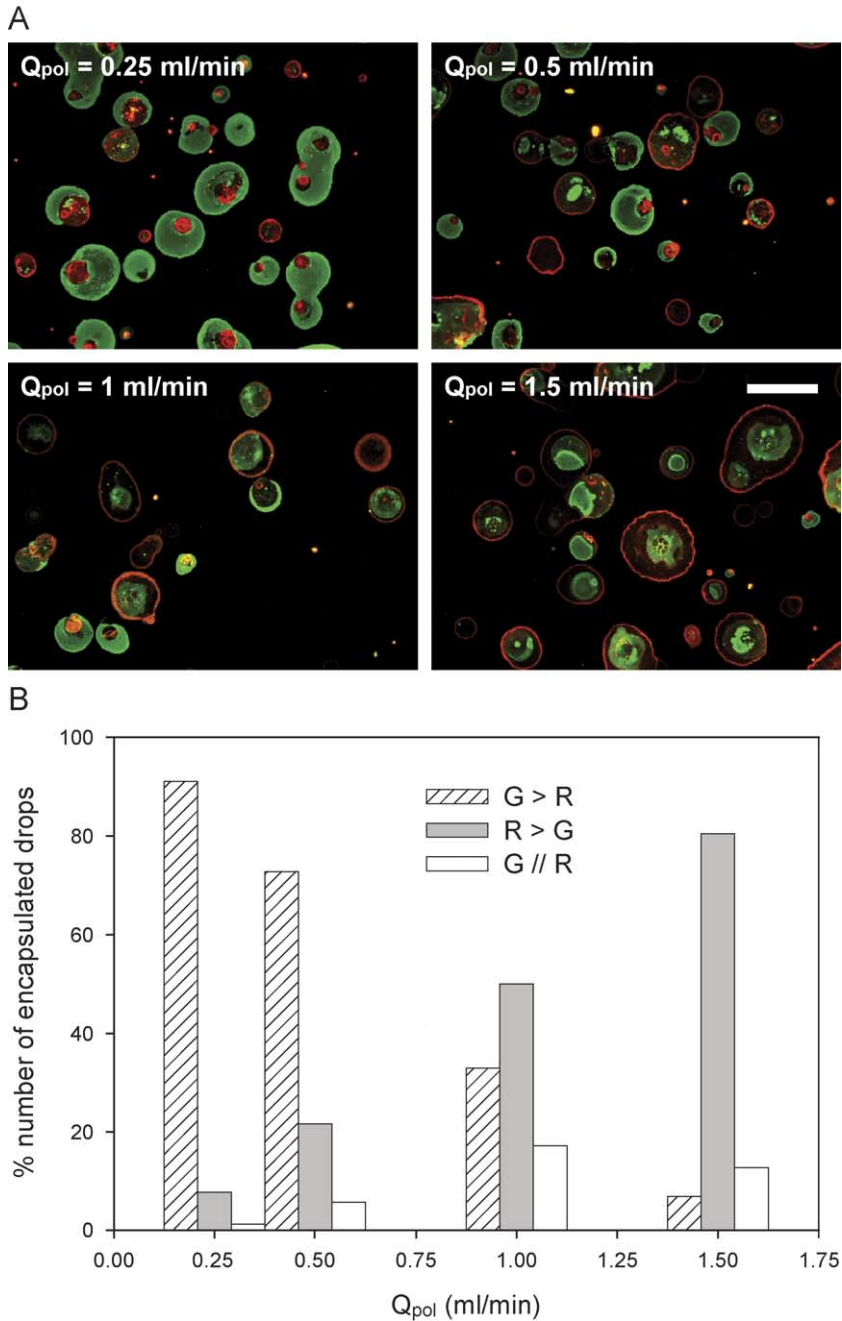
Fig. 5. (A) Fluorescence microscopic image of microdrops captured on a glass plate. Microdrops emerging from the coaxial ultrasonic atomizer were captured on the glass plate under varying Q_{Pol} at a fixed level of Q_{Aq} (0.25 ml/min). The PLGA phase is red due to the presence of Nile Red. The alginate solution is green due to the FITC-dextran. Scale bar=100 μm. (B) The percent number of encapsulated drops out of 70–100 randomly selected drops. Green aqueous drops are noted as G and red polymer drops R. G>R indicates polymer drops encapsulated by aqueous drops; R>G indicates aqueous drops encapsulated by polymer drops; and G//R indicates unencapsulated aqueous drops.

π are surface tension and density of the liquid, and f is the vibration frequency, the sizes of the individual water drop and ethyl acetate drop produced at 60 kHz are expected to be 27 and 19 μm in diameter, respectively. In addition, considering that the surface tension of ethyl acetate (24 dyn/cm) is lower than that of water (72 dyn/cm), it seems natural that the polymer drops require relatively higher flow rates, i.e., higher population density of the polymer drops, to form a comparable size to that of its aqueous counterpart. In the underwater system, it was noticed that most capsules formed in water were smaller than those produced in air. This suggests that the collision among drops was more restricted in water than in air; thus, the collision occurred among a limited number of drops.

When multiple drops participate in making one microcapsules, it would be possible to estimate the number of participating drops based on the volume of resulting microcapsules shown in Fig. 2. It is expected that 60-μm diameter core requires 11 aqueous drops of 27-μm diameter, and 5- to 10-μm-thick shell requires 18–43 polymer solution drops of 19-μm diameter. When these numbers are converted to the volume of each liquid, the ratio of aqueous solution to polymer solution becomes 1:0.6–1:1.4. However, this is the case only when the volume of polymeric layer does not change in the collection bath. In reality, the polymeric layer undergoes shrinkage as the solvent exchange proceeds [17], and Fig. 2 shows the membrane which is left after the shrinkage. This means that in fact the microencapsulation involves higher numbers of polymeric drops than those expected based on apparent volume of the core and wall thickness, and this is consistent with our observation in Fig. 6 where the normal capsules begin to form only when the volumetric flow ratio (Q_{Aq}/Q_{Pol}) is less than 1/4. This also agrees well with our previous observation on the wall thickness in the dual microdispenser approach [19].

Once the polymer drops encapsulated the aqueous drops successfully, the polymer layer became phase-separated (i.e., precipitated) on the surface of the aqueous drop through the interfacial mass transfer between the two liquids (i.e., solvent exchange). It is thought that the solvent exchange would begin as soon as the collision of two drops forms an interface between the two liquids. In this study, the completion of the phase separation is, however, mainly driven by the solvent extraction into the surrounding water because the microcapsules were collected in the water bath shortly after the collision. This would be
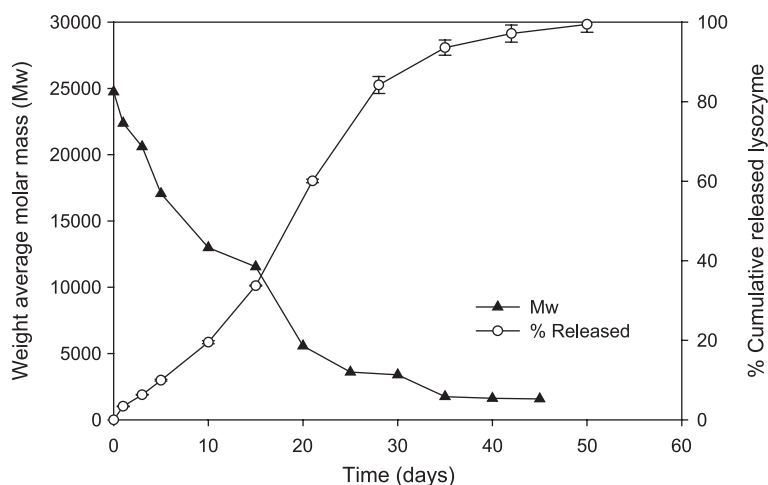

Fig. 6. In vitro release profile of lysozyme and polymer degradation profile from the prepared microcapsules. Error bars indicate the standard deviation of three replicates. For fabrication of the microcapsules, a 5% PLGA-EA solution, 3% lysozyme in 0.1M phosphate buffer (pH 2.5), and 0.5% PVA solution were used as the polymer solution, aqueous solution, and collection bath, respectively. The PLGA polymer was 50:50 blend of 44 and 13 kD polymers. Q_{Pol}=2 ml/min and Q_{Aq}=0.2 ml/min. Microcapsules were collected for 2.5 min in a 200 ml collection bath. The weight average molar mass was determined by gel permeation chromatography. Despite the bimodality of the molecular weight distribution, the molecular mass of PLGA polymers in degrading microcapsules was presented as the weight average for simplicity of representation.

Table 1
Enzymatic activity of the released lysozyme

Time (days)	Lysozyme (μg/ml)[a]	$k_{theoretical}$	$k_{apparent}$	% RBA[b]
1	23.0	−0.0209	−0.0172	82.3
3	35.3	−0.0289	−0.0281	97.4
5	48.3	−0.0372	−0.0451	121.1
10	98.8	−0.0700	−0.0580	82.8
15	163.4	−0.1119	−0.1014	90.7
21	292.1	−0.1954	−0.2428	124.3
28	362.9	−0.2412	−0.2981	123.6
35	304.5	−0.2034	−0.2298	113.0
42	227.4	−0.1534	−0.1897	123.6
50	170.1	−0.1162	−0.1271	109.4

[a] Measured by BCA assay.
[b] Percent retained biological activity.

particularly true at relatively low Q_{Aq}/Q_{Pol} ratios, because a very small amount of ethyl acetate is enough to saturate the small amount of aqueous core solution, and the majority of polymer phase would stay as a solution when it enters the collection bath.

The prepared microcapsules were tested for in vitro release behavior using lysozyme as a model protein. Because the drying process was not optimized at the time of the release study [24], the microcapsules were tested without drying. Lysozyme was slowly released from the microcapsules at the rate of 0.46% of the total encapsulated lysozyme per day (r^2=0.9947) for 35 days following the polymer erosion profile (Fig. 6). It is likely that the continuous release is due to heterogeneity of the membrane thickness and the microcapsule size. Otherwise, the entire payload might have been burst released at once as soon as polymer degradation induced minor defect on the membrane. The released lysozyme remained functionally intact as shown from the enzymatic activity test (Table 1), which suggests that the protein survived the microencapsulation process without losing its biological activity.

4. Conclusion

Reservoir-type microcapsules can be produced using a coaxial ultrasonic atomizer based on midair collision among multiple drops of component liquids and subsequent mass transfer at their interface. The reported process has several advantages over traditional emulsion microencapsulation techniques, espe-

cially in the encapsulation of protein drugs. First, the microcapsules form as a result of collision between different species of liquid drops; hence, the proteins are not subjected to damagingly strong mechanical stresses, such as prolonged emulsification [25,26]. The encapsulated materials are only briefly exposed to the mild ultrasonic vibration during atomization. The energy that is applied to the ultrasonic atomization is also less than a few watts, which is far below a damaging level [23]. Second, the interfacial area between the aqueous phase and the hydrophobic polymer phase is minimal in reservoir-type microcapsules, which could otherwise cause extensive denaturation of the encapsulated proteins. Third, the solvent exchange method is a single-step process which is clearly simpler than the existing microencapsulation techniques. This simple microencapsulation technique has the potential to develop various protein formulations under mild processing conditions.

References

[1] R. Langer, J. Folkman, Polymers for the sustained release of proteins and other macromolecules, Nature 263 (1976) 797–800.

[2] S.P. Schwendeman, H.R. Constantino, R.K. Gupta, G.R. Siber, A.M. Klibanov, R. Langer, Stabilization of tetanus and diphtheria toxoids against moisture-induced aggregation, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 11234–11238.

[3] S.P. Schwendeman, Recent advances in the stabilization of proteins encapsulated in injectable PLGA delivery systems, Crit. Rev. Ther. Drug Carr. Syst. 19 (1) (2002) 73–98.

[4] M. van de Weert, W.E. Hennink, W. Jiskoot, Protein instability in PLGA microparticles, Pharm. Res. 17 (10) (2000) 1159–1167.

[5] C. Perez, I.J. Castellanos, H.R. Costantino, W. Al-Azzam, K. Griebenow, Recent trends in stabilizing protein structure upon encapsulation and release from bioerodible polymers, J. Pharm. Pharmacol. 54 (3) (2002) 301–313.

[6] S.D. Putney, P.A. Burke, Improving protein therapeutics with sustained-release formulations, Nat. Biotechnol. 16 (1998) 153–157.

[7] H. Okada, T. Heya, Y. Ogawa, T. Shimamoto, One-month release injectable microcapsules of luteinizing hormone-

releasing hormone agonist (Leuprolide acetate) for treating experimental endometriosis in rats, J. Pharmacol. Exp. Ther. 244 (2) (1988) 744–750.

[8] A.M. Dlugi, J.D. Miller, J. Knittle, Lupron depot (leuprolide acetate for depot suspension) in the treatment of endometriosis: a randomized, placebo-controlled, double-blind study. Lupron Study Group, Fertil. Steril. 54 (3) (1990) 419–427.

[9] G. Zhu, S.R. Mallery, S.P. Schwendeman, Stabilization of proteins encapsulated in injectable PLGA, Nat. Biotechnol. 18 (2000) 52–57.

[10] G. Zhu, S.P. Schwendeman, Stabilization of proteins encapsulated in cylindrical PLGA implants: mechanism of stabilization by basic additives, Pharm. Res. 17 (3) (2000) 351–357.

[11] J. Heller, J. Barr, S.Y. Ng, K.S. Abdellauoi, R. Gurny, Poly(ortho esters): synthesis, characterization, properties and uses, Adv. Drug Deliv. Rev. 54 (7) (2002) 1015–1039.

[12] C. Berkland, E. Pollauf, D.W. Pack, K.K. Kim, Uniform double-walled polymer microspheres of controllable shell thickness, J. Control. Release 96 (2004) 101–111.

[13] A. Sanchez, R.K. Gupta, M.J. Alonso, G.R. Siber, R. Langer, Pulsed controlled-release system for potential use in vaccine delivery, J. Pharm. Sci. 85 (6) (1996) 547–552.

[14] N. Wang, X.S. Wu, A novel approach to stabilization of protein drugs in PLGA microspheres using agarose hydrogel, Int. J. Pharm. 166 (1998) 1–14.

[15] M. Tobio, S.P. Schwendeman, Y. Guo, J. McIver, R. Langer, M.J. Alonso, Improved immunogenicity of a core-coated tetanus toxoid delivery system, Vaccine 18 (2000) 618–622.

[16] M.D. Blanco, M.J. Alonso, Development and characterization of protein-loaded poly(lactide-co-glycolide) nanospheres, Eur. J. Pharm. Biopharm. 43 (1997) 287–294.

[17] G. Crotts, T.G. Park, Preparation of porous and nonporous biodegradable polymeric hollow microspheres, J. Control. Release 35 (1995) 91–105.

[18] Y. Yeo, O.A. Basaran, K. Park, A new process for making reservoir-type microcapsules using ink-jet technology and interfacial phase separation, J. Control. Release 93 (2) (2003) 161–173.

[19] Y. Yeo, A.U. Chen, O.A. Basaran, K. Park, Solvent exchange method: a novel microencapsulation technique using dual microdispensers, Pharm. Res. 21 (8) (2004) 1419–1427.

[20] Y. Yeo, K. Park, New microencapsulation technique using an ultrasonic atomizer based on the solvent exchange method, Polym. Mater. Sci. Eng. 89 (2003) 143–144.

[21] H. Sah, A new strategy to determine the actual protein content of PLGA microspheres, J. Pharm. Sci. 86 (11) (1997) 1315–1318.

[22] R. Ghaderi, J. Carlfors, Biological activity of lysozyme after entrapment in PLGA-microspheres, Pharm. Res. 14 (11) (1997) 1556–1562.

[23] H.L. Berger, Ultrasonic Liquid Atomization, Partridge Hill Publishers, Hyde Park, NY, 1998.

[24] Y. Yeo, K. Park, Characterization of reservoir-type microcapsules made by the solvent exchange method, AAPS PharmSciTech (2004) in press.

[25] M. Morlock, H. Koll, G. Winter, T. Kissel, Microencapsulation of rh-erythropoietin, using biodegradable PLGA: protein stability and the effects of stabilizing excipients, Eur. J. Pharm. Biopharm. 43 (1997) 29–36.

[26] M.F. Zambaux, F. Bonneaux, R. Gref, E. Dellacherie, C. Vigneron, Preparation and characterization of protein C-loaded PLA nanoparticles, J. Control. Release 60 (2ã3) (1999) 179–188.