Fabrication of hollow biodegradable microcapsules from microbubble templates

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Hollow poly-lactic acid (PLA) microcapsules and hollow polyelectrolyte microcapsules made of poly-allylamine hydrochloride (PAH) were synthesized by directly adsorbing these polymers to N_2 (air) and CO_2 microbubbles respectively, using the bubble template method. To manufacture PLA microcapsules, droplets of a solution of PLA in methylene chloride (CH_2Cl_2) were emulsified in water. Then by solvent diffusion, N_2 microbubbles nucleated inside the droplets and PLA adsorbed to the bubble surface to form microcapsules. Likewise, for PAH microcapsules, when an aqueous solution of Na_2CO_3 including PAH is titrated with HCl, within a specific range $7.5 < pH < 9.0$, colloidal PAH particles are formed and then adsorbs to the nucleated CO_2 microbubbles. Zeta potential value for these capsules at pH 8.5 is positive, allowing PSS adsorption onto the PAH capsule to attain a bilayer capsule. Herein we present on size control and shell formation kinetics of these two types of microcapsules.

Key words: Hollow microcapsule, biodegradable polymer, PAH, PLA, PSS, bubble template method, bilayer, size control.

1. Introduction

Microbubbles have the potential to become great echo enhancers, since the acoustic impedance between the blood and the gas in the bubble is high, so incident waves are completely reflected. However, if microbubbles were to be used as ultrasound contrast agents by themselves, several difficulties must be addressed. They have a very short lifespan in the system. The gas pressure in the bubble is the result of the equilibrium pressure (Henry's law), the Laplace pressure, and the blood pressure[1]. Thus as soon as they are infused intravenously, they cannot remain stable for a long time. Even if lipid bilayers are used to stabilize them, they rapidly disintegrate since the interaction between lipid molecules is weak[2]. In addition microbubbles' optimum size to easily pass through the capillary blood circuit and assure a longer circulation time must range between 1 – 4 μm. A more rigorous constraint is that they must be uniform in size since their resonant frequency depends on its radius. This requirement allows attaining a narrow backscatter signal. Therefore if microbubbles are to be employed as ultrasound contrast agents, the solution is to functionalize the microbubbles (1) with an enhanced lifespan, (2) with high uniformity in size and (3) within the size range of interest for ultrasound applications. To comply with these requirements hollow biodegradable microcapsules are synthesized.

Hollow microcapsules are defined as microbubbles (CO_2, N_2, perfluorocarbons "PFC's") encapsulated in a thin polymer or protein shell.[3] In order to synthesize hollow biodegradable microcapsules, current manufacturing techniques rely on the use of sacrificial cores (liquid droplets or solid particles) onto which a biodegradable polymeric layer can be formed via interfacial polymerization or adsorption. After shell formation the shell is intentionally degraded so that the cores can be removed by dissolution, evaporation, or thermolysis and finally attain a hollow capsule. But, if instead a microbubble is employed as template, the complexities in the current fabrication methods can be avoided.

We focus on the synthesis and size control of two different types of hollow microcapsules: 1. Polyelectrolyte (PAH/PSS) microcapsules and 2. PLA microcapsules. We employed the bubble template method to synthesize these two types of hollow capsules. In this method a bubble (the template) is nucleated inside a solution and immediately stabilized by a polymer without the need of surfactants. In addition we succeeded to synthesize a bilayer capsule by adsorbing PSS onto the PAH capsule. By doing so, PAH microcapsules were able to remain undamaged at pH = 7.0.

2. Experiments

2.1 Materials

PLA (molecular weight is 2,45, and 100 KDa) (Polyscience, U.S.); CH_2Cl_2 with 99.99% purity (Wako, Japan); and an aqueous phase 2% poly-vinyl alcohol (Gohsenal T-350, Nippon, Gohsei, Japan), PAH (molecular weight: ca. 56 000) (Sigma-Aldrich, US), FITC-PAH (molecular weight: ca. 56 000) (Sigma-Aldrich, US). 1 M HCl (Wako, Japan), and Na_2CO_3 (Wako, Japan) were employed. All chemicals used in this study were reagent grade.

Water from a Milli-Q Advantage A10 water purification system was employed. Images were obtained and analyzed using an inverted microscope system (Eclipse Ti-E, Nikon Japan). The zeta potential measurement was performed using a zeta potential analyzer (ZEECOM, Microtec Corporation, Chiba, Japan). The FTIR measurement was performed using an FTIR spectrometer (Nicolet 6700 FTIR, Thermo Scientific, US) with a diamond attenuated total reflectance (ATR) smart accessory.

2.2 Methods

2.2.1 PAH microcapsules

Hollow PAH microcapsules were synthesized at atmospheric pressure using the method described in reference 4. When CO_2 microbubbles were nucleated, these microbubbles were stabilized when the colloidal PAH adsorbed to the bubble surface. If pH is further decreased, $R\text{-}NHCOO^-$ concentration decreases and that of $R\text{-}NH3^+$ increases, which makes capsules disappear at pH lower than 7.5. Further, several 0.05 M solutions of Na_2CO_3 with different concentrations of PAH, c_{PAH} = 0.1, 1.0, 2.0, 3.0, 7.0, 15.0, were employed to analyze the effect of c_{PAH} on the final microcapsules size at pH= 8.5. In addition bilayer capsules were synthesized by adsorbing PSS onto FITC-PAH-stained PAH. The concentration of PAH solutions was kept at 0.5 g/L, and the concentrations of the PSS solution were set to be 0.5, 1.0, and 2.0 g/L. the mixing volume ratios of PAH to PSS solution was V_{PAH}:V_{PSS} = 1:1. All measurements were performed 2h after capsules were fabricated.

2.2.2 PLA microcapsules

The general fabrication method of hollow PLA microcapsules is the same as the one reported in reference 5. The effect of using different PLA initial concentrations, c_{PLA} = 1, 2, 3, 5, 10, 20g/L, the use of surfactants, and increasing the molecular weight on the final capsule size was analyzed as well.

3. Results and Discussion

3.1 PAH microcapsules

3.1.1 Size control of PAH microcapsules

In a Na_2CO_3, because the system is oversaturated with CO_2, nucleated microbubbles will keep growing due to bubble coalescence or heterogeneous nucleation on the CO_2 microbubble surface. This unstable microbubbles must be stabilized by PAH colloidal particles before they reach the bulk surface and escape into the environment.

Once microcapsules are synthesized, it was found that regardless of the concentration, they shrink because of the leakage of CO_2 gas. The required time to achieve a stable equilibrium radius is independent of the initial PAH concentration and it was found to be aprox. 1 hour. This suggest that it took about 1 hour to completely form a PAH layer on the surface of the nucleated CO_2 microbubbles. Further increasing the initial concentration of PAH yields to bigger microcapsules. Not only the final radius but also standard deviation increased with increasing the PAH concentration. The top panel of Fig. 1 shows that the proportionality of the final radius to the PAH concentration is given by a power function of exponent 1/2.

For our system, it was assumed that, (1) PAH colloidal particles are uniform in size, and (2) the total number of nucleated bubbles is the same and is independent of the PAH concentration; it was also assumed that all microcapsules present a unique average radius. Thus, since all PAH colloidal particle adsorbs to the microbubble surface and furthermore if the number of adsorbed particles is proportional to the microbubble surface area, this implies that the mean surface area of the microcapsules is proportional to the concentration of PAH. In addition as time goes on, the number density of colloidal particles reduces because it adsorbs to the microbubble surface and it also increases if PAH concentration increases. Ergo, both the mean radius and the standard deviation of the PAH microcapsules increased when PAH concentration was increased. It can also be seen that for an initial PAH concentration of 20 g/L, no microcapsules can be synthesized. Table 1sumarizes the results of changing PAH concentration as a parameter for changing capsule radius.

3.1.2 Bilayer microcapsules.

If PAH microcapsules were to be used as ultrasound contrast agents, it won't be possible since as pH decreases, the concentration of R-NHCOO$^-$ decreased and that of R-NH_3^+ increased resulting in nonequimolar particles. In other words, colloidal particles are not present and therefore microcapsules cannot exist at low pH (i.e. human pH = 7.0).

The electrical properties of the PAH microcapsules were examined and it was found that at pH = 9.0 the zeta potential is negative, however, at pH = 8.5, the zeta potential is positive. This result implied that PSS can be sequentially adsorbed, as we did. We observed that the hollow PAH/PSS bilayer microcapsules tended to form aggregates with decreasing the mass ratio of PAH and PSS. Thus when the mass ratio of PAH and PSS is large,the hollow PAH microcapsules are completely covered with PSS and the independent hollow PAH/PSS bilayer microcapsules can be formed. Fig. 2 shows fluorescent and bright field images of hollow bilayer microcapsules at pH 7. Further, Table 2 summarizes the measured zeta potential for PAH and PAH/PSS microcapsules.

Fig. 3 shows the FTIR measurements performed on both types of

Table 1. The effect of PAH concentration on final microcapsule size.[4]

PAH conc. (g/L)	Mean radius (μm)	Standard dev. (μm)	PI%
0.1	1.32	0.26	20.41
1.0	1.77	0.45	25.30
2.0	1.84	0.55	30.11
3.0	2.23	0.63	28.40
7.0	3.21	1.04	32.46
15.0	4.08	1.62	39.85

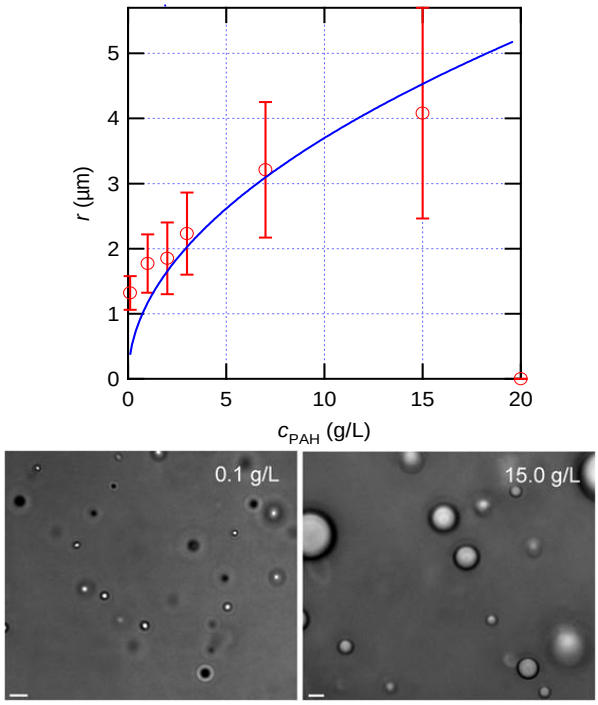

Fig1. (top) Mean radius of hollow PAH microcapsules as a function of PAH concentrations. (bottom) bright field images of hollow PAH microcapsules as a function of PAH concentrations. Inset bar is 5μm. The pH of the solutions was 8.5.[4]

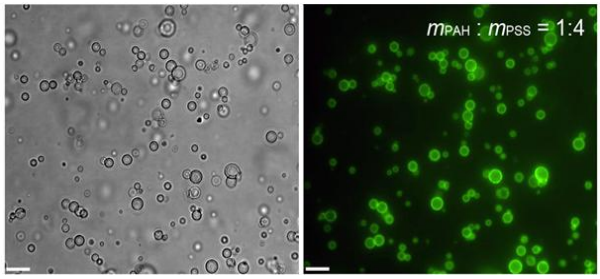

Fig. 2 Bright field (left) and epifluorescent (right) images of hollow PAH/PSS bilayer microcapsules. The scale bar is 5 μm. The mass ratios of PAH and PSS was 1:4. The pH of the solution was 7.0.[4]

Table 2. measured zeta potential of hollow PAH, PAH/PSS micro capsules.

Type of microcapsules	pH	Zeta potential (mV)
PAH	9.0	-64.8
PAH	8.5	21.2
PAH	7.5	63.9
PAH/PSS	8.5	-92.9
PAH/PSS	7.0	-83.3

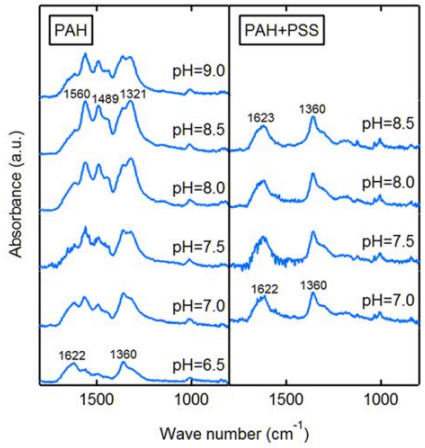

Fig. 3 FTIR-ATR spectra of 0.05-M aqueous Na_2CO_3 solutions with PAH (left) and with PAH + PSS (right) titrated with 1-M HCl. The initial concentrations of PAH and PSS were set to be 0.5 and 1.0 g/L, respectively[4]

microcapsules. The band around 1500–1600 cm^{-1} is of the so-called amide II and is attributed mainly to the carbamate group. The band at 1320 cm^{-1} can also be attributed to the carbamate. The FTIR-ATR spectra of the solution with PAH at pH = 6.5 shows the bicarbonate bands due to the stretching vibrations of the carbonate groups at 1360 and 1622 cm^{-1}.[6] The FTIR-ATR spectra of the solutions with PAH + PSS is similar to the spectra for the PAH solution at pH = 6.5. It can be understood that R-$NHCOO^-$ changes to R-NH_3^+ in aqueous PSS solutions. Therefore the negative PSS can firmly adsorb on the positive PAH capsule shell. The hollow PAH microcapsules were stable in the solution for several hours in a closed bottle, while, the hollow PAH/PSS bilayer microcapsules were stable more than a week in the same condition.

3.2 PLA microcapsules

3.2.1 Size control of PAH microcapsules

Three parameters were employed to tune PLA capsule radius: (1) initial concentration of PLA in CH_2Cl_2, (2) the use of PVA_{aq} solution as continuous phase and (3) increasing molecular weight.

The effect of increasing the initial concentration of PLA is shown in Fig. 4. Increasing the initial concentration leads to smaller microcapsules and less uniform capsules. Further, the standard deviation is above 0.1um when the initial PLA concentration is below 2g/L and above 3g/L. Also the amount of bridged capsules and viscosity of the PLA solution increased when the initial PLA concentration was increased.

As shown in Fig. 5 (top), the inner and outer radius of hollow PLA microcapsules fabricated in pure water were smaller than those of a 2% (w/w) PVA aqueous solution. In addition, for a PLA of molecular

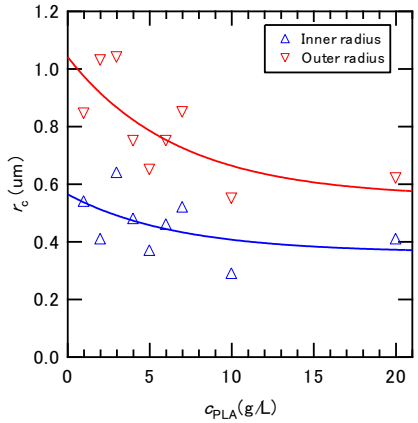

Fig. 4 The mean inner and outer radiuses of hollow PLA microcapsules fabricated in a 2% (w/w) PVA aqueous solution as a function of PLA concentration

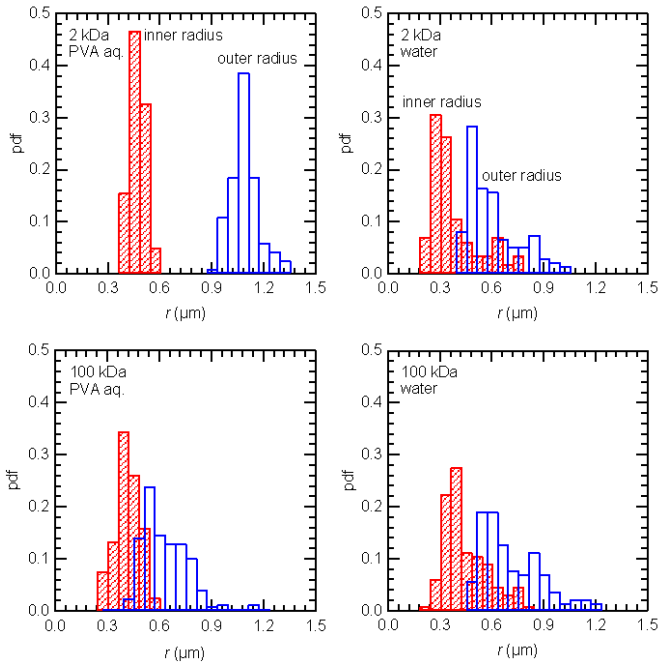

Fig. 5 Probability density function (PDF) of inner and outer radius of hollow PLA microcapsules fabricated in 2% (w/w) PVA aqueous solution (top) and water (bottom). c_{PLA} = 2 g/L

weight of 2 kDa, the standard deviation for the outer radius was larger in pure water than in a 2% (w/w) PVA aqueous solution when the PLA concentration was 2 g/L.

Increasing the molecular weight also yields to a reduction in the final microcapsule size. Table 3 shows the effect of PLA molecular weight for capsules synthesized in either water or 2% (w/w) PVA_{aq} solution. In a 2% (w/w) PVA_{aq} solution, as the molecular weight increases, the outer radius of the microcapsules and the shell thickness decreases. Uniformity of the final capsule size decreased as well.

As the initial PLA concentration increases, PLA can absorb to the liquid-liquid interface (droplet-surrounding liquid) and some other PLA remain dissolved in the solution. Further, methylene chloride keeps diffusing into the aqueous medium and the PLA concetration

Table 3. PLA outer capsule size as a function of PLA Molecular Weight synthesized in either 2% PVA_{aq} or water.

MW	Mean (μm)	Stdev (μm)	PI(%) = (Stdev/mean*100)
2 kDa in PVA_{aq}	1.03	0.08	7.70
45 kDa in PVA_{aq}	0.58	0.15	26.7
100 kDa in PVA_{aq}	0.58	0.14	25.0
2 kDa in H_2O	0.55	0.15	27.5
45 kDa in H_2O	0.57	0.13	23.2
100 kDa in H_2O	0.65	0.17	26.0

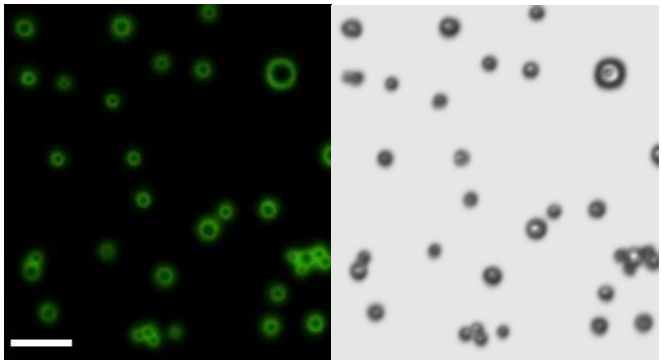

Fig. 6 Fluorescent (left) and bright field (right) images on fabricated hollow PLA microcapsules. The scale bar is 5 μm. Several bridged capsules were observed. Droplets of 1 g/L PLA methylene chloride solution were formed in 2% (w/w) PVA aqueous solution. The molecular weight of PLA was 2 kDa.

increases forming a film that sorround the droplet and a higly concentrated region underneath the interface. Due to this barrier microbubbles reach the interface and stay there. Meanwhile more microbubbles are nucleated and start to agglomerate at the interface as well. In this condition coalescence takes place, the internal pressure of microbubbles releases and the pressure wave breaks the PLA adsorption layer locally, and then bubbles and/or aggregates of bubbles near the interface erupt into a surrounding aqueous medium. When the bubbles erupt they can break into smaller bubbles and thus form smaller capsules. In addition, upon bursting, the film of methylene chloride solution of PLA can break into droplets which then yield to PLA microparticles.

Inside the droplet, PLA is in a good solvent (methylene chloride), thus the PLA polymer chains are completely swollen[7]. Outside the droplet, i.e. in an aqueous medium, instead of an extended configuration, the polymer should adopt a globular configuration because of the hydrophobicity of PLA. However, when PVA is present in an aqueous medium, PVA hinders this PLA–H_2O interaction[8]. Therefore bigger capsules can be attained. In addition PVA acts as a surfactant. It reduces the energy barrier at the CH_2Cl_2-PVA_{aq} interface allowing for a smooth microcapsule release from the droplet. This explains why do capsules synthesized in PVA are bigger and uniform when compared to their water counterparts as shown in Fig. 5. Further, those fabricated in pure water displayed a high standard deviation regardless of the molecular weight and the final size remained the same.

It can be observed that the final microcapsule size decreases as molecular weight increases. Fig. 6 shows a representative image of fluorescent capsules synthesized in water and its corresponding bright field image for a PLA (MW of 45kDa and concentration of 2g/L) capsule synthesized in water. If the PLA concentration remains constant (in our case 2g/L), as the PLA molecular weight increases, PLA has a larger PLA coil but the number of coils decreases. As a result, the possible surface area covered with PLA coils decreases, which yields to smaller hollow PLA microcapsules.

Our results implied that a combination of a low PLA concentration, low PLA molecular weight, and the use of PVA yields to bigger and uniform hollow capsules.

4. Conclusions

In our studies, we succeeded not only to synthesize hollow biodegradable capsules using microbubble as templates, but also attained uniformity and size control over these microcapsules. Furthermore the required conditions for capsule formation and the kinetics of shell formation were elucidated as well for both, PLA and PAH/PSS hollow microcapsules.

PAH capsules size was increased when PAH concentration was increased, yet standard deviation increased as well. Furthermore, from zeta potential measurements, it was known that PAH microcapsules are positively charged at pH = 8.5, this allowed PSS to successfully adsorb on the PAH microcapsule surface to form a bilayer microcapsule. The PAH/PSS microcapsule solution pH was adjusted to 7.0 and it was confirmed that capsules were able to remain undamaged at human pH.

Increasing PLA concentration decreases PLA microcapsules size. In addition, the presence of surfactant (PVA) yields to bigger microcapsules compared to those fabricated in water. Furthermore, increasing initial polymer concentration decreases capsule size. It was found that the optimum concentration for highest uniformity is at 2 and 3g/L; increasing the PLA MW decreases the final capsule size. Changing these three parameters namely PLA molecular weight, initial PLA concentration and the addition of PVA, allowed us to tune final capsule size.

5. References

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