1. Introduction

Osmotic pressure is a crucial factor for determining the stability of microcapsules and biological systems enclosed by lipid bilayers. The pressure difference between the interior and exterior of such systems induces water flux through semipermeable membranes, deflating or inflating the compartments. In particular, the high osmotic pressure difference frequently disintegrates delicate capsules and cells by imposing significant stress on the membrane. Therefore, measuring the osmotic strength is very important for a wide range of research and applications that use semipermeable membranes, ranging from the fundamental study of cell functions to drug delivery systems. To estimate the strength, colligative properties of aqueous solutions, such as freezing-point depression, vapor-pressure lowering, or boiling-point elevation, have been measured and analyzed. However, such indirect measurements require elaborate machines to measure the properties. Moreover, additional analysis is sometimes required for non-ideal solutions such as colloidal systems.
as concentrated solutions of polymers or biomaterials, which can exhibit nonlinear behavior.\[^4\] By contrast, the osmotic pressure can be directly measured by a setup composed of manometers and semipermeable membranes.\[^5\] However, this measurement requires relatively large quantities of samples. Moreover, these conventional methods are not available for \textit{in-vivo} or \textit{in-situ} measurement of the osmotic strength. Therefore, a facile and direct measurement of the osmotic strength using an injectable kit remains an important challenge.

Double-emulsion drops have provided useful templates to produce microcapsules because of their core–shell geometry. A variety of microcapsules have been prepared through solidification of the shell phases, resulting in a membrane enclosing the cores.\[^2a,6\] The membranes can be semipermeable due to formation of very small pores that allow diffusion of water molecules through it but not solutes. Microfluidics has enabled the production of such double-emulsion drops with unprecedented controllability and very high efficiency of encapsulation.\[^7\] Therefore, double-emulsion drops produced in microfluidic devices can be useful to make semipermeable microcapsules that are sensitive to the osmotic pressure difference across the membrane.\[^8\]

In this paper, we report a microfluidic approach to produce microcapsules with ultrathin and semipermeable membranes or osmocapsules, providing a facile and direct measurement of the osmotic strength. Using capillary microfluidic devices, we prepare water-in-oil-in-water (W/O/W) double-emulsion drops with an ultrathin middle phase, which transform into polymeric microcapsules that contain an aqueous solution with standard osmotic strength upon solidification of the middle phase. Either UV-induced photopolymerization or evaporation-induced consolidation of the middle phase is employed. The resultant microcapsules are highly sensitive to osmotic pressure differences due to the semi-permeability and very small thickness of the membrane. Therefore, a small positive pressure can lead to buckling of the capsules by an outward flux of water through the membrane. Therefore, when a mixture of distinctly labeled microcapsules, containing different standard osmotic solutions, are dispersed in an unknown solution, the capsules are selectively buckled when subjected to positive pressure, thereby enabling an estimation of the osmotic strength. In addition, the capsules potentially enable \textit{in-vivo} measurement of the osmotic strength by injecting them. Moreover, the capsules can be ruptured when they are subjected to a high osmotic pressure difference, providing the osmotic pressure-triggered release of encapsulants.

\section*{2. Results and Discussion}

\subsection*{2.1. Preparation of ETPTA Capsules}

The microfluidic device comprised two tapered cylindrical capillaries that were coaxially aligned in a square capillary as shown schematically in Figure 1a, where the left cylindrical capillary has a 70-µm diameter orifice and a hydrophobic surface, whereas the right cylindrical capillary has a 190-µm diameter orifice and a hydrophilic surface. A small tapered capillary was inserted into the hydrophobic cylindrical capillary. We injected the aqueous solution of poly(vinyl alcohol) (PVA) and NaCl through the small tapered capillary to form innermost drops and a photocurable monomer of ethoxylated trimethylolpropane triacrylate (ETPTA) through the hydrophobic cylindrical capillary to form hydrophobic cylindrical capillary to form ultra-thin shells. These two immiscible fluids flow through a single capillary channel of the hydrophobic injection capillary. Because of the hydrophobic nature of the capillary, ETPTA flows along the inner...
wall of the capillary, whereas the aqueous solution flows in the shape of plug-like drops without contacting the wall, forming a discontinuous core–sheath stream.[6b,9] We injected the same aqueous solution through the interstices of the hydrophobic capillary and the square capillary to form a continuous phase and this emulsified the core–sheath stream at the tip of the hydrophobic capillary into double-emulsion drops with an ultra-thin shell, as shown in Figure 1b and Movie S1 of the Supporting Information.[6b] Although excess ETPTA between the plug-like drops produced large oil blobs, separation of the double emulsion was simply accomplished by exploiting their density difference. This discontinuous emulsification generated a small fraction of double-emulsion drops with a relatively thick shell when both ends of the plug-like drops were emulsified at the tip of the injection capillary; the fraction therefore depends on the length of the drops and is typically less than 10% of the total double-emulsion drops. The ETPTA monomers in the ultra-thin shell were polymerized in a collection bath by UV irradiation for 2 seconds over a 1 minute interval. Using this in-situ polymerization technique, all double-emulsion drops generated in the microfluidic device were converted into stable microcapsules. The resultant microcapsules with an average diameter of 122 µm, containing the aqueous solution of different osmolarity and dye, are shown in Figure 1c, where the capsules appearing in red, orange, yellow, and green colors contain aqueous solutions of 33, 342, 649, and 950 mOsm L\(^{-1}\), respectively. The thickness of the ETPTA membrane was characterized by scanning electron microscopy (SEM), as shown in Figures 1d and e where we broke deflated capsules by applying a mechanical force to observe the cross-section of the membrane, and resulted in a thickness of 1.05 µm and 1.34 µm for the thin and thick parts, respectively. Therefore, the ratio of average thickness to capsule radius, \(h/R\), is 0.0196. The membrane can be designed to be magneto-responsive by adding magnetic nanoparticles. The magnetic capsules can be easily separated from solutions and non-magnetic capsules by applying a magnetic field as shown in Figure S1 of the Supporting Information, where capsules contained food-coloring pigments instead of fluorescent dyes. This magnetic response is useful for the separation of the capsules after measurements.

**2.2. Estimation of Osmolarity Using ETPTA Capsules**

When semipermeable capsules are subjected to positive osmotic pressure, the capsules shrink due to the outward flux of water through the membrane. The capsules exhibit an isotropic shrinkage for small volume reduction and they begin to buckle by forming one indentation at the weakest point for further volume reduction than a certain value, \(\Delta V^*\), as shown schematically in Figure 2a.[8,10] Therefore, the osmotic pressure difference that reduces the volume of the capsules more than \(\Delta V^*\) can be confirmed by the formation of an indentation on the capsule membrane. In particular, sets of such semipermeable capsules of which the aqueous cores have different osmolarity can be used to estimate the osmolarity of the continuous phase. We demonstrate this by using semipermeable ETPTA capsules. The ETPTA membrane is permeable for water molecules, whereas impermeable for PVA and sodium and chloride ions; the permeability of water through the ETPTA membrane is estimated as \(2 \times 10^{-24} \text{ m}^2\) in previous work.[6b,8] For example, a mixture of four distinct ETPTA capsules, confining aqueous solutions with 33, 342, 649, and 950 mOsm L\(^{-1}\) respectively, was dispersed in an aqueous solution with 475 mOsm L\(^{-1}\). This resulted in the selective buckling of two capsules, namely those with 33 and 342 mOsm L\(^{-1}\), because of the positive osmotic pressure, whereas the other two capsules, with 649 and 950 mOsm L\(^{-1}\), remained unchanged in size and shape, as shown in Figures 2b and c. Although the latter capsules experienced a negative pressure, they remained without inflation or release of

![Figure 2](https://www.small-journal.com/1157)
encapsulants because of the high modulus of ETPTA, $E_{\text{ETPTA}}$ (as high as 600 MPa).[11] In a similar fashion, the mixture was dispersed in aqueous solutions with 175, 767, and 1068 mOsm L$^{-1}$, resulting in a selective buckling of capsules when subjected to positive osmotic pressure, whereas the others remained, as shown in Figure S2 of the Supporting Information. It is shown that only red capsules buckled in aqueous solutions with 175 mOsm L$^{-1}$, whereas red, orange, and yellow capsules buckled in aqueous solutions with 767 mOsm L$^{-1}$, and all capsules buckled in aqueous solutions with 1068 mOsm L$^{-1}$. Therefore, we can estimate the osmolarity of the continuous phase by observing the buckling of a set of ETPTA capsules containing aqueous solutions with different osmolarity. However, buckling of the ETPTA capsule is not highly sensitive to the osmotic pressure difference, thus lowering the precision of this estimation. To evaluate the sensitivity, four distinct ETPTA capsules with 412, 456, 492, and 550 mOsm L$^{-1}$ were prepared as shown in Figure S3 of the Supporting Information, and they were dispersed in an aqueous solution with 475 mOsm L$^{-1}$. Although both red and orange capsules were subjected to positive osmotic pressure, only the red capsules buckled as shown in Figures 3a and b. The orange capsules were subjected to an osmotic pressure difference, $\Pi = \Delta CRT$, of 47 kPa ($\Delta C$ ca. 19 mOsm L$^{-1}$) through the membrane, which was not enough to buckle the ETPTA capsules, whereas the red capsules were subjected to 156 kPa ($\Delta C$ ca. 63 mOsm L$^{-1}$), where $R$ is the gas constant and $T$ is the temperature. Similarly, the mixture dispersed in an aqueous solution with 592 mOsm L$^{-1}$ exhibited buckling of the red, orange, and yellow capsules, as shown in Figures 3c and d, although all capsules were subjected to positive osmotic pressure; the green capsules were only subjected to 104 kPa ($\Delta C$ ca. 42 mOsm L$^{-1}$). Therefore, we can estimate the threshold osmotic pressure difference needed to buckle the ETPTA capsules to be approximately 125 kPa ($\Delta C$ ca. 50 mOsm L$^{-1}$).

2.3. Preparation of PLA Capsules

For a spherical capsule with uniform thickness of the membrane, the threshold pressure difference, $\Pi$, for buckling is proportional to $E(h/R)^2$ and $\Delta V/V_0$ is proportional to $h/R$, where $V_0$ is the initial volume of the capsule.[10] Therefore, if the ratio of membrane thickness to radius of the capsule, $h/R$, is reduced, the capsule becomes more sensitive to the osmotic pressure difference and buckling begins earlier. To accomplish this, we used evaporation-induced consolidation of the middle phase, enabling shrinkage of the membrane during evaporation.[6b,12] In a fashion similar to that of the ETPTA capsules, we produced double-emulsion drops as templates, where 12 wt% chloroform solution of PLA was employed as the oil phase, instead of ETPTA. The double-emulsion drops were collected in a glass petridish and incubated at 35 °C for 1 hour to completely evaporate the chloroform. During the evaporation, the middle phase shrunk and finally became a solid membrane made of PLA which is biocompatible and biodegradable; more than 95% of double-emulsion drops generated were converted into stable PLA capsules during evaporation. Monodisperse PLA microcapsules with an average diameter of 125 µm are shown in Figure 4a. We dried the capsules and observed them with SEM, as shown in Figure 4b. During drying, all capsules deflated on the substrate, forming a thin film with a weak trace of the capsules. To characterize the thickness of the membrane, we made scars on the film using a razor blade, which enabled the measurement of the membrane thickness (150 nm) as shown in the inset of Figure 4b. Therefore, $h/R$ is 0.0024 which is reduced by a factor of 8 in comparison with the $h/R$ of the ETPTA capsules, which was 0.0196. This shows that buckling of the PLA capsules is much more sensitive to the osmotic pressure difference than that of ETPTA capsules even with the higher modulus of PLA (2050 MPa).[13] Although PLA is biodegradable, the capsules remain intact for one month if they are kept in an osmolarity-matched aqueous solution.[6b] The relative sensitivity is clearly shown in Figure 4c, where both PLA and ETPTA capsules were subjected to a positive osmotic pressure of 22.3 kPa ($\Delta C$ ca. 9 mOsm L$^{-1}$) across their membranes and approximately 100 capsules were used to plot this time-dependence of the fraction of buckled capsules. The buckling of capsules could be confirmed by the sudden formation of indentations during observation with an optical microscope. Although the rate of buckling is not fast under this low osmotic pressure difference, more than 70% of the PLA capsules were buckled within 12 hours, whereas less than 10% of the ETPTA capsules were buckled; we attribute
this buckling of ETPTA capsules to the concurrent formation of inhomogeneous capsules in small portions, making them more sensitive to the osmotic pressure difference.\[8\] We also attribute the distribution of the response time of the PLA capsules under low osmotic pressure difference to the inhomogeneity of the membrane thickness. Approximately 90\% of the PLA capsules buckled during 1-day incubation, whereas 10\% remained unbuckled. This is because of the co-generation of double-emulsion drops with a relatively thick shell that convert into less-sensitive capsules to osmotic pressure difference.

2.4. Estimation of Osmolarity Using PLA Capsules

We characterized the time- and osmotic pressure-dependence of the buckling of PLA capsules by observing them in various osmolarity conditions whereby the difference in osmolarity was 0, 5, 9, 16, 30, and 43 mOsm L\(^{-1}\), as shown in Figure 5. Images of the PLA capsules, taken at 10 minutes and 210 minutes after they were subjected to the osmotic pressure difference of 74.3 kPa (\(\Delta C \approx 30\) mOsm L\(^{-1}\)) across their membranes.

Figure 4. a) Optical microscopy image of microcapsules with a diameter of 125 \(\mu\)m whose membrane is made of PLA. b) SEM image of the dried PLA capsules; the capsules are completely deflated, forming a thin film on the substrate. The inset shows the thickness of the membrane, 150 nm. c) Time-dependence of the fraction of buckled capsules, where both ETPTA and PLA capsules are subjected to a positive pressure difference of 22.3 kPa (\(\Delta C = 9\) mOsm L\(^{-1}\)) across their membranes.

Figure 5. a) Optical microscopy images of PLA microcapsules, taken at the denoted times in the images after they were subjected to a positive osmotic pressure of 74.3 kPa. b) Time-dependence of the fraction of buckled PLA capsules, whereby the PLA capsules were subjected to a series of positive pressure differences of 0, 12.4, 22.3, 39.6, 74.3, and 106.5 kPa across their membranes; these pressure differences correspond to 0, 5, 9, 16, 30, and 43 mOsm L\(^{-1}\) differences, respectively.

buckled after 184, 113, and 42 minutes, respectively. For small pressure differences such as 12.4 kPa (\(\Delta C = 5\) mOsm L\(^{-1}\)) and 22.3 kPa (\(\Delta C = 9\) mOsm L\(^{-1}\)), it takes 840 and 500 minutes, respectively. Therefore, the precision of the osmolarity measurement is smaller than 5 mOsm L\(^{-1}\); however, the measurements take a long time, which can lead to problems, such as evaporation, for practical applications and therefore the time needs to be further reduced by making thinner membranes with a higher permeability.

Folding of the PLA membrane with high curvature during buckling can lead to rupture of the capsules, forming cracks or holes on the membrane surface. Subsequently, PVA and ions can diffuse through the scars, thereby eliminating any osmotic pressure difference. This absence of stress enables the deformed capsules to recover their spherical shape. For example, the capsules subjected to high osmotic pressure difference of 106.5 kPa (\(\Delta C = 43\) mOsm L\(^{-1}\)) initially buckled by forming an indentation and then, recovered their spherical shape, as shown in Figure 6a. Although the scars are not visible under an optical microscope, we confirmed the diffusion of materials by weakening the optical contrast of the capsules after they recovered their spherical shape, as shown in the last image of Figure 6a. Diffusion of materials leads to the reduction of the refractive index contrast between the interior and exterior of the capsule, thereby resulting in a low optical contrast. The time-dependence of the buckling and rupturing of the capsules, subjected to a positive osmotic pressure of 106.5 kPa (\(\Delta C = 43\) mOsm L\(^{-1}\)), is shown in Figure 6b. The fraction of buckled capsules quickly increases as they are
subjected to the osmotic pressure difference, whereas the fraction of ruptured capsules slowly increases because of the high degree of membrane folding required to induce rupture. The fraction of ruptured capsules was always smaller than the fraction of buckled capsules and it decreased with decreasing applied osmotic pressure difference.

The release of encapsulants from osmocapsules is not always favored. To make inert osmocapsules without release function, we prepared capsules using a middle phase of 10 wt% toluene solution of poly(lactic-co-glycolic acid) (PLGA); PLGA is also a biocompatible and biodegradable polymer but it is less brittle than PLA. Most PLGA capsules, subjected to a positive osmotic pressure of 106.5 kPa (ΔC = 43 mOsm L⁻¹), buckled and remained deformed. In addition, the capsules retained the encapsulants during the buckling as shown in Figure S4 of the Supporting Information.

The buckling behavior of PLA microcapsules is much more sensitive to osmotic pressure differences because of the smaller h/R compared to that of ETPTA capsules, thereby providing higher precision in measurement of osmotic strength. We demonstrate this with a set of PLA capsules encapsulating aqueous solutions of 94, 118, 129, and 148 mOsm L⁻¹, as shown in Figures S5 and S6, where the capsules are labeled with red, orange, yellow, and green colors in the same manner to the ETPTA capsules. The mixture of these four distinct capsules was dispersed into five different aqueous solutions with 92, 110, 124, 147, and 163 mOsm L⁻¹, as shown in Figure 7. All PLA capsules subjected to positive osmotic pressure were buckled, whereas the other capsules remained spherical. Therefore, it proves that the mixture can be used to estimate the osmotic strength of unknown

Figure 6. a) Optical microscopy images of a PLA microcapsule, taken at denoted times in the images after subjection to a positive osmotic pressure difference of 106.5 kPa; the capsule buckled first (middle image) and then recovered its spherical shape (right image) after rupture of the membrane. The optical contrast becomes weaker upon rupture. b) Time-dependence of fractions of buckled (red line) and ruptured (black line) PLA capsules, where the PLA capsules are subjected to a positive pressure difference of 106.5 kPa across their membranes.

Figure 7. Confocal microscopy images of a mixture of four distinct PLA microcapsules dispersed in an aqueous solution of 92, 110, 124, 147, and 163 mOsm L⁻¹, whereby the red, orange, yellow, and green capsules contain aqueous solutions of 94, 118, 129, and 148 mOsm L⁻¹, respectively; all capsules subjected to positive osmotic pressure are buckled as denoted with arrows, whereas others remained spherical. Some capsules were ruptured under high positive pressure, appearing transparent due to the release of the dye.
aqueous solutions. Some PLA capsules dispersed in aqueous solutions with high osmolarities of 147 and 163 mOsm L\(^{-1}\) exhibited rupture of their membranes because of the high degree of buckling, and they appeared transparent without color due to the release of the dye molecules, whereas most PLA capsules when dispersed in an aqueous solution with low osmolarity of 92, 110, and 124 mOsm L\(^{-1}\) retained the dye. To estimate the osmolarity of an unknown solution, we can apply two or three sets of these osmocapsules, where the first set can be used to find a rough range of the osmolarity and the second and third sets will be used to estimate the value more precisely. For example, to cover a wide range of osmolarity of 0–1000 mOsm L\(^{-1}\), three steps of measurements with five distinct osmocapsules at each step can be used, which will provide an error smaller than 8 mOsm L\(^{-1}\). If 10 capsules are employed for each osmolarity to have enough statistics and avoid mismeasurement, a total of 150 capsules (ca. 100 nL) would be required per single measurement. Although the capsules can buckle in a sample whose volume is similar to that of the capsules, in practice a volume of 100 times that of the capsules (i.e. 10 µL), will be required; which is still much smaller than the volume required for conventional micro-osmometers.

3. Conclusion

In this work, we report a method to estimate the osmotic strength of aqueous solutions using microcapsules with an ultra-thin membrane. Microfluidic emulsification of biphasic flows enables the production of monodisperse double-emulsion drops with an ultra-thin middle phase, providing a stable template to produce microcapsules containing an aqueous solution with standard osmotic strength. By employing photocurable monomers as the middle phase, microcapsules with a ratio of membrane thickness to capsule radius of approximately 0.02 were prepared by UV-induced polymerization. These resultant capsules exhibited buckling when subjected to a positive osmotic pressure as high as approximately 125 kPa. Highly sensitive microcapsules to osmotic pressure difference were prepared by evaporation-induced consolidation of the middle phase. Because of shell shrinkage during evaporation, the resultant capsules have a ratio of membrane thickness to capsule radius of approximately 0.002. These sensitive microcapsules buckled under positive osmotic pressures as small as 12.5 kPa, thereby enabling the precise estimation of the osmotic strength using a set of distinct microcapsules containing aqueous solutions of different standard osmotic strengths. This approach directly measures the osmotic strength using semipermeable membranes without complex setup or analysis. In addition, it requires a very small volume of the sample liquid. Therefore, it has great potential as an alternative method to measure the osmotic strength. Furthermore, this capsule-based method can be potentially used for in-vivo measurement of the osmotic strength by implanting biocompatible and degradable PLGA or PLA capsules into tissue. At the same time, we expect that the PLA capsules can be used as smart delivery vehicles that sense osmotic pressure and release encapsulants by rupture of their membrane.

In addition, the capsules can be inserted into reactors or microfluidic channels, which enables in-situ monitoring of the osmotic pressure without sampling the solution. The current approach has almost no limitations on material selection for the membrane, thereby potentially providing a wide variety of properties and applicable areas for osmotic pressure measurement.

4. Experimental Section

Materials: Aqueous solutions containing PVA (Mw 13 000–23 000, Sigma-Aldrich) and NaCl were used as the innermost and continuous phase of the double-emulsion drops. As oil phases, we used one of ETPA containing 0.2 wt% photoinitiator (2-hydroxy-2-methylpropio-phenone, Sigma-Aldrich), 12 wt% chloroform solution of poly(lactic acid) (PLA, Mw 15,000, Polyscience, Inc.), and 10 wt% toluene solution of poly(lactic-co-glycolic acid) (PLGA, Mw 120,000, 8515 DL High N, Evonik). For some experiments, we dispersed iron oxide nanoparticles (smaller than 50 nm, Sigma-Aldrich) to render our capsules magneto-responsivity and silica particles to improve the dispersion stability.[14] To distinguish the capsules containing aqueous solutions with different osmolarity, we dissolved red dye, sulforhodamine B (Sigma-Aldrich) and green dye, 8-hydroxy-1,3,6-pyrenetrifluorosonic acid, trisodium salt (Spectrum Chemicals) in the innermost phase with four different mixing ratios; confocal microscopy showed four different colors of red, orange, yellow, and green in overlay images. For the same purpose, we also used common food-coloring pigments with red and green colors.[15] The osmolarity of aqueous solutions was measured by an osmometer based on depression of the freezing point (Model 3300, Advanced Instrument, Inc.) before injection.

Device Fabrication: Two cylindrical capillaries (World Precision Instruments, Inc., 1B100–6) were tapered by a micropipette puller (Sutter Instrument, P-97) to have a 70-µm diameter orifice and 190-µm diameter orifice, respectively. The cylindrical capillary with smaller orifice was treated with n-octadecyltrimethylsilane (Aldrich) to render the surface hydrophobic, whereas the other cylindrical capillary with larger orifice was treated with 2-[methoxy(polyethyleneoxy)propyl] trimethoxy silane (Gelest, Inc.) to render the surface hydrophilic. These two cylindrical capillaries were coaxially aligned in a square capillary and a small tapered capillary was inserted into the untapered opening of the hydrophobic cylindrical capillary.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgments

This work was supported by the KAIST HRHRP (Project No. N10130008), the International collaboration grant (No. Sunjin-2010–002) and the Industrial strategic technology development
program (No. 10045068) of the Korea Evaluation Institute of Industrial Technology funded by the Ministry of Trade, Industry, & Energy (MI, Korea). We dedicate this article to late Professor Seung-Man Yang for his lifelong contribution to colloid and interface science.


Received: July 27, 2013
Revised: October 3, 2013
Published online: January 30, 2014