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Enhanced Encapsulation of Actives in Self-Sealing Microcapsules by Precipitation in Capsule Shells

Yuanjin Zhao,[†,‡] Ho Cheung Shum,[*,†,§] Laura L. A. Adams,[†] Bingjie Sun,[†] Christian Holtze,[⊥] Zhongze Gu,[‡] and David A. Weitz[*,†]

[†]School of Engineering and Applied Sciences and Department of Physics, Harvard University, Cambridge, Massachusetts 02138, United States

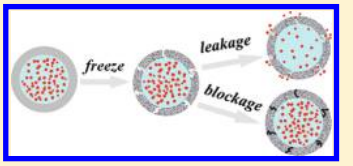
[‡]State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, China

[⊥]BASF SE, 67056 Ludwigshafen, Germany

[§]Department of Mechanical Engineering, The University of Hong Kong, 7/F Haking Wong Building, Pokfulam Road, Hong Kong, China

Supporting Information

ABSTRACT: Microcapsules with core−shell structures are excellent vehicles for the encapsulation of active ingredients; however, the actives often leak out of these structures over time, without observable damage to them. We present a novel approach to enhancing the encapsulation of active ingredients inside microcapsules. We use two components that can form solid precipitates upon mixing and add one each to the microcapsule core and to the continuous phase. The components diffuse through the shell in the same manner as the actives, but upon meeting, they precipitate to form solid particles within the shell; this significantly reduces leakage through the shell of the microcapsules. We show that the reduction in the leakage of actives is due to the blockage of channels or pores that exist in the shell of the capsules by the solid precipitates.



INTRODUCTION

Microcapsules hold great potential for applications involving the encapsulation, delivery, and release of actives in the fields of agriculture, health care, and foods and beverages.[1−3] A variety of physical and chemical methods, such as spray drying, coextrusion, interfacial polymerization, and complex coacervation, have been used for the high-throughput preparation of microcapsules.[4−6] However, the sizes, mechanical properties, and delivery and release characteristics of microcapsules obtained with these techniques vary significantly, even among capsules prepared in the same batch.[7] Microfluidic emulsification technologies can overcome the limitations of the variability in the production and are thus a promising alternative technique for the fabrication of microcapsules.[8−18] Because of the excellent flow control achieved in the microfluidic devices, emulsion drops are generated with exquisite precision, albeit only one drop at a time; this enables the formation of highly monodisperse emulsions. The degree of control afforded by microfluidics is highlighted by the ability to generate controlled double, triple, and even higher-order multiple emulsions, where the size and number of encapsulated droplets can be manipulated with high accuracy.

Using microfluidic technologies, a variety of actives have been encapsulated into monodisperse water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) double emulsions; these emulsions can then be solidified to form stable solid microcapsules by interfacial polycondensation or polymerization of the middle shell phase.[19−21] Alternatively, solid microcapsules can also be formed by freezing the shell phase of double emulsions; this approach enables the subsequent release of actives simply by heating the microcapsules above the melting temperature of their shell phase.[22,23] However, regardless of how the capsules are prepared, the leakage of actives from the capsules is often observed. This undesired leakage is exacerbated when there is a gradient in the concentration of the active ingredients across the shell, which causes an osmotic pressure difference. This imposes a tensile stress on the shell, which enhances the rate at which actives diffuse out of the capsules. This diffusion has been shown to be an activated process in which the tensile stress imposed on the shells lowers the activation energy for such diffusion.[24] This decreases the encapsulation efficiency and shortens the shelf life of the encapsulated actives. Therefore, effective strategies for avoiding such leakage are essential if these structures are to find practical applications.

In this article, we present a novel approach to alleviating the leakage of active ingredients from microcapsules during their storage. We achieve this by using two components that can form solid precipitates upon mixing; we introduce one to the core of the microparticles and the second to the continuous phase. We show that the two components diffuse across the shell the same way that the actives do and, upon mixing, form solid precipitates that block the path through which actives leak. Our results provide new insights into the process of leakage of actives during storage

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and suggest new strategies for preventing such leakage; this is particularly important to applications that require the long-term storage of actives by encapsulation.

EXPERIMENTAL SECTION

Materials. The inner phase used in microfluidics consisted of 1 wt % Allura Red AC or Tartrazine (Sigma-Aldrich Co.) and 1 wt % sodium carbonate. The middle oil phase was a molten Suppocire AIM oil (mixture of glycerides of saturated fatty acids from C8−C18, mp 33−35 °C, Gatefosse) or Witepsol H15 (mp 33.5−35.5 °C, fatty glyceride saturation C10−C18, Sasol) maintained at a constant temperature of 70 °C. The outer phase was a 10 wt % poly(vinyl alcohol) (PVA; M_w = 13 000−23 000 g·mol^{-1}, 87−89% hydrolyzed, Sigma-Aldrich Co.). Solutions were all filtered before introduction into glass microcapillary devices. Water with a resistivity of 18.2 MΩ·cm^{-1} was acquired from a Millipore Milli-Q system.

Microcapsule Fabrication. The microcapsules were templated from W/O/W double emulsions. These uniform double emulsions were prepared by using microcapillary devices. The round capillaries, with inner and outer diameters of 0.58 and 1.0 mm, respectively, were purchased from World Precision Instruments, Inc. and tapered to desired diameters with a micropipet puller (P-97, Sutter Instrument, Inc.) and a microforge (Narishige International USA, Inc.). The tapered round capillaries were fitted into square capillaries (Atlantic International Technology, Inc.) with an inner dimension of 1.0 mm for alignment. During the fabrication of the double emulsion, a typical set of flow rates for the outer, middle, and inner phases was 15 000, 2000, and 1000 μL/h, respectively. All fluids were pumped into the capillary microfluidic device using syringe pumps (Harvard PHD 2000 series). The generated double emulsions were collected in bottles that were filled with ice−water mixtures or a 1 wt % calcium chloride solution.

Characterization. The double-emulsion generation process in the microfluidic device was monitored using an inverted optical microscope (DM-IRB, Leica) connected to a high-speed camera (Phantom V9, Vision Research). Bright-field images were obtained with 5×, 10×, and 20× objectives at room temperature using an automated inverted microscope with fluorescence (Leica, DMIRBE) equipped with a digital camera (QImaging, QICAM 12 bit). The release profile of Allura Red AC and Tartrazine was monitored using a UV−vis spectrophotometer (Nanodrop, ND 1000) at room temperature (about 25 °C) without agitation. Scanning electron microscope (SEM) images of dried microcapsules coated with a thin layer of platinum and palladium were taken using a Zeiss Supra 55VP field emission scanning electron microscope (FESEM, Carl Zeiss, Germany) at an acceleration voltage of 3 kV.

RESULTS AND DISCUSSION

In this study, W/O/W double emulsions with a molten middle phase are used as templates for the fabrication of microcapsules. These monodisperse double emulsions are generated with a glass microcapillary microfluidic device that combines the coflow and flow-focusing geometry shown in Figure 1a. The inner droplets containing the actives are formed in the dripping regime using a small injection tube in the coflow geometry while the molten middle oil phase that encapsulates the inner droplets is flow-focused by the outer continuous phase from the opposite end. As a result, the jet breaks up into double-emulsion droplets. Because the inner phase is in contact only with the immiscible middle oil phase, coalescence between the inner phase and the continuous phase is prevented; thus no leakage of the actives to the outer continuous phase is observed during emulsion generation. The overall size of the double emulsions and the thickness of the shell can be adjusted by

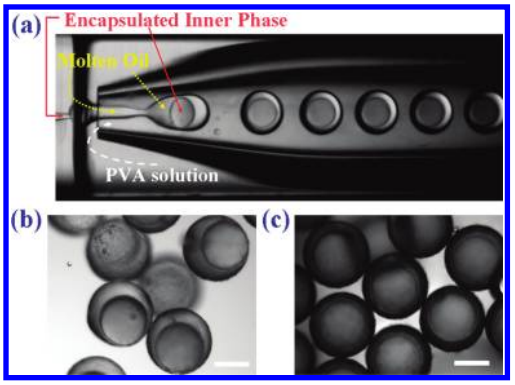


Figure 1. (a) Formation of W/O/W double emulsions with a molten middle oil phase in a glass microcapillary device. (b) Optical micrograph of the solid microcapsules obtained after collection and delayed solidification (about 20 s) in vials containing a 10% PVA solution at 4 °C. The inner drops are driven off-center by their buoyancy mismatch, leading to a very thin section of the shell. (c) Optical micrograph of the solid microcapsules after collection and solidification within about 5 s in cold water. The rapid solidification ensures that the inner drops remain centered. The scale bars denote 100 μm.

tuning the flow rates of the fluid phases and the diameters of the capillaries in the device. The uniformity in the size and shape of the double-emulsion droplets formed inside the collection tube makes them ideal templates for microcapsules.

Solid microcapsules are obtained by cooling the molten middle phase of the double emulsions below their melting temperature. Because the double emulsions are thermodynamically unstable, they must be cooled as quickly as possible and the osmotic pressure across their shells must be minimized. Because the innermost phase and the shell phase are often not density-matched, delaying the solidification of the shell can result in inner droplets that are significantly off-center, as shown in Figure 1b. The encapsulated actives tend to leak quickly from these microcapsules, particularly through the thin regions of the shell, significantly reducing their encapsulation efficiency. Therefore, we prepare the microcapsules by cooling the double emulsions immediately inside the collection tube and collecting them in an ice−water mixture or a cooled salt solution with osmolarity matched to that of the innermost phase. Because the microcapsules are solidified almost immediately after their formation, the off-centering of the inner droplets is minimized (Figure 1c) and there is no loss of encapsulation efficiency due to leakage through the nonuniform shell.

Even though the dye is well encapsulated by the glycerides during emulsion generation and the shells are not disrupted throughout the observation period, dye leakage is still observable. To visualize this leakage, we encapsulate a model compound, Allura Red AC food dye, in the fatty acid glyceride microcapsules and monitor the leakage of the dye from the microcapsules by detecting the UV−vis absorbance of the continuous phase.We find an average leakage of 16.3% in 4 weeks, as shown by the gradual coloring of the continuous phase in the photographs of the capsule suspension and by the increase in UV−vis absorbance in Figure 2. By increasing the thickness of the glyceride shells of the microcapsules, the leakage of the encapsulated food dye is reduced, as shown in Supporting Information Figure S1. However, this improvement inevitably reduces the loading of the encapsulated actives in the capsules.

To reduce this undesired leakage effectively, we add two reactants of a precipitation reaction, one to the inner phase of

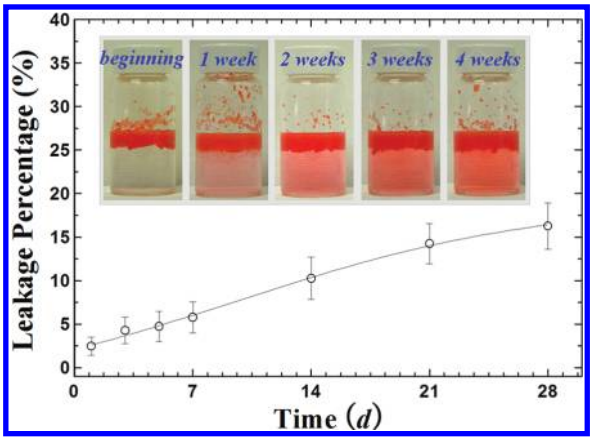
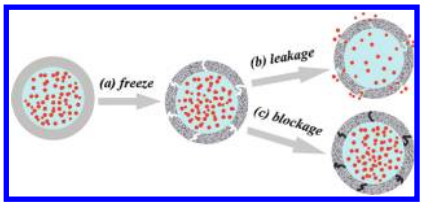


Figure 2. Plots of the Allura Red AC food dye leakage percentage from the plain microcapsules as a function of preserving times. The line is a guide to the eye. The inset shows pictures of the microcapsules in the vessels after different storage times.

the microcapsule and the second to the continuous phase. These reactants presumably diffuse across the capsule shells in the same way as do the actives. Upon meeting in the shell layer of the capsules, the two reactants form solid precipitates that effectively block the pathways through which the actives diffuse, as indicated in Scheme 1. Therefore, the microcapsules are self-sealing. To implement this concept, two common salts, sodium carbonate and calcium chloride, are dissolved in the inner phase and the continuous solution. To assess the leakage correctly, we prepare all microcapsules with a 2:1 volume ratio of the glycerides and the encapsulated inner droplets. The resultant microcapsules have a core diameter of 130 μm and a shell thickness of 30 μm. After the glyceride shells of the microcapsules are solidified, the salts in the solutions of the inner droplets and the outer collection can diffuse across the shells. When the salts meet, they react to form solid calcium carbonate. Using this approach, the leakage of the dye in the microcapsules is significantly reduced, from 16 to only 3% in a month, as shown by the reduction in the UV−vis absorbance in Figure 3. As a control, we repeat the experiments by replacing the reactants with nonreactive salts at the same concentration. When only sodium carbonate is added to the inner phase without calcium chloride added to the continuous phase, 35% of the dye leaks out over 4 weeks. In this case, the leakage of actives is exacerbated by the large difference in osmolalities between the inner and continuous phases. However, even when sodium carbonate and sodium chloride are added to the two phases with no osmotic pressure across the capsule shells, 22% of the dye leaks out over 4 weeks. These profiles confirm the efficacy of the precipitation strategy in preventing the leakage of the actives from the microcapsules. To validate our hypothesis further, we look for the precipitates in the shell of the microcapsules using elemental analysis; indeed, calcium is detected in the solid shell of the microcapsules (as shown by the energy-dispersive X-ray (EDX) spectroscopy data in Supporting Information Figure S2). Our results suggest that dye molecules leak out of the capsules through small pores that may form upon the rapid solidification of the shell and that are blocked with the precipitates. Pores can indeed be observed on the surface of the capsules, as confirmed by SEM images of the microcapsules in Supporting Information Figure S3. To demonstrate the generality of this approach for different active molecules and shell materials, we repeat the

Scheme 1. Illustration of the Encapsulation and Leakage of Actives in a Microcapsule[a]



[a] (a) Small channels form in the solid shell of the microcapsules during freezing-induced solidification. (b) The encapsulated actives leak from pores and channels in the shell layer. (c) Introduction of the reactants for precipitation reaction leads to blockage of the pores and channels, thus enhancing the encapsulation of actives.

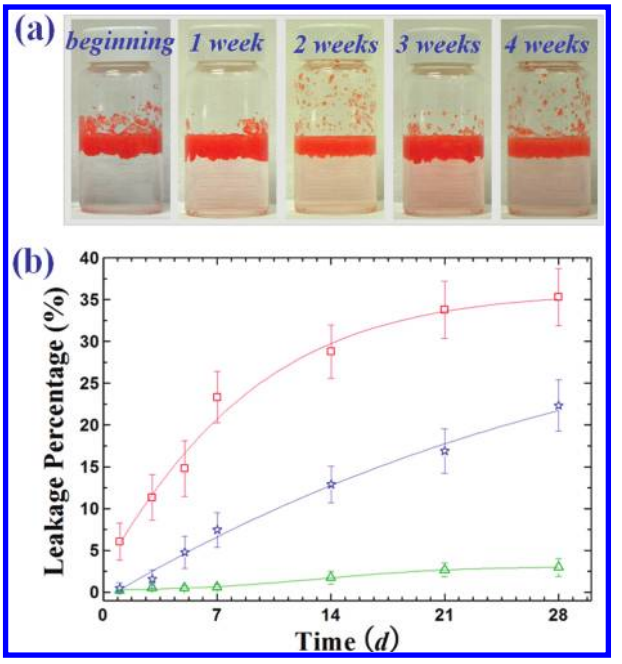


Figure 3. (a) Photographs of the microcapsules encapsulating Allura Red AC dye in the vessels after preserving different times. Reactants for precipitation have been added to the inner and continuous phases in all capsules. (b) Plots of the food dye leakage percentage from the glyceride microcapsules as a function of storage time. Squares represent the microcapsules containing sodium carbonate solution and pure water in the inside and outside of the capsules; pentagons represent the microcapsules with sodium carbonate solution and sodium chloride solution in the inside and outside of the capsules; and triangles represent the microcapsules with sodium carbonate solution and calcium chloride solution in the inside and outside of the capsules. The lines are guides to the eye.

experiments using Tartrazine and Witepsol H15 oil as an alternative encapsulant and shell material, respectively. In this case, the leakage of actives is reduced significantly, as shown by the reduction in the intensity of the yellow color in the continuous phase in the pictures in Supporting Information Figure S4. Even though we demonstrate the concept of enhancing the encapsulation using 1 wt % sodium carbonate and calcium chloride for subsequent precipitation in the shell, leakage of the encapsulated species was significantly reduced even with a low concentration (0.1 wt %) of sodium carbonate and calcium chloride in the system. Our approach for enhancing the actives

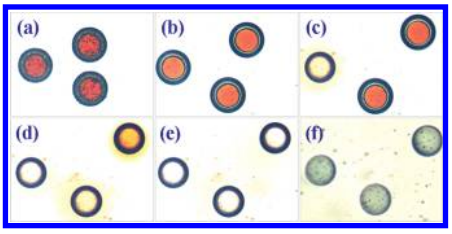


Figure 4. Release of Allura Red AC from the solid glyceride microcapsules after heating to trigger the release. (a−e) Bright-field microscope images showing the dye release from three microcapsules during heating; the whole process of release takes about 2 min. (f) Bright-field image of the refrozen microcapsules after the triggered release of the red dye, showing the solid particles that remain.

encapsulation should also be applicable to capsules fabricated using polymerization methods.

Despite the good encapsulation and preservation stability, the actives in the precipitate microcapsules can nevertheless be easily released upon triggering. After the microcapsules are heated to above their melting temperature, their shell phase melts and the microcapsules are no longer stable, thus the inner droplets coalesce with the continuous phase, releasing the actives, as shown in Figure 4. The complete release of actives is confirmed by the formation of solid wax spheres rather than microcapsules after refreezing, as shown in the optical micrographs in Figure 4f. These results confirm that whereas the incorporation of the precipitation reaction enhances the encapsulation of actives, the release of actives is not compromised.

CONCLUSIONS

We present a novel approach to enhancing the encapsulation of active ingredients inside microcapsules. The monodisperse microcapsules are generated in glass microcapillary microfluidic devices. By adding reactants for a precipitation reaction separately to the inner and continuous phases of the microcapsules, the leakage of actives can be significantly reduced. The formation of precipitates within the shell blocks pores, slowing the leakage rate. Our approach to improving the performance of solid microcapsules does not require any additional processing steps yet enables fabrication of self-sealing microcapsules with the highly efficient encapsulation of actives.

ASSOCIATED CONTENT

Supporting Information. Plot of the leakage percentage of the actives as a function of storage time for different shell thickness microcapsules, EDX spectroscopy and SEM images of the microcapsules, and pictures of the microcapsules encapsulating Tartrazine after storage for 3 months. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: ashum@hku.hk (H.C.S.); weitz@seas.harvard.edu (D.A.W.).

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