J|A|C|S
COMMUNICATIONS

Published on Web 05/30/2008

One-Step Multicomponent Encapsulation by Compound-Fluidic Electrospray

Hongyan Chen,[†,‡] Yong Zhao,[*,†] Yanlin Song,[†] and Lei Jiang[*,†]

Beijing National Laboratory for Molecular Sciences (BNLMS), Center for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, P. R. China, and Graduate University of Chinese Academy of Sciences, Beijing 100080, P. R. China

Received March 11, 2008; E-mail: jianglei@iccas.ac.cn; zhaoyong@iccas.ac.cn

Microencapsulation is a highly sought-after technique for its extensive applications in fields like pharmaceutics, food science, material science, and others.[1] So far, various elegant physical or chemical encapsulation methods have been developed, such as microfluidic technique,[2a] self-assembly,[2b] emulsion,[2c] flow focusing technique,[2d] selective withdrawal technique,[2e] and a promising electrified coaxial cone jet generating technique.[3,4] However, most of these encapsulation methods generally operate only two kinds of materials (core and shell materials); in other words, they can only encapsulate one content at a time. If more kinds of core contents, especially active ingredients, need to be encapsulated, it inevitably falls into a multistep, troublesome, and inefficient process by routine strategies. Controlled integration of multiple components into one entity is of considerable significance in many fields. Recently, some scientists have devoted efforts to manipulate several kinds of materials into multiphase particles or capsules. Lahann et al. reported a side-by-side electrospray technique that could fabricate anisotropic particles with Janus or triphase structure.[5] Lately, a shell-in-shell capsule was fabricated by layer-by-layer (LbL) self-assembly of polyelectrolytes in which two different biomolecules could be inhibited in two concentric compartments respectively.[6] Nevertheless, how to simply and effectively encapsulate multiple contents into one shell and keep them independent is still a very important and challenging issue.

Here we report a compound-fluidic electrospray method that could one-step enclose multiple components into a single microcapsule without contact. The as-prepared microcapsules have multiple compartments inside, in each of which different content can be addressably loaded. This method is based on a multifluidic compound jet generating system aided by electrostatic force. The experimental setup is sketched in Figure 1a; here the simplest multicomponent capsule−bicomponent microcapsule fabrication system is taken for example. It is characterized by the hierarchical compound nozzle which is assembled by embedding two metallic capillaries separately into a blunt metal needle. We first fabricated a solid microcapsule in which two liquid components were enveloped. $Ti(OBu)_4$ sol, a precursor for titania, labeled with red fluorescent dye was chosen as shell fluid, and paraffin oil worked as two core fluids with one portion labeled with blue fluorescent dye while the other portion was without any dye. After the electrospray process, a layer of fine particles was collected on the counterelectrode. The as-fabricated particles exhibit spherical morphologies (Figure 1b) with diameter ranges from the micrometer to submicrometer scale (Figure 1S). Laser scanning confocal microscopy (LSCM) image discloses an interesting structure of a single particle (Figure 1c), which has two compartments inside with one filled with blue core content and the other filled with dark core content (without dye). It indicates that the two core fluids have been inhibited into individual compartments free of contact. Here,

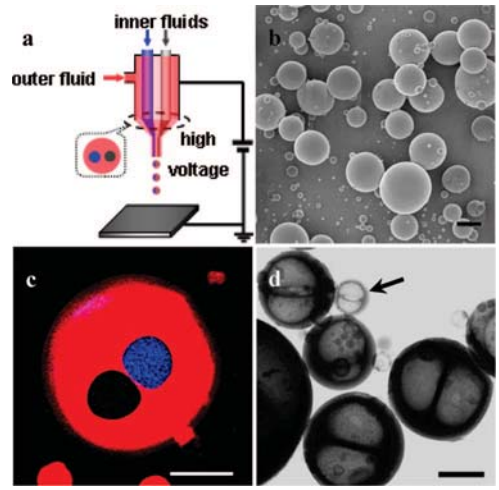


Figure 1. (a) Illustration of bicomponent microcapsule fabrication system. Two core liquids (blue and gray) were pumped out from two inner metal capillaries, respectively, and shell liquid (red) flowed through gaps between inner capillaries and outer needle. The immiscible core fluids and shell fluid formed a compound but not a mixed pendent drop under the exit of the nozzle. The compound jet was stretched to a meniscus (called Taylor cone) first in which shell fluid surrounded the two core fluids entirely and individually, and then fragmented into a spray of charged droplets when a proper high voltage was applied. The products were collected on the substrate below. The inset shows the cross-section of compound fluid at the outlet of nozzle. (b) SEM image of titania composite capsules, which range from submicrometer to several micrometers. Scale bar: 2 μm. (c) LSCM overlay image of titania composite capsules. The two core contents have been inhibited into individual compartments without contact. Scale bar: 10 μm. (d) TEM image of "θ" structured titania bicompartment microcapsules after organics have been removed by calcination. The two compartments correspond to the vacancies of the inner fluids. The smallest capsule is only hundreds of nanometers as arrow directed. Scale bar: 1 μm.

capsules with diameters above 10 μm were fabricated to clearly show the inner structure of capsules using a microscope, and they were obtained using a relatively higher flow rate of shell fluid than the case in Figure 1b. Furthermore, the TEM image (Figure 1d) also confirms this novel dual-compartment structure which has a consecutive dissepiment embedded, liking a Greek character "θ". The samples have been calcined to remove the organic materials for better imaging result, and two hollow compartments correspond to the vacancies of the paraffin oil. In addition, almost all of the products are of this dual-compartment structure except for the very smaller ones, which may result mainly from coulomb fission of charged drops during the jetting process. It should be noted that no operations were undertaken here to optimize the size distribution of products. Actually, the experimental parameters could be controlled further to obtain capsules with relative uniform size.[7a–d]

Microcapsules with more components encapsulated are also available by this method by rationally designing the configuration of the compound nozzle. As mentioned before, the nozzle for

[†] Institute of Chemistry, Chinese Academy of Sciences.
[‡] Graduate University of Chinese Academy of Sciences.

10.1021/ja801803x CCC: $40.75

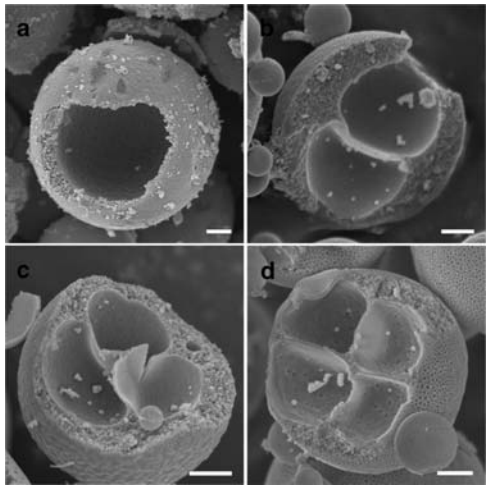


Figure 2. Cross-section SEM images of titania microcapsule with well-tailored one (a), two (b), three (c), and four (d) compartments, respectively. They correspond to the original compound nozzle with same number of inner capillaries. Scale bar: 500 nm.

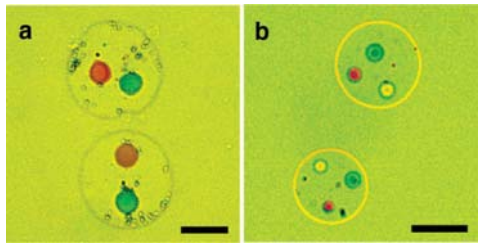


Figure 3. Micrographs of glycerol-in-Somos liquid microcapsules. (a) Two different glycerol fluids are loaded separately. (b) Three glycerol fluids are independently loaded. Scale bar: 20 μm.

bicomponent capsules was assembled by inserting two slender capillaries into a thick outer needle as illustrated in Figure 1a. Analogously, if more core contents are needed, the corresponding numbers of inner capillaries could be inserted into the outer needle to assemble the required compound nozzle, and each capillary would be fed with selected core fluids, respectively. In fact, titania composite capsules with one, two, three, and four core fluids loaded have been fabricated following this method, which corresponds to capsules with one to four cavities after organics have been removed (Figure 2a−d). The inner structures show good fidelity to the original compound nozzle which validates the capability and handleability of this versatile technique.

Besides hybrid or organic microcapsules, organic multicomponent microcapsules were also fabricated using photopolymer Somos 14120 as shell material and glycerol as core material. Figure 3a and 3b indicate that two and three core contents (blue, red, and yellow dyed) have been expectably encapsulated into one microcapsule independently, which verified the reliable practicability of such proposed approach.

In traditional electrospray[7] or coaxial electrospray[3] processes, the final morphologies of products are mainly affected by electrohydrodynamic parameters (electric field strength, flow rate) and properties of the jetting liquids (concentration, viscosity, conductivity, surface tension, et al.), and all of parameters can be controlled with relative ease. These parameters also play similar roles in our experimental system. Besides these, however, the rational design of the compound nozzle is the key point for the successful fabrication of capsules with the novel multicompartment structure. To do this job, the outer shell liquid must surround each inner core fluid completely to form a liquid "jacket".[4k] Therefore, each inner capillary should be kept at noncontact with other inner capillaries or even with the outer needle. By this way, shell fluid flows through the gaps between inner capillaries and outer needle, ensuring a completely envelope of core fluids. If the inner capillaries made contact with each other, multiple inner fluids would get mixed, making the multicompartment structure unavailable. In addition, an immiscible core and shell fluids pair was also important for the precise compartmentalization of multiple core contents, for its ability to obtain clear boundary between the core and shell materials.

We have proposed a very facile and effective compound-fluidic electrospray technique to produce multicomponent microcapsules with a novel multicompartment structure in a single step. Such microcapsules can not only promise to enlarge the functionality of a single entity by loading multiple ingredients simultaneously, but also protect each of the ingredients from each other and from the environment, which is critical for the effective encapsulation of active ingredients, such as sensitive and reactive materials. The general technique could be readily extended to produce diverse microcapsules with many other functional materials. This multi-function-in-one, high efficiency technique may establish an avenue for a wide range of applications, such as multicomponent drug delivery and as a microreactor.

Acknowledgment. We thank the NSFC (G.90306011, G.20571077 and G.20774101), National Basic Research Program (2007CB936403), MoST Research Program 2007AA03Z327, and Innovation Foundation of CAS for continuing financial support.

Supporting Information Available: Detailed experimental information, size distributions of products, additional CLSM data, and solution properties. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Sah, H.; Toddywala, R.; Chien, Y. W. *J. Controlled Release* **1994**, *30*, 201–211. (b) Champagne, C. P.; Fustier, P. *Curr. Opin. Biotechnol.* **2007**, *18*, 184–190. (c) Comiskey, B.; Albert, J. D.; Yoshizawa, H.; Jacobson, J. *Nature* **1998**, *394*, 253–255. (d) Turkenburg, D. H.; Antipov, A. A.; Thathagar, M. B.; Rothenberg, G.; Sukhorukov, G. B.; Eiser, E. *Phys. Chem. Chem. Phys.* **2005**, *7*, 2237–2240.

(2) (a) Okushima, S.; Nisisako, T.; Torii, T.; Higuchi, T. *Langmuir* **2004**, *20*, 9905–9908. (b) Peyratout, C. S.; Dähne, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 3762–3783. (c) Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. *Science* **1994**, *263*, 1600–1603. (d) Utada, A. S.; Lorenceau, E.; Link, D. R.; Kaplan, P. D.; Stone, H. A.; Weitz, D. A. *Science* **2005**, *308*, 537–541. (e) Cohen, I.; Li, H.; Hougland, J. L.; Mrksich, M.; Nagel, S. R. *Science* **2001**, *292*, 265–267.

(3) (a) Loscertales, I. G.; Barrero, A.; Guerrero, I.; Cortijo, R.; Márquez, M.; Gañán-Calvo, A. M. *Science* **2002**, *295*, 1695–1698. (b) Larsen, G.; Velarde-Ortiz, R.; Minchow, K.; Barrero, A.; Loscertales, I. G. *J. Am. Chem. Soc.* **2003**, *125*, 1154–1155. (c) Marín, Á. G.; Loscertales, I. G.; Márquez, M.; Barrero, A. *Phys. Rev. Lett.* **2007**, *98*, 014502. (d) Chen, X.; Jia, L.; Yin, X.; Cheng, J. *Phys. Fluids* **2005**, *17*, 032101.

(4) (a) Li, D.; Xia, Y. *Nano Lett.* **2004**, *4*, 933–938. (b) Sun, Z.; Zussman, E.; Yarin, A. L.; Wendorff, J. H.; Greiner, A. *Adv. Mater.* **2003**, *15*, 1929–1932. (c) Yu, J. H.; Fridrikh, S. V.; Rutledge, G. C. *Adv. Mater.* **2004**, *16*, 1562–1566. (d) Zussman, E.; Yarin, A. L.; Bazilevsky, A. V.; Avrahami, R.; Feldman, M. *Adv. Mater.* **2006**, *18*, 348–353. (e) Zhang, Y.; Huang, Z.-M.; Xu, X.; Lim, C. T.; Ramakrishna, S. *Chem. Mater.* **2004**, *16*, 3406–3409. (f) Dror, Y.; Salalha, W.; Avrahami, R.; Zussman, E.; Yarin, A. L.; Dersch, R.; Greiner, A.; Wendorff, J. H. *Small* **2007**, *3*, 1064–1073. (g) Reznik, S.; Yarin, A. L.; Zussman, E.; Berkovici, L. *Phys. Fluids* **2006**, *18*, 062101. (h) Yarin, A. L.; Zussman, E.; Wendorff, J. H.; Greiner, A. *J. Mater. Chem.* **2007**, *17*, 2585–2599. (i) Bazilevsky, A. V.; Yarin, A. L.; Megaridis, C. M. *Langmuir* **2007**, *23*, 2311–2314. (j) Li, X. H.; Shao, C. L.; Liu, Y. C. *Langmuir* **2007**, *23*, 10920–10923. (k) Zhao, Y.; Cao, X.; Jiang, L. *J. Am. Chem. Soc.* **2007**, *129*, 764–765.

(5) (a) Roh, K.-H.; Martin, D. C.; Lahann, J. *Nat. Mater.* **2005**, *4*, 759–763. (b) Roh, K.-H.; Martin, D. C.; Lahann, J. *J. Am. Chem. Soc.* **2006**, *128*, 6796–6797.

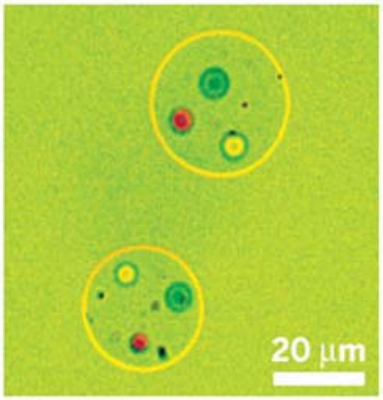
(6) Kreft, O.; Prevot, M.; Möhwald, H.; Sukhorukov, G. B. *Angew. Chem., Int. Ed.* **2007**, *46*, 5605–5608.

(7) (a) Berkland, C.; Pack, D. W.; Kim, K. *Biomaterials* **2004**, *25*, 5649–5658. (b) Xie, J.; Marijnissen, J. C. M.; Wang, C.-H. *Biomaterials* **2006**, *27*, 3321–3332. (c) Fantini, D.; Zanetti, M.; Costa, L. *Macromol. Rapid Commun.* **2006**, *27*, 2038–2042. (d) Xu, Y.; Hanna, M. A. *Int. J. Pharm.* **2006**, *320*, 30–36. (e) Jiang, L.; Zhao, Y.; Zhai, J. *Angew. Chem., Int. Ed.* **2004**, *43*, 4338–4341.

JA801803X

Multiple Components Individually Wrapped

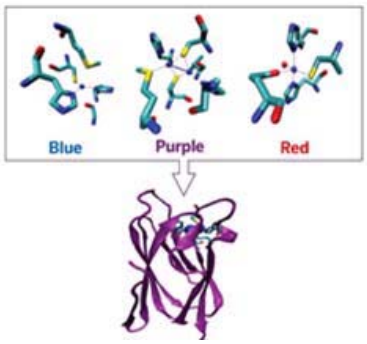
An electrospray method developed by Yong Zhao, Lei Jiang, and coworkers at the Chinese Academy of Sciences in Beijing can trap and keep separate multiple components in a single cell-like capsule in one step (*J. Am. Chem. Soc.*, DOI: 10.1021/ja801803x). This technique could lead to applications in drug delivery, materials science, and food science. The researchers first embedded multiple capillaries into a needle to form a compound nozzle. They then fed a viscous inorganic "shell" fluid through the needle and up to four different organic "core" fluids through the capillaries. The shell and core fluids formed a compound fluid as they met at the nozzle's outlet. Under an applied voltage to the nozzle, the compound fluid stretched and fragmented into a spray of charged droplets that formed into larger capsules. Droplets of each of the core fluids ended up with a "jacket" of shell fluid, which kept them separate from the other components in each capsule. In one test, the researchers used their one-step apparatus to separately encapsulate three different glycerol-based fluid droplets in a photopolymer shell. Previous methods to enclose multiple components in this manner required numerous steps and couldn't always keep the components separate, the researchers note.



J. Am. Chem. Soc.

THREE GLYCEROL FLUIDS SEPARATELY LOADED INTO PHOTOPOLYMER CAPSULES.

Three Protein Copper Sites Interconnected



The copper sites in cupredoxins, a family of proteins that facilitate a variety of redox activities, are structurally and functionally diverse. As little as 10% of their amino acid sequence is identical. Nevertheless, the copper sites are always found within a similar β-barrel protein structure and may be evolutionarily linked. Studies on the binuclear Cu_A (purple) site of nitrous oxide reductase (N_2OR) by Masha G. Savelieff, Yi Lu, and coworkers at the University of Illinois, Urbana-Champaign, now provide experimental evidence for that connection (*Proc. Natl. Acad. Sci. USA*, DOI: 10.1073/pnas.0711316105). The researchers added copper to metal-free protein and observed by electronic absorption and electron paramagnetic resonance spectroscopy that copper was first incorporated almost equally in separate type 1 (blue) and type 2 (red) sites. Over time the separate sites converted to the binuclear Cu_A structure. "These observations suggest that the purple Cu... site contains the essential elements of type 1 and type 2 copper