A facile approach to synthesize uniform hydrogel shells with controllable loading and releasing properties†

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We present a facile and straightforward method to synthesize uniform poly(vinyl amine) hydrogel shells with excellent loading capability for active materials and controllable responsiveness to applied stimuli, providing tunable releasing properties.

Encapsulation techniques have become of great interest for a variety of fields of fundamental research as well as industrial applications; they have provided efficient systems for protection and controlled release of encapsulated materials. Typical systems include micelles,[1] liposomes,[2] polymersomes,[3] colloidosomes,[4] polyelectrolyte microcapsules,[5] hydrogel capsules,[6] and polymeric micro- or nanoparticles.[7] Among them, there have been considerable studies on hydrogel-based capsules due to the bio-friendly encapsulation process, their high loading capacity, tunable permeability, and controllable phase properties by using external triggers. These advantages of hydrogel capsule systems can allow us to explore new substrates for biochemical processes and probing materials for diagnostics; there have been efforts to develop new types of capsules, which are suitable for these applications, by using sophisticated techniques, such as layer-by-layer assembly[8] or microfluidics.[9] However, to make them more widely useful, it is essential to develop techniques that enable fabrication of hydrogel capsules with controllable morphologies as well as uniform sizes, since these are critical to fine-tuning of the release kinetics of encapsulates.[10] Moreover, they should be produced using a simple process, thus making them more practical in their ultimate applications.

In this communication, we introduce a facile method for synthesizing hydrogel shells, which are hollow-structured microcapsules. The procedure, carried out using precursor polymer particles dispersed in a strong basic solution, is based on *in situ* hydrolysis and subsequent creation of covalent bonds between the hydrolyzed polymer chains, as shown schematically in Fig. 1a. The technique that is essential in our approach is to use polymer chains that are both hydrolyzable and disconnectable and to covalently crosslink the hydrolyzed polymer chains to each other. Here it is truly important to notice that the additional crosslinks are not homogeneously distributed in the hydrogel particles but are instead concentrated at the periphery. Selectively removing the core part in the hydrogel particles enables us to generate uniform hydrogel shells. In this study, we rationalize this approach and characterize the morphology of the hydrogel shells and experimentally demonstrate their applicability to load and release active materials.

In a typical procedure, we first produce poly(vinyl formamide) (PNVF) particles by using the dispersion polymerization technique,[11] in which *N*-vinyl formamide (19 g, NVF, Aldrich) and *N*,*N*′-methylene-bis-acrylamide (1 g, BIS, Aldrich) are polymerized at 70 °C for 24 h in a methanol solution (215 ml) in the presence of an initiator (azobis(isobutyronitrile), 0.2 g, Junsei) and a stabilizer (poly(2-ethyl-2-oxazoline), 2 g, $\sim 5 \times 10^4$ g mol^{-1}, Aldrich). The stirring speed is set to 100 rpm.

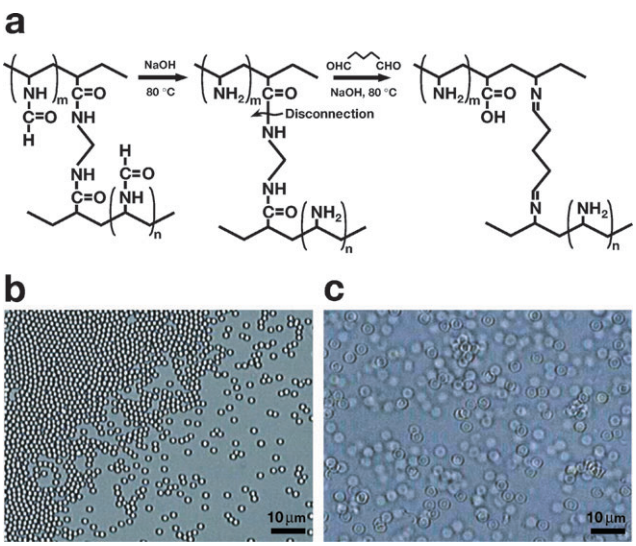

Fig. 1 The process for synthesizing uniform poly(vinyl amine) (PVAm) hydrogel shells. (a) Schematic for the hydrolysis of *N*-vinyl formamide groups and the crosslinking reaction of PVAm chains. The crosslinking was achieved by adding glutaraldehyde that can covalently link the PVAm chains. (b) Uniform poly(vinyl formamide) (PNVF) particles produced by using the dispersion polymerization technique. (c) The PVAm hydrogel shells with single voids in the center, produced after the hydrolysis and crosslinking in the strong base solution. This sample was fabricated using ~0.3 mol glutaraldehyde. The images of the samples were observed using a bright field microscope (Olympus BX-51).

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After the polymerization, all unreacted monomers and additives are removed by repeated centrifuges with methanol. The diameter of the resulting PNVF particles is ~2.3 μm and the coefficient of variation in size is less than 10% (Fig. 1b). Then, we re-disperse the PNVF particles (1 g) in 76 ml of an ethanol solution containing glutaraldehyde (0.1 to 0.7 mol, TCI). While stirring the PNVF particle dispersion, we slowly add a 2 N NaOH aqueous solution (40 g) and carry out the reaction at 80 °C for 12 h. In this step, the vinyl formamide unit is hydrolyzed to form the vinyl amine unit that is covalently linked again by glutaraldehyde,[12] thus creating a poly(vinyl amine) (PVAm) network. The importance of this step in determining the microgel structure is that another hydrolysis reaction indeed occurs at the amide linkages coming from BIS,[13] and then the cleaved PVAm chains diffuse out of the hydrogel particles. After washing the by-products produced by hydrolysis, we can generate uniform PVAm hydrogel shells, as shown in Fig. 1c.

To better characterize the exact morphology of the resulting PVAm hydrogel shells, we use confocal laser scanning microscopy after labelling the amine groups of the PVAm with rhodamine B. The result is shown in Fig. 2a. As can be seen, the structure retains the spherical hollow capsules. Also, the microgel shells are still dispersible in water, since the PVAm wall at neutral pH has positive charges, providing sufficiently

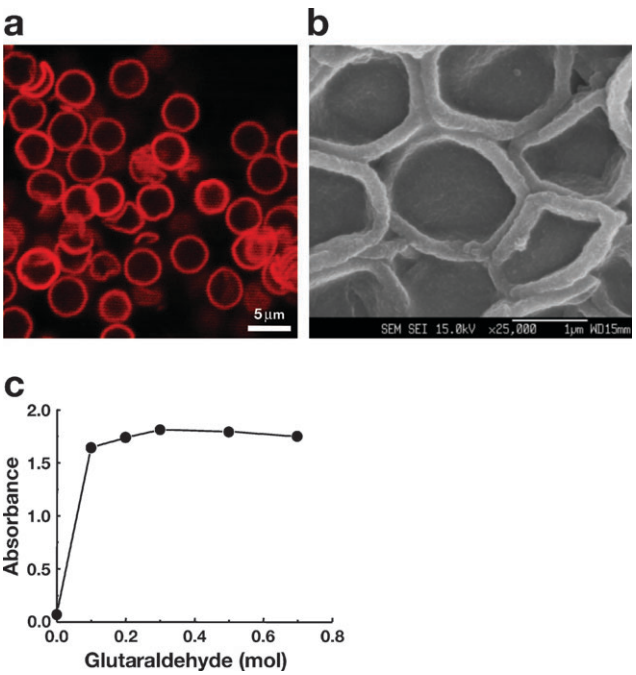

Fig. 2 Characterization of PVAm hydrogel shells. (a) The structure of the PVAm hydrogel shells observed using confocal laser scanning microscopy (CLSM, MRC-1024) after labeling the amine groups linked in the chains of the PVAm with rhodamine B isothiocyanate (RBITC, Fluka). (b) A scanning electron microscope (JSM-6300, JEOL) image of the PVAm hydrogel shells. These samples were fabricated using ~0.2 mol glutaraldehyde. (c) A quantitative analysis of the amine groups incorporated in the PVAm hydrogel shells by using the ninhydrin assay method, in which the reaction of the ninhydrin reagent with the amine groups is characterized by using a UV-Vis spectrophotometer at 570 nm.

high hydrophilicity, which favors a stable dispersion of the shells in water. The thickness of the shells at pH 7 is ~250 nm (see ESI†). A perfect hollow structure can be confirmed again by observing their dried shapes with a scanning electron microscope, as shown in Fig. 2b (see also ESI†); evaporation of water, placed in the hollow, during a drying procedure leads to complete collapse of the shells. Usually, ionic hydrogels show different degrees of charge dissociation depending on the pH of the medium, enabling control over their volume transitions; our hydrogel shells also show a ~50% decrease in volume when we change the pH of the dispersion medium from 3 to 11. These volume changes with pH occur reversibly.

We note in our synthetic approach that there is a competition between the disconnection of the PVAm network in the center and the additional crosslinking by glutaraldehyde at the periphery of the hydrogel particles. To maintain the macroscopic topology of the shell during hydrolysis and to achieve a rigid shell, the additional crosslinking should be more favored compared to the disconnection of the PVAm network. For this, we add a high concentration of glutaraldehyde, thereby resulting in very small mesh sizes. Once the shell is formed, it seems that the PVAm network formed by glutaraldehyde is indeed dense so that we observe a negligible increase in the crosslinking density even at high concentrations of glutaraldehyde. Further support for this comes from directly detecting the concentration of amine groups in the shell by using the ninhydrin method (see ESI†),[14] as shown in Fig. 2c; the amount of amine groups in the shells almost remains constant as a function of the concentration of glutaraldehyde, meaning the microgel shells have almost the same crosslinking density irrespective of the concentration of glutaraldehyde.

The advantage of fabricating PVAm microgel shells is the ability not only to load a variety of active ingredients in either the shell or the hollow, but also to control their release through changes in pH. To experimentally demonstrate this, we exploit a probing molecule, Chromotrope 2R (CR), and load it in the shell, as shown in Fig. 3a. We observe the CR can be encapsulated in the hollow by diffusion and loaded in the shell due to the electrostatic interactions with the amine groups of the shell (see ESI†).[15] This shows the hydrogel shell can serve as a pH-sensitive reservoir for the CR. The pH of the medium is critical to the loading capacity of the microgel shells; we obtain higher loading at a low pH, because low pH increases the degree of ionization of the amine groups, thus providing stronger electrostatic interactions with the CR molecules; in our study, we are able to load the CR up to ~40 wt%. Using the insights gained from loading the CR enabled manipulation of the releasing properties. We demonstrate this by quantitatively measuring the release of CR from the shells at different pH values, as shown in Fig. 3b; changing the pH allows us to control the profiles of CR release out of the shells.

In summary, this study introduces a facile framework for synthesizing hydrogel shells that have the ability of loading active ingredients and releasing them through pH changes. The key to our synthesis approach is to selectively cleave the chains in the center of the supporting microgel particles without affecting their overall topology during the hydrolysis procedure, thus generating a hollow capsule structure. Future

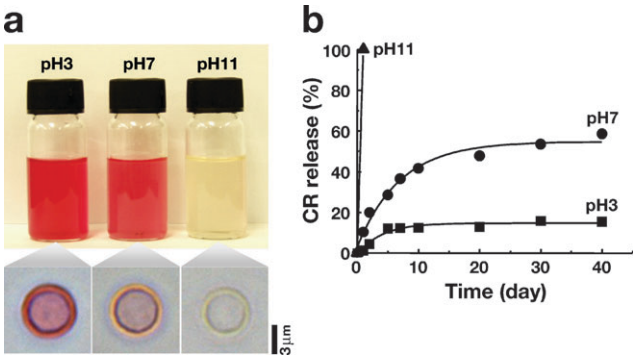

Fig. 3 Accumulated release of a probing dye from the PVAm hydrogel shells. For this, we first dispersed the PVAm hydrogel shells in three different buffer solutions, pH 3, 7, and 11, containing anionic probe (0.005 g, CR, Chromotrope 2R, Aldrich). This microgel shell was fabricated using ~0.2 mol glutaraldehyde. (a) After equilibrating for 24 h at room temperature, the samples were completely washed with fresh identical buffer solutions and their images observed with a bright field microscope. (b) CR release from the PVAm hydrogel shells into the buffer solutions at 37 °C. In these measurements, we loaded the CR into the PVAm hydrogel shells at pH 3, then we detected the concentration of the CR released from the shells at different pH values using a UV-Vis spectrophotometer at 510 nm.

studies will focus on treating the periphery of the shell with counter-charged either polymers or nanoparticles to provide better control over the permeability through changes in pH as well as to improve the mechanical strength of the microgel shells, eventually allowing us to more precisely manipulate the releasing properties of encapsulated materials with different length scales. Using these types of microgel shells will enable the design of novel capsules for applications in drug delivery.

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