

Preparation of Zeolite Microspheres and their Application in Biology

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ABSTRACT

Hollow zeolite microspheres were prepared by growing a thin zeolite membrane shell on seeded polystyrene beads. The removal of the organic growth directing agent and polystyrene bead was successfully performed without damaging the thin zeolite shell. The storage and delivery of sodium pervanadate, a potent tyrophosphatase inhibitor to MDCK cells was successfully carried out. Thus, demonstrating the potential use of these materials for biological studies of cell signaling.

Keywords: hollow zeolite microspheres, storage, delivery, water-soluble organic compounds, bioactive molecules.

1 INTRODUCTION

Chemical storage and delivery system is an important technology not only for drug delivery, but also for biological research. It is a critical technology for the emerging "laboratory-on-a-chip" devices that include μ -TAS, DNA-Chips and Bio-MEMS. These microdevices are expected to duplicate and automate the procedures used in chemical analysis, biological assay and medical diagnosis. This means that chemical reagents needed for the separation, purification, isolation, conversion, amplification and detection must be stored in the microdevice and made available on demand during the operation. Chemical storage and delivery system that respond to changes in environmental conditions, such as temperature [1], pH [2], light [3], electric field [4], and certain chemicals [5] have been the focus of many researches. The selection of an appropriate method for preparing drug-delivery vehicles depends on the physicochemical properties of the polymer used for fabricating the vehicles and the drug itself. However, the methods available for incorporating the hydrophilic compounds are few and difficult to implement. One major challenge is to produce delivery vehicle that can carry water soluble drugs and does not tend to aggregate. Membrane-enclosed microspheres based on nanoporous zeolites and molecular sieves are potential candidate for chemical storage and release, either alone or onboard a lab-on-a-chip device. The transport through zeolites is governed by the structure and chemistry of the pore channels. The zeolite pore structure restricts the size and shape of the molecules that can access the zeolite pores giving rise to molecular sieving properties. The narrow

channels also restrict the movement of bulky molecules and constrain their orientation within the pore. The chemical environment within the zeolite pore channel is governed by the structure of the zeolite framework, the nature of the framework substitution ions and the presence of counterions. The interactions between the diffusing molecules and these adsorption sites have resulted in many unique separation properties. Here, we report on the fabrication of hollow zeolite microspheres that possess ordered pores size and with long term chemical and biological stability. The growth directing agent and polymer core are removed by ozone treatment, which is carried out in solution under low temperature. The procedure avoids the problem of agglomeration and the need for redispersion that plagued the other methods.

2 METHODOLOGY

2.1 Construction of zeolite-coated hollow capsule

Assembly of polyelectrolytes was started by adding 1 ml of Poly(diallyldimethylammonium chloride)PDADMAC (1mg/ml in 0.5 M NaCl) to a 100 μ l suspension of 4.5 μ m polystyrene (PS) particles (1 wt%, $\sim 10^{11}$ particles mL⁻¹). The suspension was stirred at room temperature for 15 minutes to allow adsorption, and then centrifuged at 6,000g for 5 minutes. Following the removal of supernatant, the coated particles were washed three times with 0.5 M NaCl to remove excess polyelectrolyte. A 1 ml aliquot of (polystyrene sulfonate) PSS (1 mg mL⁻¹ in 0.5 M NaCl) was next added to form a second polyelectrolyte layer of negative charge on the particle surface. The centrifugation and washing steps and the sequential adsorption of oppositely charged polyelectrolytes were repeated to obtain PS particles with three layers of polyelectrolyte coating, herein referred to as PS/(PDADMAC/PSS/PDADMAC). The coated particles were re-suspended in 900 μ l of double distilled, deionized (DDI) water and 100 μ l of 100 nm TPA-Sil-1 seeds (2 wt %) was added. The suspension was stirred at room temperature for overnight. The consecutive assembly of polyelectrolyte multilayer onto PS particles was examined by Zeta Potential Analyzer (Brookhaven, Zetaplus), Scanning Electron Microscope (SEM, Philips XL-30) and Transmission Electron Microscope (TEM, Philips CM-20). Zeolite seed-coated PS particles were

transferred to a synthesis solution containing molar ratio of 40 SiO₂: 5 TPA₂O: 20,000 H₂O. The synthesis was conducted at 403 K for 24 h. The zeolite microspheres were recovered and purified by a series of centrifugation and washing steps. The organic growth-directing molecules and the polymer core were removed by bubbling ozone to the microsphere suspension at 353 K for 20 h. The suspension was then centrifuge to recover the hollow zeolite microspheres. The entire preparation process for hollow zeolite microspheres is depicted in Fig. 1.

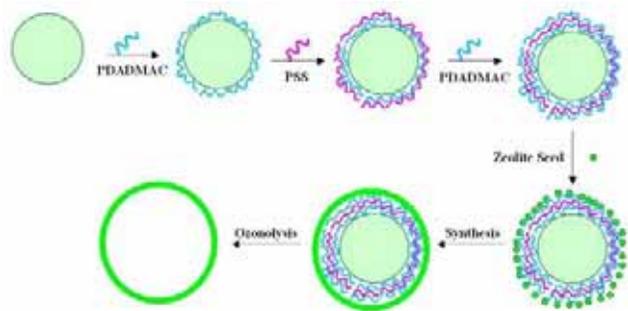


Figure 1. Illustration of the procedure for the preparation of hollow zeolite microspheres.

2.2 Use of zeolite microspheres to study cell signaling

A 1 M sodium orthovanadate was mixed with 1.7% H₂O₂ at a 30:1 ratio to produce sodium pervanadate, which known to suppress the phosphokinase activity in cells. Hollow zeolite microspheres were equilibrated with the solution for half an hour, spin down and washed. This procedure was repeated three times. The loaded microspheres were applied to Madin-Darby canine kidney (MDCK) cells and incubated for 1 h. The MDCK cells were then fixed with 96% ethanol at 253 K for 5 minutes and then stained with anti-phosphotyrosine antibodies (mAb4G₁₀, Upstate Biotechnology) and FITC-conjugated anti-mouse secondary antibodies.

3 RESULTS AND DISCUSSIONS

Polystyrene (PS) beads were selected as the template for preparing hollow zeolite microspheres. Polystyrene beads with narrow size distributions are readily available commercially. The sulfate-stabilized 4.5 μm PS beads have a measured surface zeta potential of -74.1 mV (Fig. 2). This means that the beads have the same negative charge as the zeolite seeds. Seeds were needed to provide the nucleation sites for zeolite growth. The beads were seeded with 100 nm-sized TPA-Sil-1 using the established layer-by-layer (LBL) method. The polymer beads were coated with PDADMAC to change surface from a negative to a positive charge (Fig. 2). However, to ensure a uniform charge

distribution over the beads' surface, a second layer of PSS was added. This resulted in a negatively charged surface. A final layer of PDADMAC gives the beads a uniform positive charge needed for seed deposition. Figure 2 plots the changes in the surface Zeta potential of the beads after each coating steps. Precaution must be taken to wash away the excess polyelectrolyte species between coating steps. These make sure that a monolayer is formed with each coating [6,7].

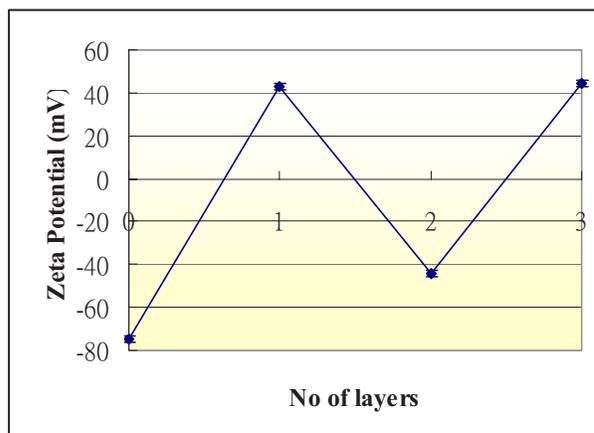


Figure 2. A plot of Zeta Potential for 0-uncoated PS beads, 1-PS/PDADMAC, 2-PS/(PDADMAC/PSS) and 4-PS/(PDADMAC/PSS/PDADMAC)

Figure 3a displays the PS beads seeded with 100 nm zeolite seeds. The seeds form a uniform, densely packed layer on the polyelectrolyte-coated bead as shown in a higher magnification SEM picture in Fig. 3c. The zeolite shell was then grown from a synthesis solution containing both the silica source, tetraethyl orthosilicate (TEOS) and the growth directing agent, tetrapropylammonium hydroxide (TPAOH). The TPAOH also served as a source of hydroxide ions for maintaining the alkalinity of the solution. The morphology of the Sil-1 zeolite grain depends on the composition of the synthesis solution. Larger amount of TPAOH in the solution led to a larger grain size and square shape. Dilute synthesis solutions often resulted in elongated zeolite grains. A smooth and well-intergrown Sil-1 zeolite shell was obtained from synthesis composition of 40 SiO₂: 5 TPA₂O: 20,000 H₂O as shown by the zeolite microspheres in Fig. 3b & 3d.

The TPA⁺ molecules trapped with the zeolite pores and the polystyrene core enclosed by the thin zeolite shell were removed using a novel method. Ozone gas was bubbled into the microsphere suspension at 353 K. The ozone oxidizes and removes the TPA⁺ molecules freeing the zeolite pores. The ozone then diffuses and reacts with the polymer core, breaking the polymers into shorter and more soluble carboxylic acid chains that could easily dissolve in water and diffuse out of the zeolite shell. The whole process is conducted at low temperature, minimizing

the damage to the microspheres. Unlike the current high temperature treatment process, less than 1 percent of the hollow zeolite microspheres were damaged compared to the usual 10-20 % values reported in the literature [8-10]. Also by keeping the microspheres in suspension throughout the preparation prevented their agglomeration and dispersed particles were obtained.

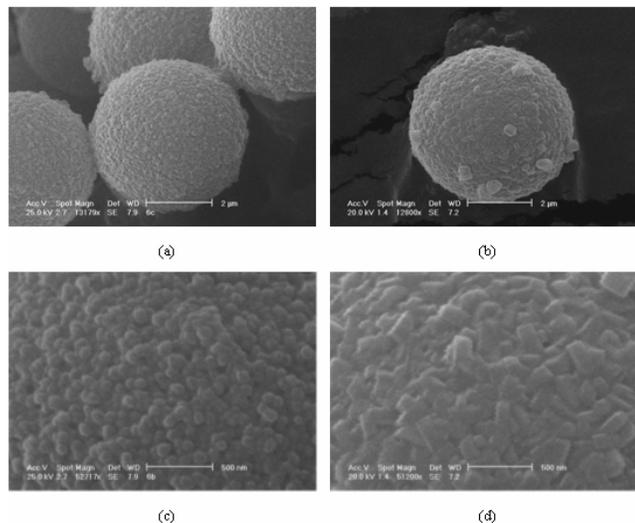


Figure 3. Scanning electron micrographs of (a, c) zeolite seeded microsphere and (b,d) zeolite microsphere.

Figure 4a shows a SEM picture of a hollow zeolite microsphere. The average sphere diameter is 4.9 μm from the original 4.5 μm PS beads. This means that the zeolite shell is roughly 200 nm thick. Transmission electron microscopy was used to confirm that the polymer core was successfully removed from the hollow zeolite microspheres as shown in Fig. 4b.

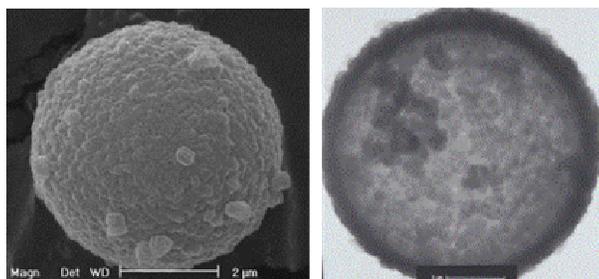


Figure 4. Hollow zeolite microspheres.

The potential use of hollow zeolite microspheres for biological studies was illustrated in the study of cell signaling. The hollow zeolite microspheres carry and locally deliver signalling molecules that mimicks the *in vivo* signalling process between chemicals and regulating enzymes. The hollow zeolite microspheres were loaded with sodium pervanadate (i.e., Na-orthovanadate + H₂O₂). Sodium pervanadate (PV) is known to inhibit the activity of

tyrosine phosphatase that leads to the accumulation of tyrosine in phosphorylated form. The PV loaded zeolite microspheres were applied on Madin-Darby canine kidney (MDCK) cell as shown in Figure 5. The marker used to assess the action of the pervanadate PTPase inhibitor was anti-phosphotyrosine (PY) antibody. The increases in the PY staining at microsphere-cell contacts indicate a successful local delivery of PV.

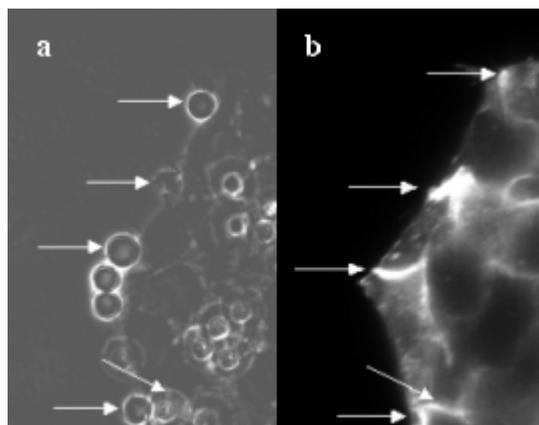


Figure 5 (a) Optical and (b) fluorescent microscope pictures of microspheres deposited on MDCK cells. It is clear that there is an increase in the phosphotyrosine (PY) labelling at the point of contacts in (b).

4 CONCLUDING REMARKS

Hollow zeolite microspheres can carry and deliver small, water-soluble molecules such as sodium pervanadate, a potent inhibitor of the cellular signaling proteins called tyrosine phosphatase. Local delivery of PV resulted in a clear increase in phosphotyrosine at the microsphere-cell contacts. These microspheres could prove to be an invaluable tool for investigating cell signaling such as the post-synaptic formation of neuromuscular junction (NMJ). An understanding of NMJ is critical for combating many serious diseases including NMJ disorder, muscular dystrophy and Myasthenia Gravis. It may also provide insights for curing botulism and snakebite that act on NMJ.

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