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Microspheres

Amphiphilic Hollow Carbonaceous Microspheres with Permeable Shells**

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Hollow microspheres have stimulated great interest because of their growing applications in targeted delivery, artificial cells, catalysis, energy storage, and enzyme immobilization.[1] Quite a few of these promising applications profit from the efficient encapsulation of active ingredients (e.g., dyes, drugs, inks, cells, and proteins)[2] into the interior cavities. For easy access of guest materials to the interior of the materials, fine control of shell porosity and surface permeability is necessary. Up to now, several effective strategies, such as emulsion process, surface-protected etching, supramolecular self-assembly, as well as the replication method, have been developed to prepare hollow spheres with porous shells.[3] However, controlling the size of the shell pores has proved to be a hard work.

It is well known that surface characteristics are of paramount importance in determining the dispersion behavior of microspheres in various media and thus their practical applications. The surface characteristics of microspheres are mainly determined by their chemical compositions. With specific functional groups, the surface layer molecules can endow the microspheres with improved physical and chemical properties (e.g., superhydrophobicity, superhydrophilicity, and amphiphilicity).[4] However, the as-obtained hollow spheres are usually not provided with the desired characteristics, and further surface functionalization is often needed. Generally, control of the surface functionality of microspheres can be realized through two main approaches. The first one, especially for polymer microspheres, is to incorporate reactive block comonomers with desired functional groups into the polymer chains during the polymerization process.[5] The presence of these reactive comonomers on microsphere surfaces can alter the surface characteristics. Another commonly applied approach is chemical modification of the microsphere surfaces by a posttreatment process.[6] By taking advantage of covalent bonds, hydrogen-bonding interactions, and electrostatic effects, functional agents can anchor onto the surfaces of preformed microspheres. However, additional chemical modification of these structures makes the synthesis procedure multistep and troublesome. Therefore, a direct and efficient synthesis of spherical materials with hollow core/porous shell structures is still a challenge, especially when customized surface properties are expected.

Budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*), a low-cost and widely applied industrial microorganism for baking and ethanol production, is one sort of elliptical unicellular fungus with sturdy cell wall, which mainly consists of a polysaccharide layer constructed from coiled β-1,3-glucan chains. By offering physical protection, the cell wall sustains the whole cell system even in relatively harsh environments.[7] It is known that through mild hydrothermal carbonization, biomass consisting of polysaccharides can be transformed into highly carbonaceous materials with better strength and stability.[8] Thus, we were inspired to speculate that carbonaceous hollow spheres may be obtained from the yeast cells by a controlled carbonization process.

Herein, we present a facile method for fabricating hollow carbonaceous microspheres with controlled shell porosity from *S. cerevisiae* cells. Through mild hydrothermal treatment (180–200 °C) of these tiny unicellular organisms, hollow microspheres with controllable meso- and macroporous shells were synthesized. Most interestingly, the surface of these hollow spheres was found to be covered with both hydrophobic and hydrophilic functional groups, endowing the as-obtained microspheres with amphiphilic property. Moreover, the amphiphilic property allows these porous hollow carbonaceous spheres (PHCSs) to exhibit attractive phase transfer, protein auto-enrichment, and pH-controlled release behavior.

To synthesize the PHCSs, the yeast cells were firstly pretreated by acetone to make sure that the protoplast inside the cells was removed.[9] In preliminary experiments, we found that hydrothermal treatment of the as-obtained cell walls with pure water as solvent led to only irregular broken fragments (Figure S1a in the Supporting Information), rather than integral hollow spheres. To overcome this problem, glutaraldehyde (GA) was added as a protecting agent. Through covalently bonding to aldehyde or amide groups in the cell-wall polysaccharide networks (CWPNs), GA could enhance the strength of the polysaccharide networks, thus avoiding the undesired breaking. SEM images of the as-

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obtained products are shown in Figure 1. It was found that all the products were preserved as integral hollow microspheres in the size range 2.0–4.0 μm, indicating the disruption of CWPNs could be well restricted when GA was added. Pore

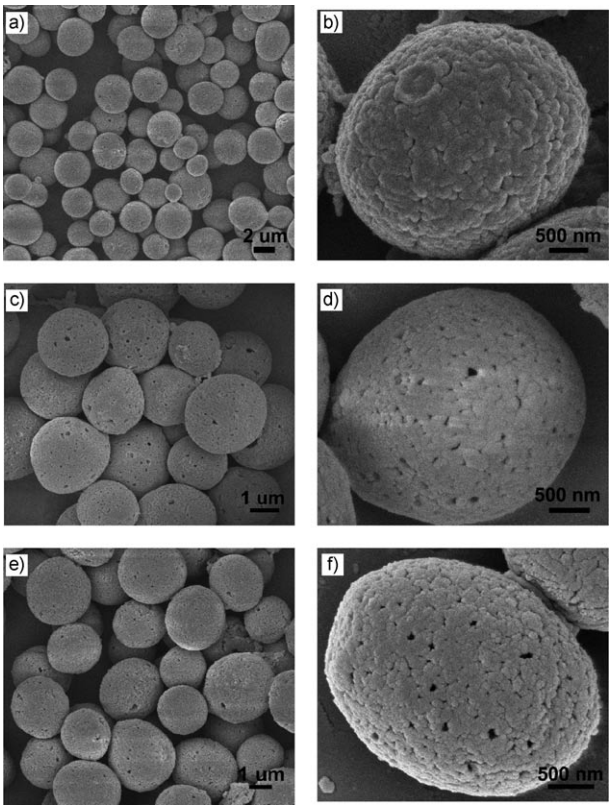


Figure 1. SEM images of PHCS-I (a, b); PHCS-II (c, d); and PHCS-III (e, f).

size analysis of the PHCSs revealed that mesopores existed in the shells (Figure S2 in the Supporting Information). Moreover, by altering the synthesis conditions, the pore sizes of PHCSs could be adjusted. Data calculated using the BJH (Barret–Joyner–Halenda) method showed that the average size of the shell pores could be restricted to below 5 nm by using 5% (v/v) GA aqueous solution (Figure 1 a,b, noted as PHCS-I). When 2% (v/v) GA aqueous solution was adopted, the average size of the shell pores was about 43 nm (Figure 1 c,d, noted as PHCS-II). The size increased to 50 nm after further acid etching (the corresponding products are noted as PHCS-III, Figure 1 e,f). Meanwhile, macropores with size exceeding 100 nm could be seen clearly in some samples. The BET surface areas of PHCS-I, PHCS-II, and PHCS-III are 17.7, 9.7, and 9.2 $m^2 g^{-1}$, respectively.

To clarify the formation mechanism of these porous hollow carbonaceous spheres, analysis of the hydrothermal treatment products, that is, both the PHCSs and the separated supernatant were carried out. In the separated supernatant, soluble mono- and oligosaccharides were detected by high-performance liquid chromatography (HPLC), which indicated hydrolysis of glucan molecules took place during the hydrothermal process.[10] Several other techniques were employed to characterize the PHCSs to acquire more information. Figure 2 a shows the ^{13}C solid-state nuclear magnetic resonance (NMR) spectrum of the PHCSs. Peaks at $\delta = 26$ and 31 ppm can be attributed to methyl and

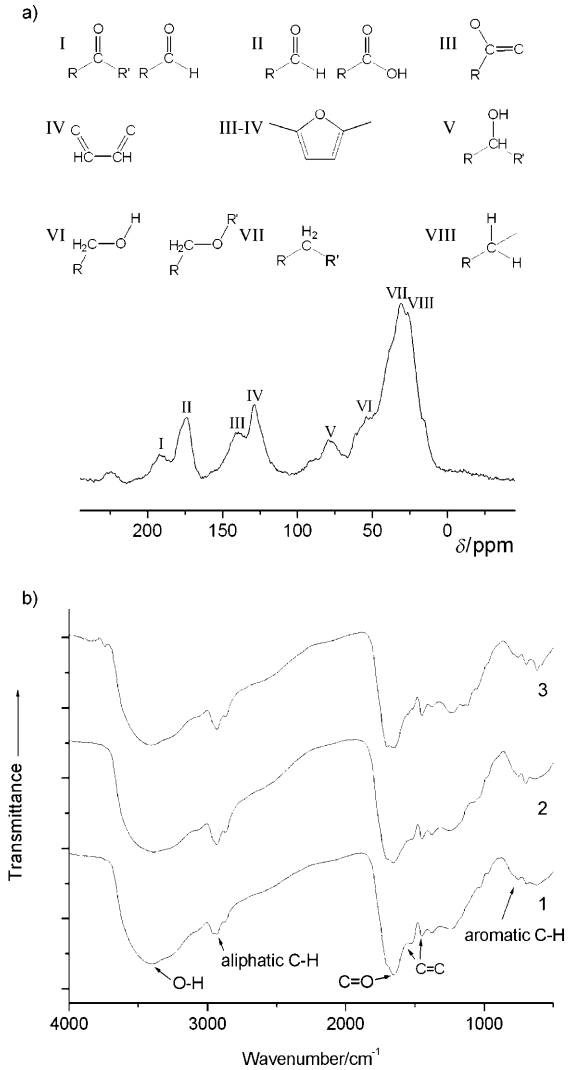


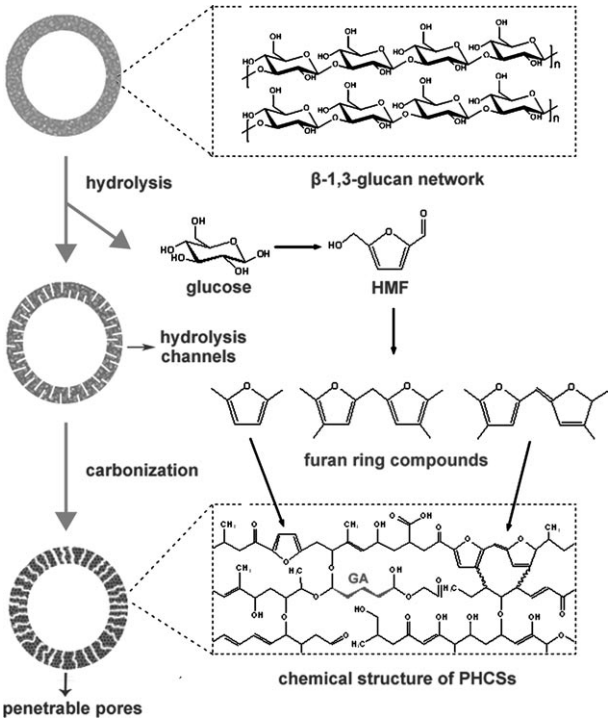
Figure 2. a) Solid-state ^{13}C NMR spectrum of PHCS-III; b) FTIR spectra of PHCS-I (1), PHCS-II (2), and PHCS-III (3).

methylene, respectively, and those in the $\delta = 120$–150 ppm region can be assigned to long-range conjugated C=C bonds and oxygen-substituted C=C bonds, revealing the existence of aromatic furan ring compounds.[11] Moreover, oxygenated functional groups, including carbonyl, carboxy, hydroxy, ether, and ester groups were also detected. Corresponding infrared adsorption bands could also be observed in Fourier transform infrared (FTIR) spectra of PHCSs. As shown in Figure 2 b, the appearance of infrared adsorption bands corresponding to C=C and aromatic C–H out-of-plane bending vibrations demonstrates the aromatization of CWPN during hydrothermal treatment. Besides that, the adsorption band of O–H bending vibrations implies the existence of large numbers of residual hydroxy groups. The data obtained from XPS analysis (Figure S3 in the Supporting

Information) further testified to the existence of these oxygenated functional groups. The appearance of abundant C=C and aliphatic carbon atoms as revealed above indicated that CWPNs underwent carbonization reactions during the hydrothermal treatment. The chemical composition analysis of PHCSs was also determined. It was found that while the carbon content of the yeast cells was 41.82 wt%, that of the PHCSs could reach 71.01 wt% after 36 h of hydrothermal carbonization (Table S1 in the Supporting Information).

On the basis of the above analysis, it was believed that both carbonization and hydrolysis reactions occurred during the hydrothermal process. In fact, the CWPNs possess an inhomogeneous structure consisting of two kinds of components, namely, an amorphous matrix and a fibrillar network.[12] Although the amorphous matrix is sensitive to hydrolysis, the fibrillar network is more resistant to decomposition. Therefore, under the hydrothermal conditions, the fibrillar network was inclined to in situ inter- and intramolecular dehydration to form the scaffold of the PHCSs.[10] In contrast, the amorphous matrix underwent drastic decomposition by hydrolysis, and transformed into mono- and oligosaccharides. According to Baccile and co-workers,[11] the mono- and oligosaccharides derived from hydrolysis could undergo further decomposition in the medium to produce 5-hydroxymethylfurfural (HMF) as the main intermediate. Through a series of reactions including decomposition, polymerization, and condensation, furan ring compounds could be formed from HMF and then bound to the surface of PHCSs. The anchoring of abundant furan ring compounds can increase the surface aromaticity of PHCSs.

Thus, it is the competition between carbonization and hydrolysis that determines the final morphology of PHCSs. As a result of unequal hydrolysis resistances, first, some small hydrolysis channels appeared in the shells. Then, individual hydrolysis channels kept enlarging and incorporating with each other during further hydrothermal treatment. Finally, penetrable meso- and macropores emerged in the microsphere shells. The introduction of GA into the synthesis medium could enhance the hydrolysis resistance of CWPNs. Through covalent binding with glucose residues of adjacent glucan chains, GA molecules could firmly link the glucan chains of CWPNs. Therefore, the hydrolysis etching of CWPNs, that is, the pore formation, could be well restricted when adequate GA was added. On the basis of the above discussion, the pore formation mechanism and surface molecular structure of PHCSs are illustrated in Scheme 1.



Scheme 1. Pore formation mechanism and the chemical structure transformation of PHCSs.

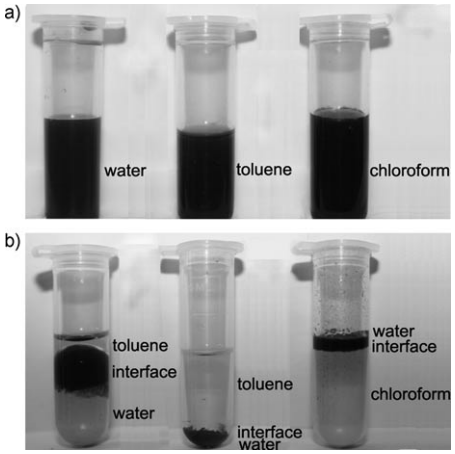


Figure 3. Photographs of PHCSs in different solvent systems: a) dispersing in water (left), toluene (middle), and chloroform (right); b) congregating at the water–toluene interface (left and middle) and chloroform–water interface (right).

Hydrothermal treatment changed the chemical composition of the CWPNs to a large degree. Owing to the partial loss of the hydrophilic groups and aromatization of the molecule networks, the wettability characteristics of PHCSs were supposed to be very different from that of the hydrophilic CWPNs. Actually, we found that the PHCSs could be well dispersed not only in water but also in nonpolar solvents such as toluene and chloroform (Figure 3a), suggesting that PHCSs possess amphiphilic surfaces. As can be seen in Scheme 1, we attribute this to the coexistence of hydrophilic and hydrophobic groups on the surface of PHCSs. More interestingly, a phase-transfer phenomenon of PHCSs between polar and nonpolar solvents was observed. As shown in Figure 3, PHCSs were initially dispersed in nonpolar solvents (toluene and chloroform). Upon addition of only several drops of water, however, the PHCSs transferred spontaneously from nonpolar solvents to the biphase interface. Moreover, this process occurred very quickly, such that it was complete within several seconds. Depending on the density of the nonpolar solvents, the PHCSs could congregate either in the bottom or top of the solvent systems. This feature will be beneficial to the industrial applications of PHCSs, as it can help facilitate the separation and recovery of PHCSs. Similar spontaneous adsorption of colloid particles onto O/W

or W/O emulsion surfaces have been reported,[13] where minimizing the surface energy of the particle-emulsion system was proposed to be the driving force. Compared with either hydrophilic or hydrophobic particles, the attachment of amphiphilic particles to the oil–water interface is suggested to be more favorable. Thus, this mechanism could well explain the spontaneous transfer of PHCSs onto the oil–water interface observed in our study.

Besides the phase-transfer feature, the porosity of the microsphere shells is another important factor on which attention should be focused. Their controllable porosity may allow the use of PHCSs as microcapsules or carriers for loading and transferring guest molecules. In this work, we verified this possibility by testing the encapsulation of proteins. An adsorption test with bovine serum albumin (BSA) showed that larger pores could greatly enhance the protein loading. For PHCS-I and PHCS-III, their corresponding protein loadings were 2.4 and 22.0 mg g^{-1}, respectively. To obtain detailed and visible information, the encapsulation of fluorescent proteins (FITC-labeled ovalbumin) was observed by confocal laser scanning microscopy. As shown in Figure 4a, for PHCS-I with mesopores in the shells, the adsorption of proteins occurred mainly on the surface of the microspheres, and no penetration of proteins into the interior cavities could be observed. In contrast, the shells of PHCS-III were penetrable, and plenty of protein molecules had entered into the interior voids. It was notable that the penetration process occurred rapidly and was almost complete within 15 min. The different loading manners could well explain the great disparity in magnitude between the protein loadings of PHCS-I and PHCS-III. Judging from the fluorescence intensity, the protein concentration in the interior cavities of PHCS-III was much higher than that in the bulk solution, suggesting an auto-enrichment of proteins occurred inside the hollow cavities. This enrichment was rather stable under certain conditions such that it could bear several washing cycles in which drastic shaking and centrifugal separation were employed (Figure 4c). Considering the amphiphilic property of the sphere shells, we owe this stable enrichment mainly to the hydrophobic interactions between the proteins and the inner surfaces of PHCSs (Scheme S1 in the Supporting Information).[14]

Furthermore, although rather stable under the above aqueous conditions, protein adsorption to PHCSs is a reversible process. Through weakening the hydrophobicity of proteins in higher-pH buffers, the loaded proteins could desorb.[15] From an in vitro release test of BSA, it was found that by adjusting the pH value of the surrounding medium, BSA could be released from PHCS-III in a controlled manner (Figure S4 in the Supporting Information). When a medium of pH 4.8 was used, no BSA release was observed. Nevertheless, while the pH value of the medium was controlled to around 7.0 and 9.0, about 17.9 and 88.2%, respectively, of the loaded BSA could be desorbed and released into the aqueous medium.

In conclusion, porous hollow carbonaceous microspheres can be readily fabricated from *S. cerevisiae* cells by hydrothermal treatment. By controlling the synthetic conditions, the average size of the shell pores could be adjusted. Furthermore, the amphiphilic property of PHCSs allows their stable dispersion in various solvents of differing polarity, and endows them with a spontaneous phase-transfer feature in biphasic systems. Moreover, rapid and reversible auto-enrichment of proteins inside the hollow cavities of PHCSs could be achieved. These findings imply the hollow carbonaceous microspheres we prepared may have promising applications in fields such as catalysis, adsorption, drug delivery, encapsulation of active ingredients, and separation techniques.

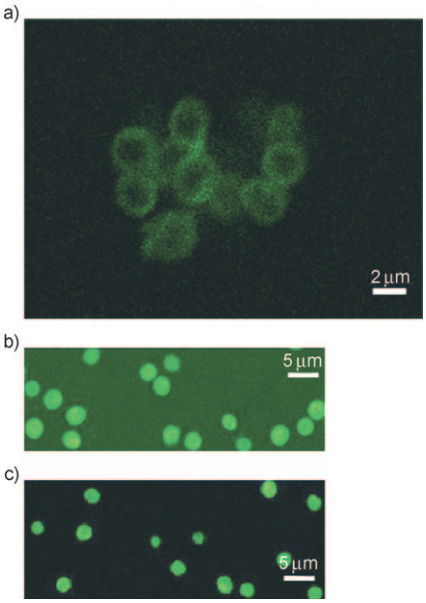


Figure 4. Representative laser scanning microscopy images of ovalbumin-loaded microspheres: a) PHCS-I, b) PHCS-III, and c) PHCS-III after several washing cycles.

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