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Rapid and high-capacity immobilization of enzymes based on mesoporous silicas with controlled morphologies†

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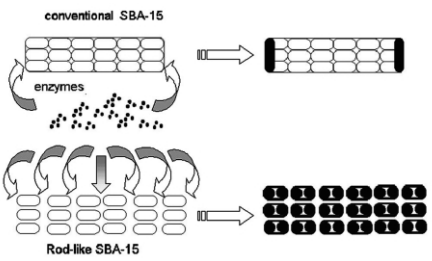
Very rapid (< 10 min to reach equilibrium) and high-capacity (up to 533 mg g^{-1}) immobilization of enzymes within mesoporous silica has been achieved by finely tuning their morphologies.

Progress in bioimmobilization has resulted in a revolution of the use of biomolecules for applications in separation, catalysis, and sensors, which typically rely in large part on the successful immobilization of the intact biomolecules onto or within a suitable host.[1,2] Recently, mesoporous silicas (MPSs) have received much attention as promising host materials for guest biomolecules because of their uniform and large pore sizes, open pore structures, and their chemical and mechanical stabilities.[3–9] It has been found that two factors may greatly influence the immobilization behavior of MPSs. One is the mesostructure: the entrance size of the mesochannels or cages should be sufficiently large for "comfortable" entrapment of biomolecules.[3,5,6] The other factor is the surface characteristic of MPSs. It has been reported that functionalized MPSs show not only improved capacity, but also enhanced bioactivity.[9]

MCM-41 and SBA-15 materials are two commonly studied MPSs in bioimmobilization. However, even for the same kind of MPSs (*e.g.* SBA-15), their immobilization behavior (*e.g.* specific capacity) of similar proteins may vary significantly according to different researchers.[10,11] Furthermore, considering the large pore volume (~1.0 cm^3 g^{-1}) of MPSs, their specific capacity for biomolecules still remains poor (< 200 mg g^{-1}).[6–8,12] Additionally, it often takes several hours, even days, for the immobilization of biomolecules to reach equilibrium.[8,12] These disadvantages may greatly restrict their future applications.

In this communication we report the correlation between the macrostructure and the bioimmobilization performance of MPSs. By carefully controlling the morphology of MPSs, it is revealed that MPSs with smaller particle sizes possess more entrances to entrap enzymes than conventional MPSs with larger particle sizes, which leads to much improved bioimmobilization abilities of MPSs (Scheme 1). The specific capacity can reach 533 mg g^{-1}, ~7 times larger than that of conventional MPSs. Moreover, ~ 200 mg g^{-1} enzymes can be completely and rapidly encapsulated within MPSs in < 10 min.



Scheme 1 Schematic illustration the super bioimmobilization ability of rod-like SBA-15 comparing with that of conventional SBA-15.

Two types of SBA-15 were used as hosts to study their bioimmobilization behaviors. Rod-like SBA-15 (denoted Rod-SBA-15) possesses discrete rod-like morphology with uniform length (1–2 μm)[13] (Fig. 1a), while conventional SBA-15 (denoted Con-SBA-15) has a fibrous macrostructure, extending tens of micrometres by stacking and coupling of rod-like SBA-15 (Fig. 1b). Lysozyme was used to examine the bioimmobilization ability of MPSs.[14] The isoelectric point of lysozyme is 11.4, thus in pH 7.0 buffer solutions the positively charged proteins may be encapsulated into MPSs with negatively charged silica walls. Fig. 2a shows the adsorbed amount of lysozyme within MPSs as a function of time when the

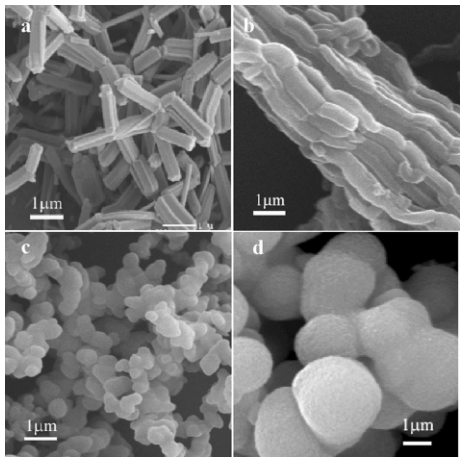


Fig. 1 SEM images of (a) Rod-SBA-15, (b) Con-SBA-15, (c) MPS-F108 and (d) MPS-F127.

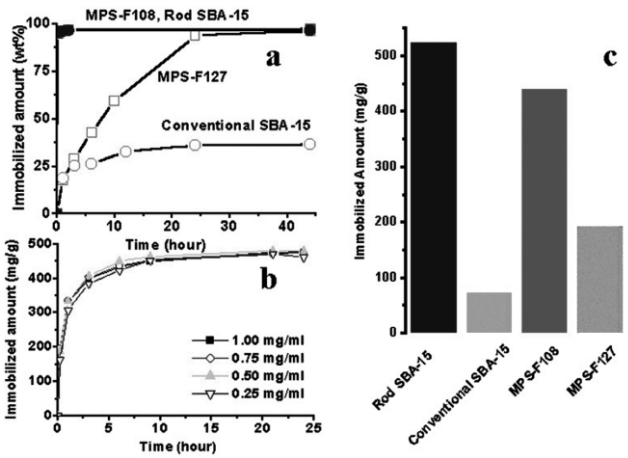


Fig. 2 a) Adsorption amount of lysozymes in different MPSs as a function of time (enzyme/silica = 0.2). b) Adsorption amount of lysozymes within Rod-SBA-15 (enzyme/silica = 0.5) as function of time with different enzyme concentrations. c) Comparison of the enzyme immobilization amount for different MPSs.

† Electronic supplementary information (ESI) available: XRD and nitrogen sorption isotherms for MPSs used in bioimmobilization. See http://www.rsc.org/suppdata/cc/b3/b304391f/
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enzyme/silica ratio is 0.2 (w/w). Adsorption of lysozymes within Rod-SBA-15 is very rapid and complete, and almost 94% enzymes can be immobilized into Rod-SBA-15 in less than 10 minutes. This rapid bioimmobilization ability is also unusual even compared with that of organic polymeric matrices, which are widely used for entrapment of proteins.[15] However, it takes more than 12 hours for Con-SBA-15 to reach equilibrium and only ~40% lysozymes are encapsulated. O'Connor and co-workers have also found that up to four days are required to reach equilibrium for lysozyme and trypsin immobilized in MCM-41 hosts.[12] Such slow adsorption behavior of conventional MPSs is believed to be due to hindered diffusion effects. Specific capacity of MPSs increases with increasing of enzyme/silica ratio until saturation. When the initial enzyme/silica ratio reaches 0.6 (w/w), Rod-SBA-15 has an ultra-high capacity (533 mg g^{-1}) for lysozyme (Fig. 2c), 7.3 times larger than Con-SBA-15. Up to date, the highest capacity is ~198 mg g^{-1} achieved by immobilizing subtilisin within large pore FSM-16 materials.[10] Previous investigations showed that an increase in initial enzyme concentration led to an increase in specific adsorption amount due to enhancement of the driving force from solution towards to the hosts.[12] In contrast, the adsorption of lysozymes within Rod-SBA-15 is almost independent of initial enzyme concentration (Fig. 2b), suggesting that the dynamic adsorption behavior in Rod-SBA-15 is quite different from that of conventional MPSs.

Rod-SBA-15 and Con-SBA-15 show similar external surface area (10 and 9 m^2 g^{-1}, respectively) based on a *t*-plot calculation (see ESI†), suggesting that the rapid adsorption behavior of Rod-SBA-15 should be explained by the "in-pore" effect instead of external surface adsorption. Moreover, Rod-SBA-15 and Con-SBA-15 possess similar physicochemical properties (Table 1); therefore, their different performance in immobilization of lysozymes may be attributed to the difference in their morphologies. When fibrous Con-SBA-15 are "cut" into short rods (Rod-SBA-15), the number of channel openings accessible to enzymes is greatly increased, resulting in very rapid immobilization of enzymes within Rod-SBA-15 materials (Scheme 1). It is believed that when large protein molecules enter the pore channels of MPSs to a certain level, localized multilayer adsorption may block the immobilization of further proteins.[8] Therefore, the specific capacity for SBA-15 should be inversely proportional to the length of particles. This explains the ultra-high immobilization amount of enzymes within Rod-SBA-15 materials.

For three-dimensional caged MPSs, reducing the particle size can also increase their bioimmobilization performance. Both MPS-F127 and MPS-F108 are caged mesoporous networks with similar structural parameters (Table 1)[16] (see ESI†) and spherical morphologies (with average diameter of ~2 and ~0.4 μm, respectively). MPS-F108 with much smaller size also shows a rapid entrapment ability for lysozymes, whereas it takes more than 20 hours for MPS-F127 to reach equilibrium (Fig. 2a). MPS-F108 has a maximal immobilization capacity of 440 mg g^{-1}, 2.3 times larger than the capacity of MPS-F127 (Fig. 2c). This difference may also be explained by the increased number of mesocages accessible to enzymes for MPS-F108 materials.

Table 1 Physicochemical properties of MPSs

Sample	Pore (entrance[a]) diameter/nm	*S*/m^2 g^{-1}	*V*/cm^3 g^{-1}
Con-SBA-15	8.0	804	1.12
Rod-SBA-15	7.9	668	1.14
MPS-F127	12.3 (8.9)	281	0.72
MPS-F108	11.7 (9.2)	372	0.88

[a] Calculated from the desorption branch of the N_2 sorption isotherms based on the BJH method. The pore size is calculated from the adsorption branch. *S*: BET surface area; *V*: total pore volume.

In conclusion, very rapid (< 10 min to reach equilibrium) and high-capacity (up to 533 mg g^{-1}) immobilization of enzymes within MPSs has been reported. More importantly, it is revealed that the macrostructures of MPSs play an important role in their bioimmobilization performance of enzymes; therefore, the morphology of MPSs must be taken into account as well as other factors (surface chemistry and mesostructures) when investigating the bioimmobilization behavior of MPSs. The rapid and high-capacity bioimmobilization ability of MPSs with controlled macrostructures makes them great candidates as new bioimmobilization hosts in future applications. It is supposed that the enzyme is active at the pH used in immobilization, and the crowding loading of biomolecules in these nanocavities/channels may induce order-of–magnitude enhancements in the rate of catalytic reactions compared to enzymes in solutions.[17] Furthermore, the improved molecule diffusion within these special MPSs (R-SBA-15 and MPS-108) is also believed to be useful for various applications involving mass transport in addition to the bioimmobilization process.

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14 In a typical adsorption experiment, lysozymes were dissolved in sodium phosphate buffer at pH 7.0 at 4 °C to make a stock solution (0.25–1.0 mg ml^{-1}). 40 mg of MPSs was added to the stock solution (4–40 ml). Kinetic experiments to determine the amount of lysozyme adsorbed as a function of contact time were conducted by contacting 8 mL of 1.0 mg ml^{-1} protein solution with 40 mg of MPSs with stirring at pH 7.0 at room temperature in a vessel covered to prevent evaporation. Samples were withdrawn periodically for immediate analysis and then returned to the mixture. Adsorbed amounts were measured by the difference of the concentration of the enzyme before and after adsorption by UV adsorption at 280 nm. To determine the adsorption capacities of the MPSs, the initial enzyme/silica ratio was changed between 0.1–0.7 (w/w).
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16 In the synthesis of MPS-F108, 2.0 g $EO_{132}PO_{50}EO_{132}$ (F108), 2.0 g 1,3,5-trimethylbenzene and 5.0 g KCl were dissolved in 120 ml of 2 M HCl. To this solution, 8.3 g of tetraethyl orthosilicate (TEOS) was added under stirring for 24 h at 40 °C. The mixed solution was transferred into an autoclave and kept at 130 °C for one day. Solid products were collected by filtration and dried at room temperature in air. The resulting powders were calcined at 550 °C for 4 h to remove the templates. MPS-F127 was synthesized by a similar method, except $EO_{106}PO_{70}EO_{106}$ (F127) was used as the template.
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