Immobilizing catalysts on porous materials

The discovery of ordered mesoporous materials (OMMs) synthesized in the presence of surfactant templates was a significant breakthrough in the field of porous materials. OMMs offer unprecedented potential for the immobilization of catalysts because of their large pore spaces, ordered pore sizes, and relatively homogeneous pore surfaces. This article briefly summarizes recent advances in the immobilization of homogeneous and enzyme catalysts on OMMs.

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Catalysis plays a vital role in many industries, such as energy and fuels, fine chemicals, pharmaceuticals, and commodity chemicals. Currently, about 90% of chemical manufacturing processes and more than 20% of all industrial products involve catalytic steps.

Heterogeneous catalysts consist, in most cases, of catalytically active component(s) carried on the surface of a solid support (normally a porous solid so as to increase reaction rate), and are widely used in the production of energy and fuels, and commodity chemicals. Heterogeneous catalysts have many advantages over homogeneous ones, such as easy catalyst separation and recovery, regeneration, and use. However, homogeneous catalysts are still used in the food, fine chemical, pharmaceutical, and agrochemical industries. On the one hand, this is because most homogeneous catalytic technologies were established in the first half of the 20th century, during which there was not the same public awareness of environmental issues as there is today. On the other, homogeneous catalysts have some attractive properties, such as high selectivity and accessibility to all catalytically active sites. However, in these environmentally conscious and economically pressured days, homogeneous catalysts are no longer acceptable because of inherent problems, such as corrosion, toxicity, difficulty in catalyst handling and separation from the reaction system, high cost, and the creation of solid waste. Consequently, it has long been appreciated that the use of alternative solid catalysts to replace homogeneous catalysts is the ultimate goal in catalysis science and engineering[1]. One strategy is to immobilize the homogeneous catalyst on an insoluble support, that is heterogenization of the homogeneous catalyst[2].

Enzymes, biological catalysts with high selectivities, have been used in the food industry for hundreds of years. Currently, enzymes are becoming increasingly important in sustainable technology and green chemistry[3]. The application of an enzyme for a given reaction is often hampered by major limitations such as high cost. If an enzyme is immobilized on a rigid support, this limitation can be overcome because the immobilized biocatalyst enables easy separation, the possibility of reuse, and simple operation[4]. Indeed, some immobilized enzymes such as glucose isomerase and penicillin G acylase have reached large-scale industrial applications[5,6], and immobilization of other enzymes has been of great interest in research[7].

The availability of ordered mesoporous materials (OMMs) such as FSM-16[8], MCM-41[9], and SBA-15[10], which are prepared in the presence

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of surfactant micelles or polymers, has opened up unprecedented opportunities for immobilizing both homogeneous and enzyme catalysts. The pore size of OMMs can be precisely controlled over a wide range (2-30 nm). In addition, OMMs allow heterogeneous single-site catalysis to be achieved[3,11,12]. Over the past ten years, research and development in using OMMs as carriers for catalysts has advanced rapidly. This topic has been comprehensively reviewed elsewhere, including general reviews[3,11,12] as well as specific articles on the immobilization of enzymes[13-15] and homogeneous catalysts[16-19] on OMMs. Here, we highlight recent advances in research and development of OMMs for catalyst immobilization.

Ordered mesoporous materials

Traditional porous materials such as silica gels possess a wide range of pore sizes, thus limiting their applications in some cases, e.g. shape-selective catalysis. Zeolites are a family of microporous crystalline materials with uniform pores. But their small pore sizes are not suitable for catalyst immobilization, especially for large catalysts like enzymes.

In the early 1990s, OMMs with uniform pore sizes in the mesopore range (2-50 nm), high surface area (~1000 m^2/g), and large pore volume (~1 cm^3/g) were reported[8-10]. Among the OMMs, FSM-16[8], MCM-41[9], MCM-48[9], SBA-15[10], and MCFs[20] have been extensively studied for catalyst immobilization. Despite their different synthesis pathways, the pore structures of FSM-16 and MCM-41 are essentially similar. Both have highly uniform, hexagonally arranged, one-dimensional cylindrical pores. MCM-48 possesses a three-dimensional, bicontinuous cubic pore structure[9]. Generally speaking, the pore sizes of FSM-16, MCM-41, and MCM-48 are similar and can be tuned in the range of 2-6 nm by using different surfactant templates or adding a pore expander. While the pore structure of SBA-15 resembles that of FSM-16 and MCM-41, the pore sizes of SBA-15 are much larger and can be controlled in the range of 6-15 nm. Importantly, the mesopores are interconnected by micropores[21,22], enabling the pore surfaces to be accessed in three dimensions. The MCF materials are synthesized in a similar protocol to SBA-15, but with oil-in-water microemulsions as templates[20]. MCFs consist of interconnecting cage-like pores with sizes ranging from 20 nm to 40 nm, and pore interconnection widths ranging from 8 nm to 25 nm.

Methods of catalyst immobilization

Four common methods for the immobilization of homogeneous catalysts can be identified, based on the interaction between the catalyst and the solid support[19]: covalent binding, electrostatic interaction, adsorption, and encapsulation.

Covalent binding is by far the most frequently used method for immobilization of homogeneous catalysts. Immobilization via electrostatic ionic interactions is conceptually simple, and is a facile method for immobilizing ionic catalysts or those catalysts that can be ionized under the immobilization conditions. While the adsorption method is simple, it tends to yield an unstable catalyst because of the weak interaction between catalyst and support. Encapsulation is the only catalyst immobilization method that does not require any interaction between the catalyst and the support, but the size of the pore-openings in the support must be smaller than the kinetic size of the immobilized catalyst.

With enzymes, cross-linking and entrapment can also be used in addition to these four immobilization methods[5-7,23-25]. The advantages and disadvantages of the different methods for enzyme immobilization have been discussed by Kennedy[26]. While this comparison was made for enzyme immobilization, the conclusions are also applicable to immobilization of homogeneous catalysts.

Covalent binding

In general, for efficient immobilization the support should first be functionalized. Ordered mesoporous silicas provide excellent opportunities for the immobilization of both homogeneous and enzyme catalysts via covalent binding because of the availability of well-defined silanol groups[27,28]. These groups provide reactive sites for functionalization[29,30] and offer tunable surface properties[31,32], allowing one to control the position and density of the immobilized catalyst precisely[33,34].

Immobilization of homogeneous catalysts via covalent binding

Two distinct approaches can be used to bind chiral homogeneous catalysts, as illustrated in Fig. 1: the sequential and convergent approaches[35]. Among the various OMMs, MCM-41 is the most frequently used support. A superior enantioselectivity has been observed with a chiral ferrocenyl catalyst when covalently confined within the inner walls of MCM-41[33,34]. The superior regio- and stereoselective properties of the molecular catalysts confined in OMMs was interpreted in terms of three possible interactions of the substrate with the pore wall, chiral ligand, and metal center, as illustrated in Fig. 2[34]. The presence of the pore wall close to the catalytic center restricts the approach of the substrate and the transition state, thus altering both activity and selectivity. This confinement effect does not occur when nonporous supports are used because they have only external surfaces where no pore constraints come into play.

Li and coworkers[18,36-38] described the immobilization of a variety of homogeneous epoxidation catalysts on OMMs including MCM-41 and SBA-15. Of most interest is the method for the immobilization of a chiral Mn(salen) complex[37]. This was achieved through the complexation of Mn by oxygen atoms of salen phenoxyl groups that had been grafted onto the surface of MCM-41. Immobilization of the Mn(salen) complex onto unmodified MCM-41 lacking the anchored phenol species was unsuccessful. While the activity of the immobilized catalyst was decreased, mainly caused by the slow diffusion of the reactant and oxidant into the mesopores of the MCM-41 support, the enantioselectivity was notably increased because of the unique spatial

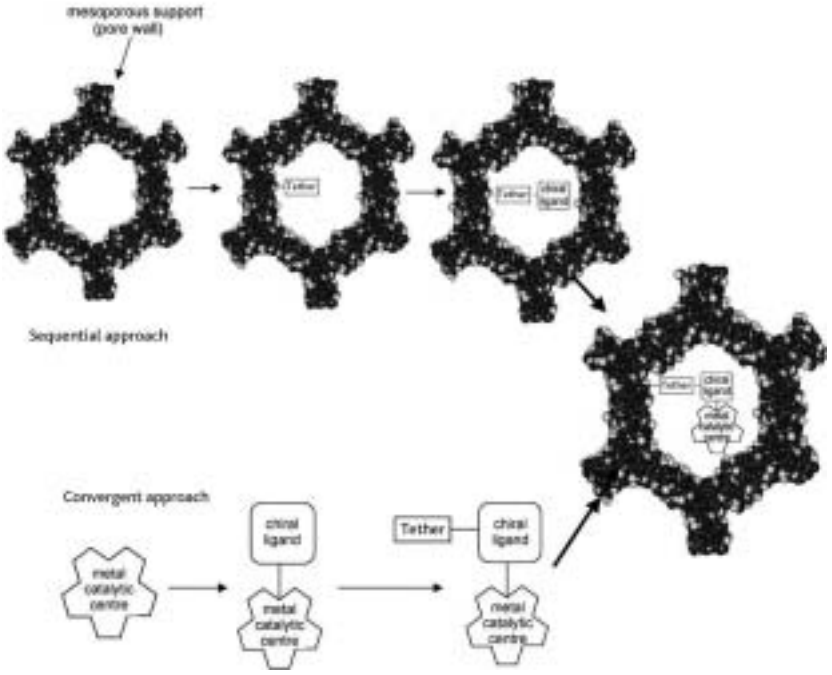

Fig. 1 Schematic of the two approaches to immobilizing an organometallic homogeneous catalyst on the surface of mesoporous silicas. (Reprinted with permission from[35]. © 2005 Wiley-VCH.)

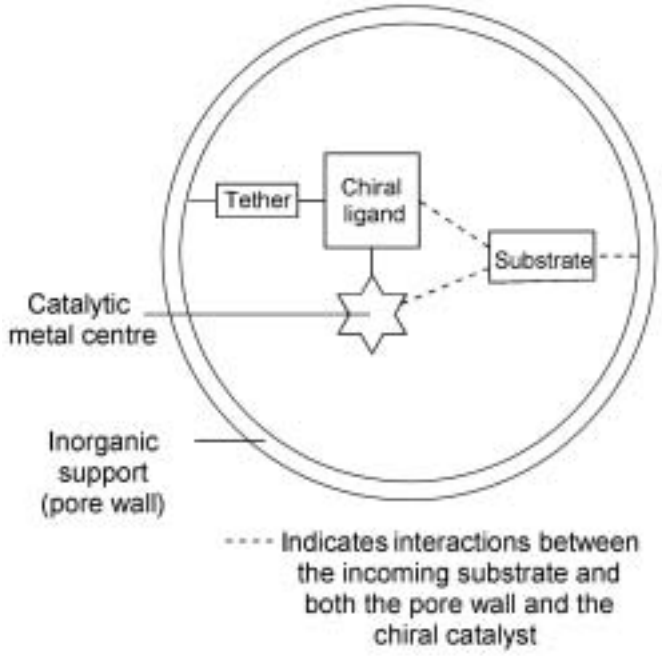

Fig. 2 Schematic showing a chiral catalyst constrained within a mesopore. (Reprinted with permission from[34]. © 2000 Royal Society of Chemistry.)

environment imposed by the MCM-41 mesopores. A further study by the same group[38] discussed factors that could result in the observed high enantioselectivity of the immobilized catalysts. Mesoporous supports have a confinement effect on heterogeneous asymmetric catalytic reactions that affects the enantioselectivity. As illustrated in Fig. 3[18], the confinement effect in the pores or on the surfaces is composed of the spatial restriction, electronic interactions, diffusion dynamics, and adsorption interactions of both the reactants and

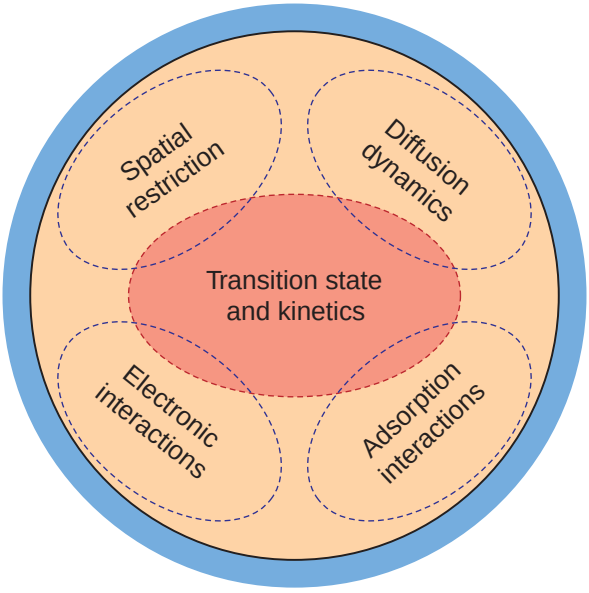

Fig. 3 Schematic of the confinement effect at the pore and surface in chiral synthesis. (Reprinted with permission from[18]. © 2004 Taylor & Francis.)

products. All these interactions can have an influence on the transition states of the chiral reactions.

Alternatively, a homogeneous catalyst can be immobilized on the surface of OMMs during hydrothermal synthesis. For example, Corma and coworkers[39] demonstrated that hydrolysis and condensation of a chiral vanadyl salen complex having two peripheral trimethoxysilyl groups in the presence of the surfactant cetyltrimethylammonium bromide, followed by removal of the surfactant using an acidified ethanol solution, leaves a mesoporous solid behind containing the metal-organic complex. The catalyst obtained displays a high turnover frequency for the room-temperature cyanosilylation reaction.

Immobilization of enzymes via covalent binding

OMMs must be functionalized for immobilizing enzymes using the covalent binding method. The most useful surface functional groups are thiols, carboxylic acids, alkyl chlorides, and amines[14]. Other functional groups, such as vinyls, have been found to modify the enzyme's environment by increasing the hydrophobicity of the support surface[40].

It should be noted that functionalization of a mesoporous silica surface by replacement of surface silanols with organic functionalities can kinetically alter the efficiency of covalent binding. For example, we compared the immobilization of penicillin G acylase (PGA) on SBA-15 silicas functionalized with different loadings of oxirane groups[41]. We found that a partially functionalized SBA-15 sample exhibited not only a high loading of PGA, but also fast binding kinetics between PGA and the oxirane groups (Fig. 4). This is attributed to the role that surface silanol groups play in the process of binding PGA. The silanol groups ensure that the solid surface is negatively charged under the experimental conditions, thus creating an electrostatic interaction between the positively charged enzyme and the support.

The major advantage of covalent binding is the stability of the immobilized enzyme, thus minimizing enzyme leaching. Wang and coworkers[42] observed that α-chymotrypsin immobilized on mesoporous silicas functionalized with trimethoxysilylpropanal exhibited a >1000 folder higher half life than the native enzyme, both in aqueous solution and organic solvents. Pandya *et al.*[43] found that immobilized α-amylase on amino-functionalized MCM-41, SBA-15, and MCF supports showed higher thermal and pH stabilities than the free enzyme. Tortajada and coworkers[44] described the covalent binding of

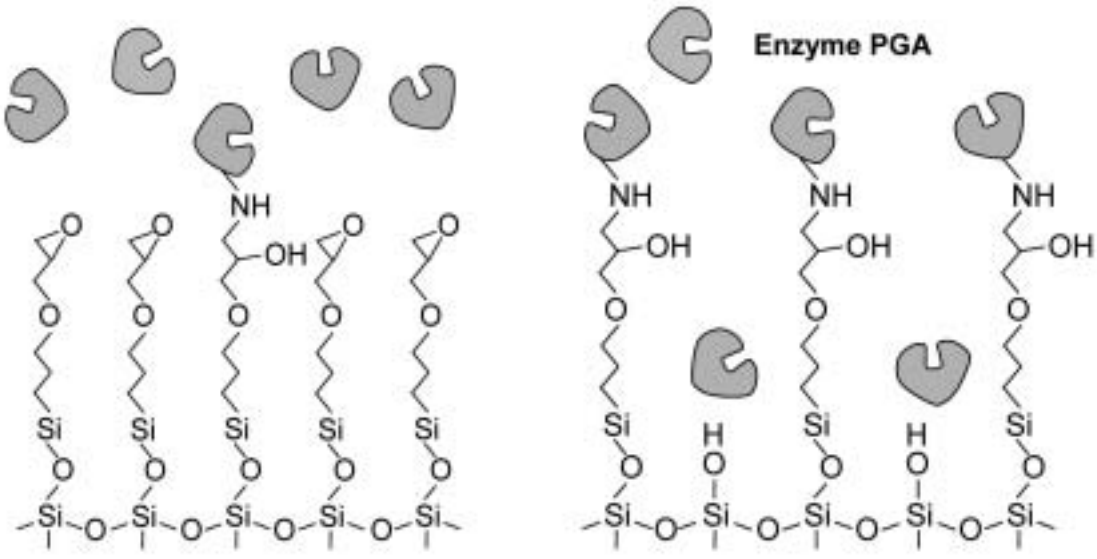

Fig. 4 Schematic showing the superior ability of nanoporous SBA-15 partially functionalized with oxirane (right) in binding enzyme PGA over completely functionalized SBA-15 (left). After functionalization, residual silanols rapidly bring PGA to the surface, which facilitates the formation of chemical linkages between PGA and the oxirane groups. The oxirane groups bind with amine or thiol groups on the surface of the enzyme.

α-L-arabinofuranosidase, an enzyme that is usually used in the wine industry but is easily inhibited under typical wine-making conditions, to an amino-functionalized, bimodal mesoporous silica support. Upon immobilization, the biocatalyst not only works under a wider range of experimental conditions (lower pH and higher temperatures), but also possesses a higher resistance toward glucose and ethanol in comparison with the free enzyme. Yiu *et al.*[45] employed SBA-15 materials with different surface functionalities (–SH, –Ph, –Cl, $–NH_2$, and –COOH) to immobilize trypsin. Leaching of the enzyme was largely solved by using SBA-15 functionalized with –SH, –Cl, and –COOH.

It must be noted that the harsh conditions employed during covalent binding can potentially alter the enzyme conformation, thus lowering the enzymatic activity. In addition, binding of the active sites of the enzyme with a support may result in a total loss of the activity. We have found that PGA physically adsorbed onto the pores of SBA-15 silica retains up to 97% of the activity of free PGA, while PGA covalently attached onto the pores of oxirane-grafted SBA-15 retains only 60% of the activity. Nevertheless, such a loss in activity can be compensated by the advantages of immobilized enzymes, such as easy separation from the reaction medium, potential reuse, and the possibility of using the immobilized enzyme in a packed-bed or fluidized-bed reactor.

Electrostatic interaction

The covalent binding method is relatively complex, involving a few preparative steps, making it unsuitable for large-scale preparations. Immobilization via ionic interaction between the catalyst and solid support has been explored for many years. Many porous solids such as zeolites are surface-charged, and the surfaces of others can be ionized.

Electrostatic interaction between the homogeneous catalyst and support has been shown to be sufficiently strong to minimize leaching[19,46]. Recently, various cationic diphosphine Rh complexes have been supported on Al-containing MCM-41 materials[46]. The catalysts were prepared by impregnating the carrier with a chloride salt of the catalyst precursor in dichloromethane. Decoloration of the solution and the yellow color of the resultant solid gave evidence that the complex had been loaded onto the support. In this way, supported Rh catalysts with Rh contents varying between 0.02 mmol/g and 0.07 mmol/g were obtained. Ligand *S,S*-Me-DuPHOS supported on Al-MCM-41 displayed the best catalytic performance in asymmetric hydrogenation of dimethylitaconate. Importantly, the immobilized catalysts can be easily recovered and reused without loss in catalytic activity.

Mn(salen) complex has also been heterogenized by using Al-MCM-41 as the support[47,48]. Ion exchange of Al-MCM-41 with aqueous $Mn(OAc)_2$ yielded Mn-exchanged Al-MCM-41. This was refluxed with chiral salen ligand (*R,R*)-(–)-*N,N'*-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediamine in CH_2Cl_2, leading to a 10% incorporation of the chiral salen ligand. Asymmetric epoxidation of (*Z*)-stilbene using iodosyl benzene oxidant showed that the heterogenized Mn(salen) catalyst gave a higher *cis*/*trans* ratio of the epoxide products than the homogeneous counterpart. The Mn(salen) immobilized in the mesopores of Al-MCM-41 restricted the rotation of the radical intermediate and increased the amount of the product *cis*-epoxide.

Immobilization of enzymes can also benefit from strong electrostatic interactions between the enzyme and the support. For instance, Lei and coworkers[49] immobilized organophosphorus hydrolase (OPH) on SBA-15 functionalized with $–NH_2$ and –COOH groups at pH 7.5. The mesoporous carrier with 2% –COOH groups exhibited the highest protein loadings (4.7% w/w) and the highest activity (4182 units mg^{-1} support). In contrast, the carrier with 20% $–NH_2$ groups was found to have a very low loading. The observed difference can be rationalized by the effect of surface charges. The isoelectric point of OPH is 8.3, so OPH possesses a positive charge at pH 7.5, while carboxylic acid groups are negatively charged and amine groups are positively charged. Thus, a net attractive interaction occurs between the enzyme and the COOH-functionalized material, and a net repulsive interaction exists between the enzyme and the NH_2-functionalized solid. Hudson *et al.*[50] measured the adsorption properties of cytochrome c and xylanase on pure silica SBA-15 and organo-functionalized SBA-15 carriers. They concluded that electrostatic forces dominate the interaction between the enzymes and pure silica SBA-15, while weak hydrophobic forces provide the major interaction between the proteins and organo-functionalized SBA-15. The morphology of OMMs also plays a significant role in the efficiency of enzyme immobilization. According to Fan and coworkers[51], an SBA-15 sample with rod-like macromorphology displays faster loading kinetics and a higher loading capacity for lysozyme than an SBA-15 sample with fiber-like morphology.

Adsorption

Catalysts immobilized by adsorption mainly rely on weak van der Waals interactions. Thus, the catalyst will readily leach into the reaction medium during use. The stability of the immobilized catalyst can be improved by modifying the catalyst and support to enable hydrogen bonding to occur. Anderson and coworkers[52,53] showed that hexagonal mesoporous silicas (HMS) with a pore size of 2.6 nm are an effective carrier for chiral Rh and Ru catalysts because the match between the size of the pore and catalyst minimizes leaching.

Adsorption is by far the most widely used method for enzyme immobilization onto OMMs because it is simple and no further treatment of the support is needed. Thus denaturation of the enzyme is avoided. The most prominent advantages of using OMMs for adsorbing enzymes include:

- High enzyme loading as a result of the high specific surface area of OMM materials (~1000 m^2/g);
- The confinement effect in the tailorable mesopores, which improves enzyme stability and activity;

- Highly ordered pore structure and uniform surface chemistry offering predictable enzyme behaviors; and
- The simplicity of the method.

For example, Vinu *et al.*[54,55] and Deere *et al.*[56] observe adsorption loadings as high as ~500 mg/g for cytochrome c on SBA-15 materials. The work of Takahashi *et al.*[57] indicates the importance of a close match between the pore size of a support and the molecular size of the enzyme. However, it should be noted that the proximity of the pore size of an OMM to the size of the enzyme imposes substantial diffusion barriers on the enzyme in entering the pores. Thus, the pore channels far from the pore openings are unlikely to be accessible to the enzyme.

Interestingly, the enzymatic activity and stability upon immobilization via physical adsorption are improved in some cases[40,56,58]. The improvement in activity is believed to be the result of enhanced interactions between the substrate and immobilized enzyme[40], or increased spin states of the adsorbed enzyme[56]. The enhancement of stability upon immobilization has been experimentally observed[58] and theoretically confirmed[59].

Encapsulation

The encapsulation method works by enclosing the catalyst in the pore space, where the size of the pore opening is smaller than the diameter of the pore space. While the small pore-opening size prevents loss of the encapsulated catalyst into the reaction medium, it also imposes a strong mass transfer resistance for the reactant and product.

Immobilization of homogeneous catalysts by the encapsulation method can be achieved using three different approaches[18,19]:

- Assembling the individual building units of the catalyst (e.g. the metal and chiral ligand for a chiral catalyst) into the pores of the support using chemical synthesis strategies (also called 'ship-in-a-bottle' synthesis);
- Forming the solid support around the catalyst; and
- Coordination of ligands for the transition metal in the framework of the support.

While the encapsulation method has been found useful for immobilizing homogeneous catalysts in zeolites[60-62], it has not been widely used for immobilization on OMMs. This is perhaps because the large pore size of OMMs does not prevent the immobilized catalyst from leaching in the course of reaction. However, the encapsulation method has been used in immobilization of enzymes on OMMs[63-65], including the first report of using OMMs for enzyme immobilization by Balkus Jr. and Díaz[63]. Wang and Caruso[64] have described the encapsulation of various enzymes through physical adsorption in mesoporous silica spheres followed by deposition of a shell of polyelectrolytes and/or silica nanoparticles onto the exteriors of the silica spheres using the layer-by-layer (LbL) technique (Fig. 5). An immobilized catalase showed a high reusability (70% activity remained after 25 successive batch reactions), high stability toward pH, and high resistance towards proteolysis. Yu *et al.*[65] fabricated novel poly(L-lysine)/poly(L-glutamic acid) microcapsules containing a high

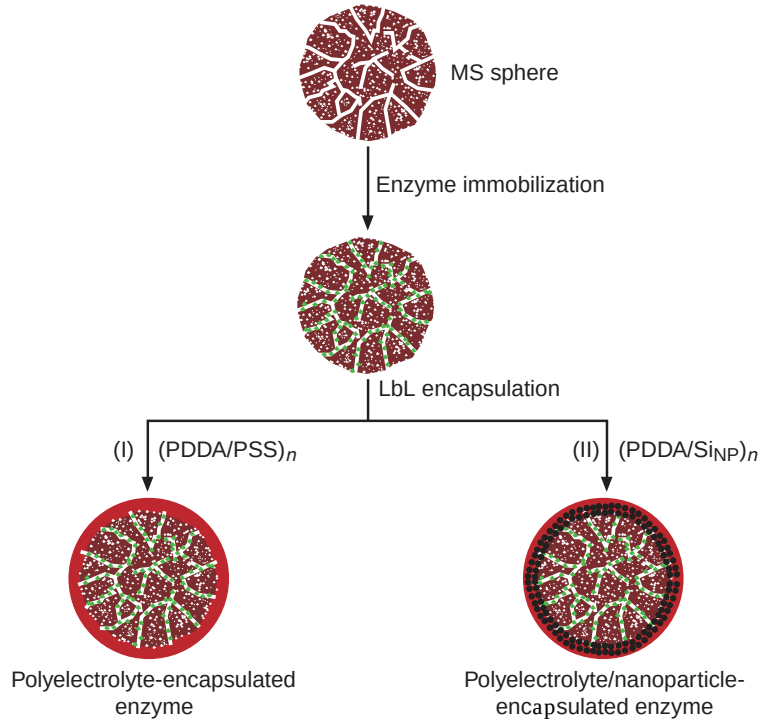

Fig. 5 Schematic of enzyme encapsulation using mesoporous silica (MS) spheres as supports. The enzyme is first immobilized and subsequently encapsulated by a multilayer shell of (I) polyelectrolytes or (II) polyelectrolyte and nanoparticles. (Reprinted with permission from[64]. © 2004 American Chemical Society.)

concentration of catalase (40 mg/ml). The porosity of the polyelectrolyte layers and, consequently, the release of the protein can be controlled by adjusting the pH or ionic strength of the solution.

Other methods for enzyme immobilization

Cross-linking

Cross-linking refers to the construction of three-dimensional enzyme aggregates by linking the enzyme molecules covalently. The major drawbacks of this methodology include the difficulty in controlling the size of the aggregates, substrate accessibility to the cores of the aggregates, and the lack of mechanical strength of the cross-linked enzyme.

These problems may be overcome by combining this approach with other enzyme immobilization techniques. For example, an enzyme can be physically adsorbed in a three-dimensional network of interconnecting cages, with diameters several times the size of the enzyme, followed by cross-linking (Fig. 6). The size of the enzyme aggregates will be controlled by the size of the cage, and the diffusion barrier for the substrate to approach the core of the enzyme aggregates may be lowered. In addition, the mechanical strength of the enzyme aggregates can be strengthened by the scaffold. Furthermore, leaching of the enzyme aggregates can be minimized by tuning the cage interconnections. Recently, Lee and coworkers[66] adsorbed α-chymotrypsin into the cages of a hierarchically ordered mesocellular, mesoporous silica material, and then cross-linked the enzyme in the cages.

Entrapment

Entrapment refers to the physical confinement of an enzyme in an environment where the substrate is able to penetrate but the enzyme cannot escape. The entrapment method suffers from the following drawbacks:

- The difficulty in controlling the enzyme confining environment may impose large diffusion barriers on the transport of the substrate or product, resulting in reaction retardation and long response times;
- If the pore size distribution of the entrapment medium is not narrow, continuous loss of activity because of enzyme leaching can occur; and
- Traditionally used polymers for enzyme entrapment often experience shrinkage and/or swelling during a reaction.

Various strategies have been developed for the entrapment of enzymes into mesoporous materials. Blin *et al.*[67] demonstrated the entrapment of glucose oxidase in mesostructured silicas using a one-step sol-gel method. Under neutral pH conditions, the enzyme solution was added into a micellar solution of a nonionic fluorinated surfactant. Gelation of tetramethyl orthosilicate around the micelles formed an enzyme-incorporated mesostructure. Improvements were made by Mureseanu *et al.*[68], who described a one-step direct sol-gel entrapment strategy for immobilizing lipase on OMMs. The immobilized biocatalyst showed a high retention of activity and low leaching.

Summary

The recent research results reviewed here strongly demonstrate the unprecedented opportunities that OMMs provide for the immobilization of homogeneous and enzyme catalysts. It is interesting to note that experimental data shows MCM-41 materials with one-dimensional cylindrical pores of smaller size perform better in immobilizing homogeneous catalysts than SBA-15 materials with a three-dimensional pore structure and larger pore size. This is believed to be related to the greater confinement effect of MCM-41. In the near future, it is likely that this highly effective confinement effect will be made the most of in the design of a heterogenized catalyst system for practical applications. Chemical engineering aspects, such as designing a highly effective and energy-saving reactor and operation under

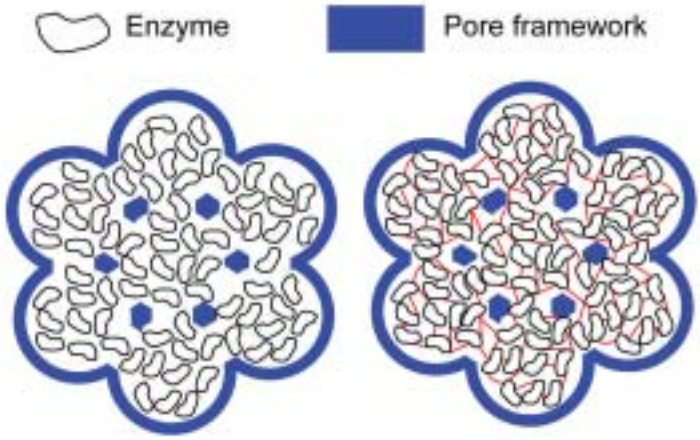

Fig. 6 Enzymes physically loaded in cages easily leach away through cage interconnections (left), whereas enzyme aggregates formed after cross-linking are encapsulated in the cage network (right).

biphasic conditions[69], should also be taken into account in the development of immobilized homogeneous catalysts.

In many cases, enzymes immobilized on OMMs exhibit not only improved stability and reusability, but also enhanced activity. Immobilized enzymes on OMMs also display some unique properties that are not observed with free enzymes. For instance, hemoglobin and horseradish peroxidase immobilized on mesoporous silicas display an excellent electrocatalytic response to the reduction of hydrogen peroxide without the aid of an electron mediator[70]. The activity of the immobilized enzyme is a complex parameter determined by a number of factors, such as the immobilization method used, the surface properties and pore structure of the support, and the molecular size of the substrate. The relationship between enzyme activity and these factors must be well established before the immobilized biocatalyst is commercialized. While the advantages of using OMMs as enzyme supports have been widely demonstrated, research on scaling up the reactions has not been done, yet such information is important in further tailoring of the support materials for industrial biocatalysis.

A number of industrial processes using immobilized homogeneous catalysts[71] and biocatalysts[5,6] are already well established. So we can be very optimistic that homogeneous and enzyme catalysts immobilized on OMMs will be used in the near future for catalytic steps with high chemo-, regio-, and stereo-specificity in the fine chemical, pharmaceutical, agrochemical, and healthcare industries. mt

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