Supporting Information

Immobilizing Penicillin G Acylase Using Silica Supported Ionic Liquids: the Effects of Ionic Liquid Loadings

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S1. Experimental Section

S1.1. Experimental Procedure for the Solvent Route The exact experimental process was as following: 2 g dried silicas and 5 g ILs were added into 100 mL pure solvents or their mixtures, 1 mL deionized water was also added as catalyst for the hydrolysis of TEOS when pure organic solvents were used,¹ and the system was first sonificated for 10 min to make the silicas dispersed well, and then kept at 50 °C for 24 h with magnetically stirring. After reaction, the silicas were collected via the centrifugation at 5800 rpm for 10 min, and washed with fresh solvents, dichloromethane, and ethanol. During the washing process, the eluent was detected by 2 M AgNO₃ solution until AgCl could not be observed and then the conductivity measurements of the eluent was conducted to make sure that the washing was complete and free ILs were thoroughly extracted. After dried under vacuum at 80 °C for 48 h with the presence of P_2O_5 , the products were kept for use.

S1.2. Experimental Procedure for Solvent-free Condition The exact process was: two grams of silicas was ground into fine powders by agate mortar and then the ILs were slowly added to the silicas with stirring until the latter lost the appearance of a dry powder and 0.5 mL water was also added as catalysts. The total mass of ILs added was 1.527 g. The mixture was kept first at room temperature for 7 h and then at 50 °C for another 17 h. After reaction, the excess liquid was removed by the same washing operation as described above.

S1.3. Factors Affecting the IL Loading

S1.3.1 Reaction time: 20 mL TEOS dissolved in 80 mL ethanol was dropped into the system containing 80 mL ethanol, 10 mL NH_3 · H_2O , and 16 mL deionized water. The mixture was kept at 55 °C for 3 h under stirring to allow the formation of silica particles. Then 5.6 g ILs dissolved in 20 mL ethanol was added, and an aliquot of 5 mL sample was taken out at different time since the addition of the ILs from 0.5 h to

45 h. The samples were centrifugated, washed and dried as described in Section S1.1.

S1.3.2 Temperature: The formation process of silica particles was the same as that described in (1). After the adding of the ILs, the mixture was divided equally into 4 aliquots, each aliquot containing approximately 1 g silicas and 1.4 g ILs. The four aliquots were allowed to react at 15, 30, 50 and 70 °C, respectively, for 24 h. The samples were thoroughly washed and dried as described in Section S1.1.

S1.3.3 The IL dosage: After the formation of silica particles as described in (1), the mixture was divided equally into 4 aliquots and different amounts of ILs, i.e., 0.04, 0.2, 2, and 4 g, were afforded to each aliquot. According to the previous experimental results, the IL loading on silicas was approximately 0.1 mmol IL/(g silicas), i.e., ~0.04 g IL/g silicas. Because each aliquot contains ~1 g silicas, the provided amounts of ILs were from 1 time to 100 times higher than the needed amounts.

S1.4. Characterization The size and morphology of the silicas were characterized using scanning electron microscopy (SEM) on JSM-6700 F (Japan). The particle size distributions were calculated using data from more than 300 particles selected from different regions of several SEM images. The Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectra were taken by using a Bruker Vecter 22 FTIR spectrometer equipped with a smart orbit assessor. The IL loading on silicas were determined via N elemental analysis, performed using a CE-440 element analyzer. Thermogravimetry analysis (TGA) was carried out on a TGA 1 analyzers (Mettler); the temperature was heated programmedly from 25 to 800 °C at a speed of 10 °C /min in the atmosphere of N₂ of 40 mL/min. Transmission electron microscopy (TEM) and energy-dispersive spectrometry (EDS) measurements were carried out by JEM-2010 transmission electron microscopy coupled with an EDS (Oxford) system. The zeta potentials were measured using DelsaTM Nano zeta potential analyzer. The results presented are the average of five independent measurements. Solid state UV-Vis was conducted on Double Beam UV-vis Spectrophotometer (TU 1901, Beijing Persee General Instrument Company).

S1.5. Immobilization and Activity Assay PGA The PGA loading (Q (mg protein/g carrier)) on carriers was calculated by mass balance. The PGA loading and the specific activity (SA, IU/mg protein) are described as follows, respectively:

 $Q \text{ (mg protein/g carrier)} = \frac{Q_1 - Q_2}{m}$

Specific activity (IU/mg) = $\frac{A}{Q}$

where Q_1 is the total amount of protein before immobilization, Q_2 (mg) is the total amount of protein in the residual solution after immobilization, m [g] is the dry weight of supports used for immobilization, and A (IU) is the apparent activity of the ImPGA.

One unit of enzyme activity (IU) was defined as the amount of enzyme required to produce 1 μ mol 6-APA per min under the assay conditions. The specific activity of enzyme is the amount of enzyme activity units contained by per milligram protein. The apparent activity of ImPGA is the amount of enzyme activity units contained by the ImPGA on 50 milligram carriers.

For detecting the reusability of ImPGA, the vials containing 10 ml 2% (w/t) penicillin G potassium solutions in 0.1 M pH 7.4 phosphate buffer were preheated to 37 °C, and 50 mg ImPGA was then added. After reaction at 37 °C for 10 min, the production of 6-APA was analyzed. After one batch, the ImPGA was separated by centrifugation, and washed once with 0.1 M pH 7.4 phosphate buffer. The fresh substrate solution was added to start the next operation, under the same reaction conditions.

S2. Results and Discussion



Figure S1. SEM images of the prepared silicas (a) and their size distributions (b). Number-average size: 284 nm.



Figure S2. SEM images of the prepared silicas (a) and their size distributions (b). Number-average size: 361 nm.

S2.1. Discussion for the Solvent [BMIM]Cl/H₂O A possible reason was that the positively charged cations in [BMIM]Cl might interact with the negatively charged surface of silicas, which disturbed or weakened the stability of H-bonds formed between water and Si-OH, and thus increased the rate of condensation reaction of IL onto silica surface. As regards as the "Solvent-free" method, a relatively high IL

loading was also observed. One of the advantages of this method was that it required much less amounts of ILs than those of reactions in certain solvents. In our experiment, only approximately one third of the amounts of ILs were used, comparing with the reaction in certain solvents, but obtaining a higher IL loading. But due to the high viscosity of ILs, the limitation in mass transport may become the bottleneck of this method.



Figure S3. Effects of the adding time (*T*) of ILs on the morphology of SILs. *T* was set at *T*=0 min since the beginning of TEOS. (a) 0 min; (b) 5 min; (c) 10 min; (d) 30 min; (e) 60 min; (f) 90 min; (g) 120 min; (h) 150 min; (i) 180 min; (j) 300 min.



Figure S4. The possible growth mechanism of silicas with (upper) and without the presence of ILs (lower).

S2.2. Effects of the Time of Adding ILs (T) on the Sizes and the IL Loading

The effects of the time of adding ILs on the sizes and the IL loading were shown in Table S1. With the delaying of the addition of ILs, the shape of silicas changed from amorphous to spherical, and once the silica cores were formed, the sizes had no obvious change with the different time of adding ILs and kept monodispersive. The IL loading exhibited a first decreasing and then increasing trend with the delaying of the addition of ILs. When the ILs were added at an early time, the IL molecules participated the formation of the -Si-O-Si- network, and thus large amounts of ILs were immobilized onto the silica precipitate, resulting in an IL loading as high as 1.918 mmol/(g silicas). With the delaying of the addition of ILs, the -Si-O-Sinetwork gradually formed, and less and less ILs participated the formation process of the -Si-O-Si- network and thus the IL loading decreased. It could also be seen that there was a decrease in the IL loading since the formation of silica cores. This could be due to the changing of the size distributions because the PDI exhibited a slight decrease from 30 to 120 min. After 120 min, a slight increase in the IL loading was observed, which may be related with the pH of the solution. With the increasing of reaction time, the consuming of NH₃·H₂O led to the decrease in the basicity of the

bulk solution. It was reported that the triethoxysilane groups could self-hydrolyze and polymerize between molecules in the basic condition.¹ Under decreasing basicity, the rate of self-polymerize of ILs decreased and thus more IL molecules were immobilized onto the silica surface.

Time (min)	Morphology	IL loading	Number-average	PDI
		(mmol/g silicas)	sizes (nm)	
0	amorphous	1.918	-	-
5	amorphous	1.631	-	-
10	amorphous	1.375	-	-
30	spherical	0.228	186±19	1.033
60	spherical	0.159	193±19	1.030
90	spherical	0.137	172±15	1.024
120	spherical	0.129	180±16	1.024
150	spherical	0.144	182±17	1.031
180	spherical	0.161	212±19	1.025
300	spherical	0.182	202±17	1.022

Table S1. Effects of the time of adding ILs on the morphology, sizes and IL loading of SILs.



Figure S5 TG profile of the SIL sample. IL loading: 0.102 (mmol/g silicas)

S2.3. Energy-Dispersive Spectrometry (EDS) Mapping

If the ILs are located onto the surface of silicas, the characteristic signals of ILs, N and Cl elements, in the EDS mapping would be uniform. If the ILs are embedded into the inner portion of silica particles, the signals of N and Cl elements in the EDS mapping will exhibit an increasing density from the edge to the inner portion of a silica particle. TEM images and EDS spectrum were shown in Figure S6. The results showed that Sol-gel 2 samples had a regular spherical shape while the Sol-gel 1 samples were aggregated severely, as shown in SEM images (Figure 4a, in the manuscript). The EDS analysis showed that both the Sol-gel 1 samples and the Sol-gel 2 samples contained Cl element, which proved the existence of ILs. The Sol-gel 1 sample was conducted the EDS mapping analysis (Figure S7). The mapping range was shown in the black pane portion in Figure S6. The distribution of Si element was given to show the border of the sample because Si had a high content which gave a strong signal (Figure 7a).



Figure S6. TEM and EDS analysis of samples prepared by the Sol-gel Route. (a) Sol-gel 1 samples and (b) Sol-gel 2 samples. The EDS spectrum was taken at the black pane portion in (a). The samples prepared by the Sol-gel 2 method possessed similar EDS spectrum (data not shown).



Figure S7. EDS mapping of the samples prepared by the Sol-gel 1 method. (a) Si element and (b) N element. The white curve in (a) and (b) was added to show the border of the sample in EDS mapping. The arrows were directed to the inner portion of samples, and the density of Si or N element increased along the arrow direction.

S2.4. X-ray Diffraction

The weak peaks at approximately $2\theta = 37^{\circ}$, 45° , 65° and 77° (shown in the inserted curve in Figure S8a) might be caused by the trace amount of absorbed materials on

the silica surfaces, such as salts containing NH_4^+ or Na^+ introduced in the preparation of silicas.² This was probable because the silica surfaces were negatively charged under basic condition and tended to absorb positively charged ions. But when ILs were immobilized onto the silicas, the amounts of the impurities were decreased and their peaks in XRD became weaker (Figure 8b) and finally disappeared (Figure S8c, d, and e).



Figure S8. XRD patterns of silicas and SILs with different IL loadings. The samples a, b, c, and d were prepared by the Sol-gel 2 method, and the sample e was prepared by the Sol-gel 1 method. a, SiO₂, b-e, SILs with IL loading of 0.129, 0.182, 0.228, and 1.918 mmol/(g silicas), respectively. The inserted curve for SiO₂ was the magnification in the range $2\theta = 35^{\circ}-80^{\circ}$.

S2.5. Diffuse Reflectance Infrared Fourier Transform (DRIFT) Spectra

Figure S9 showed the DRIFT spectrum of samples with different IL loadings or prepared by different methods. Figure 9a was the spectrum of naked SiO₂. The broad band between 3000 and 3500 cm⁻¹ could be attributed to the stretching vibrations of Si-OH, while the band at approximately 1640 cm⁻¹ corresponded to its bending vibrations. The peaks at 1400, 1100, and 470 cm⁻¹ were characteristics of Si-O bonds.³ For the SILs prepared by the Sol-gel 2 method (b-g), the peaks at 2989 and 2910 cm⁻¹ were the stretching vibrations of $-CH_3$ and $-CH_2$ - in ILs, respectively; those at 1485 and 1451 cm⁻¹ corresponded to their bending vibrations.⁴ For the SILs prepared by the

Sol-gel 1 method (h-j), firstly, the stretching vibration absorbance of $-CH_3$ and $-CH_2$ -appeared at similar positions as those of the Sol-gel 2 samples (at ~ 2989 and 2910 cm⁻¹), but with a much weaker signal though the IL loadings were higher. This may be explained by the low content of ethoxyl groups for the Sol-gel 1 samples because three of the ethoxyl groups in ILs (imbedded into silicas) were reacted, while only one or two of the three ethoxyl groups were reacted for the Sol-gel 2 method due to the steric hindrance of ethoxyl groups on the silica surface.⁵ Secondly, several new peaks or peak shifts were observed. The new peaks at 3157 and 3110 cm⁻¹ corresponded to the C-H stretching vibrations of the imidazolium ring.⁴ The peaks at 1573 and 1660 cm⁻¹ might be assigned to the C=C and C=N vibrations in the imidazole ring.⁶ The band shift of Si-O bonds from 1100 to 1066 cm⁻¹ might result from the integration of Si-O peaks in silica network and C-N peaks due to the introduction of ILs, while the peak shift from 470 to 457 cm⁻¹ could be caused by the relatively high content of Si-C bonds for the Sol-gel 1 samples due to the high IL loadings.



Figure S9. The DRIFT spectra of silicas and SILs: a, silicas; b-g: SILs prepared by the Sol-gel 2 method with IL loadings of 0.129, 0.137, 0.144, 0.161, 0.182, and 0.228 mmol IL/(g silicas), respectively; h-j: SILs prepared by the Sol-gel 1 method with IL loadings of 1.375, 1.631, and 1.918 mmol IL/(g silicas), respectively.



Figure S10 Specific activity of native PGA and ImPGA on different carriers. The ILs used in SiO₂-ILs was [C1C(S)Im]Cl.

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