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# Enantioseparation of Racemic Amlodipine Using Immobilized Ionic Liquid by Solid-phase Extraction

Min Liu[1,2], Zhong-Qian Liu [1,2], Hai Zhu [1,2], Chao-Hong He* [1,2] and Ke-Jun Wu * [3]

**Abstract:** In this paper, a novel L-glutamate based immobilized chiral ionic liquid (SBA-IL(Glu)) was prepared by chemical bonding method and applied as a solid sorbent for chiral separation of amlodipine. The performance of SBA-IL(Glu) was investigated for the absorption of (*S*)-amlodipine and separation of amlodipine enantiomer. The static experiment showed that equilibrium adsorption was achieved within 80 min, and the saturation adsorptions capacity was 12 mg/g. The complex was then packed in a glass chromatographic column for the separation of amlodipine and the enantiomeric excess (%ee) of (S)-amlodipine reached 24.67%. The immobilized ionic liquids exhibit good reusability, and the separation efficiency remains 18.24% after reused 5 times, which allows potential scale-up for the chiral separation of amlodipine.

**Keywords**: amlodipine, enantioseparation, immobilized amino ionic liquid, solid-phase extraction

## Introduction

Hypertension is the most common chronic disease, and the main risk factor of cardiovascular and cerebrovascular disease. Cardiovascular drug remains the main pharmaceutical in fatalities. [1] Amlodipine possessing (*S*)- and (*R*)- isomers (Figure 1) have been widely used in clinical treatment of hypertension and angina pectoris. As is well known, the hypotensive activity of (*S*)-amlodipine is about 2000 times higher than that of (*R*)-amlodipine, and (*R*)-amlodipine might cause side effects such as peripheral edema.[2,3] Thus, optically pure (*S*)-amlodipine is intensely demanded.

However, the direct synthesis of (*S*)-amlodipine on an industrial scale has been rarely reported. [4,5] (*S*)-amlodipine is usually obtained by the racemic synthesis of amlodipine racemate, followed by enantioselective separation of amlodipine racemate using chromatography,[6,7] preferential crystallization ,[8] capillary electrophoresis, [9,10] and liquid-liquid extraction, etc.[11,12] Amlodipine racemate can be separated by high-performance liquid chromatography (HPLC) with chiral stationary phases or chiral mobile phase additives. Using Chiralcel OJ chiral column, the resolution of amlodipine racemate was as high as 3.23, and the resolution could achieve 2.08 with $C_{18}$ column by adding SBE-β-CD to mobile phase. However, single enantiomer was mandatory for chromatography, which led to a high separation cost.[4] Preferential crystallization of (*S*)-amlodipine or (*R*)-amlodipine from the mixture of tartaric acid and racemic amlodipine in organic solvents (e.g. DMSO, DMF or DCM) is industrially feasible. However, the large amount of organic solvents used always brings about safety and environmental problems. Besides, too many operations such as mixing, crystallization, solid separation make the process complicated and time-consuming.[7] Enantioselective separation by capillary electrophoresis is performed by adding chiral additives (e.g. cyclodextrin derivatives, proteins or maltodextrin) to the running buffer. For example, the resolution of amlodipine racemate was improved from 0.93 to 2.59 after adding amino-functionalized silica nanoparticles to background electrolyte.[8] Nevertheless, the application of capillary electrophoresis is only applicable in laboratory scale due to its small sample throughput, long processing time and high cost. Liquid-liquid extraction is widely considered as an alternative approach to address the disadvantages of aforementioned methods. The commonly used chiral extractants include tartaric acid derivatives and cyclodextrin derivatives, most of which exhibit poor extraction efficiency and need to be handled at low temperature. In order to improve the extraction efficiency and reduce energy consumption, many efforts have been made to develop new extractants. Because of the outstanding thermal stability, designable structure, negligible vapor pressure and good chiral recognition ability, chiral ionic liquids (CILs) have attracted more and more attention in enantioselective liquid-liquid extraction as extractants.[5, 11-13]

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CILs were used as solvent and acceptor, and ethylacetate as donor phase when applied in extracting more L-phenylalanine through ligand-exchange mechanism. After single-step extraction, the value of %ee achieved 50.6%.[14] CILs in organic phase were used as chiral selector in extraction of flurbiprofen in aqueous phase and the maximum enantioselectivity was up to 1.20.[15] As for enantioselective separation of amlodipine racemate, enantioselectivity up to 1.35 at 298.15 K was obtained by a novel extraction system consisting of n-decanol, NaAc/HAc buffer solution and [Bmim][Glu] amino acid CILs in our previous study.[16] Although CILs serving as extractant exhibited high performance in enantioselective separation, the high pressure drop and poor mass transfer properties trigged by the high viscosity of CILs, and the high cost and environmental issues caused by the low recyclability of CILs hampered the large scale application of liquid-liquid extraction based on CILs.

To overcome the drawbacks of CILs in liquid-liquid extraction induced by liquidus nature, and take advantage of the availability of CILs in enantioselective separation, one of the most effective approaches was to immobilize CILs over solid supports. To date, a wide range of solid supports (silica, SBA-15, MCM-41) were employed. The immobilization of CILs over the surface of solid supports can be achieved through immersion, covalent anchoring and physical encapsulation methods.[17] The immersion method is carried out by directly impregnating the solid supports in CILs, and a thin film of CILs is coated on the solid supports by a physical adsorption. In this case, the leaching of CILs cannot be avoided. When CILs are connected with the surface of solid support via covalent bonds, the stability of immobilized CILs was improved, but the chemical bonding may limit the degree of freedom of cations or anions. The physical encapsulation method is established by using porous solid supports such as silica gel, NaY zeolite and aluminum oxide. The CILs are confined in matrices and form a high-concentration environment. The as-fabricated immobilized ionic liquids combine the advantages of ionic liquids and solid supports (e.g. large specific surface area, easy separation, good reusability). Therefore, the immobilized ionic liquids exhibit superior performance in comparison with their counterparts. Immobilized ionic liquids are widely used in solid phase extraction (SPE).[18,19] As the adsorbent of SPE, immobilized CILs not only exhibit good separation ability and selectivity, but also reduce the use of toxic organic solvents and realize the recycling of CILs.

In this work, immobilized L-glutamate based ionic liquid are synthesized and applied in SPE for enantioselective separation of amlodipine racemate. The morphology and structure of as-prepared immobilized chiral ionic liquid was characterized. The static adsorption performance of the immobilized chiral ionic liquid was studied to test the adsorption property. In the dynamic adsorption process, the enantiomeric recognition and separation potency were tested. In addition, the regeneration and recycle performance of the immobilized ionic liquids were also investigated. SBA-IL(Glu) is a rather stable and efficient absorbent, which could resolve the problem of toxic pollution (ILs residues in target products) and stability in previous works.

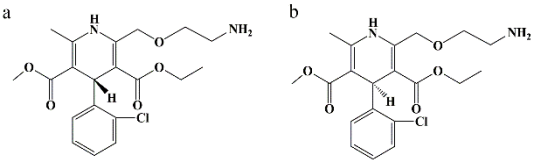



FIGURE 1 The structures of (a) (*R*)-amlodipine and (b) (*S*)-amlodipine.

## Materials and Methods

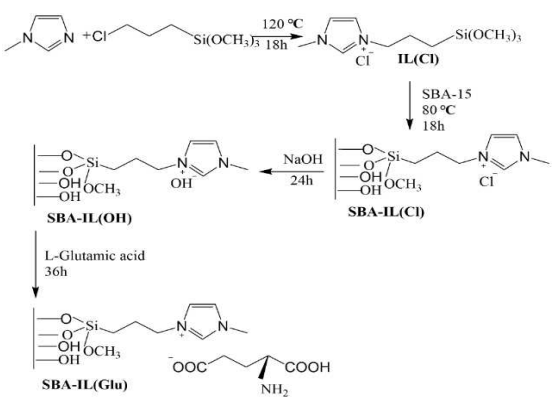
### Chemicals

SBA-15 was purchased from Xianfeng nanomaterial technology Co. Ltd (Jiangsu, China). 1-methyl-imidazole (AR, 98%), (3-chloropropyl) trimethoxysilane (AR, 98%), ethanol (HPLC grade, 99.8%), n-decanol (AR, 98%), methanol (HPLC grade, 99.5%), n-hexane (AR, 98%), and acetonitrile (HPLC grade, 98%) were purchased from Aladdin Reagent Co. Ltd (Shanghai, China). Amlodipine (racemate, HPLC grade, 99%) was purchased from Dalian Meilun Biotech Co. Ltd (Liaoning, China). Sodium acetate (AR, 99%) and acetate (AR, 99.5%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). L-Glutamic acid (AR, 99%) was purchased from Shanghai Darui fine chemicals Co., LTD (China). All chemicals were used as received without any further treatment. By mixing sodium acetate and acetate solution (both 0.3 mol/L) in different proportions, NaAc/HAc buffer solutions with different pH values were prepared.

### The preparation of SBA-IL(Glu)

First, an equimolar mixture of (3-chloropropyl) trimethoxysilane and 1-methylimidazole was stirred with a magnetic stirrer under nitrogen atmosphere at 110 °C for 16 h (Scheme 1).[20] The ionic liquid precursor generated was filtered, washed three times with ethyl acetate, dried in a rotary evaporator (40 ˚C), and denoted as IL(Cl). 0.015 mol IL(Cl) was dissolved in 300 mL acetonitrile, followed by the ultrasonic dispersion of 4 g of SBA-15 for 30 min. This suspension was kept at 80 ˚C for 24 h under magnetic stirring to obtain immobilized halogenated ionic liquid (SBA-IL(Cl)).[21-23] The as-prepared SBA-IL(Cl) was filtered, washed with acetonitrile, acetone and dichloromethane three times each and then dried for 24 h in a vacuum oven.

Subsequently, SBA-IL(Cl) was dispersed into 1000 mL of NaOH aqueous solution (0.01 mol/L) by ultrasonic treatment and stirred for 2 h to perform ionic exchange at room temperature. Then alkalized immobilized ionic liquid (SBA-IL(OH)) was collected, filtered, washed with deionized water three times and dried for 24 h in a vacuum oven.[24] The as-prepared SBA-IL(OH) was suspended in 0.1 mol/L L-glutamate aqueous solution to conduct anion exchange with stirring for 24 h at room temperature.[25] Finally, the immobilized chiral ionic liquid SBA-IL(Glu) was obtained after being washed with ethanol and deionized water in sequence.




SCHEME 1 Synthesis of SBA-IL(Glu)

**Characterization of SBA-IL(Glu)**

FT-IR spectra were recorded on Nicolet 5700 spectrometer (Thermo Electron Corporation, America) using KBr pellets. $^{29}$Si NMR spectroscopy was performed on a Bruker AVANCE III HD spectrometer (400 MHz, Bruker, Germany). Elemental analysis was carried out by using Vario Micro analyzer (Analysensysteme GmbH, Germany). The $N_2$ adsorption-desorption isotherms were measured using the AUTOSORB-IQ2-MP apparatus at 77 K (Quantachrome, America). The specific surface and pore structure were determined by Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) methods, respectively. X-ray powder diffraction (XRD) spectra were recorded on X-pert Powder diffractometer (PANalytical B. V., Netherlands) from 0.5°-10° using Cu target (voltage: 40 kV, current: 40 mA). The thermo gravimetric (TG) curves were collected on a TA-Q500 instrument. Transmission electron microscopy (TEM) was performed using a HT-7700 microscope (Hitachi Limited, Japan).

## Static adsorption of (*S*)-amlodipine

**Kinetic experiment**

A certain amount of *(S)*-amlodipine was dissolved into 10 mL NaAc/HAc buffer solution (pH = 6) to prepare *(S)*-amlodipine solution. 0.1 g of SBA-IL(Glu) was added into 10 mL (*S*)-amlodipine solution with the concentration of 250 mg/L. Several milliliters of the suspension were withdrawn at different time intervals and centrifuged to separate the solid adsorbent. An aliquot of the supernatant was taken to determine the concentration of amlodipine by high-performance liquid chromatography (HPLC, Agilent 1260 Infinity LC system). The Enantiopak SCDP column (5 μm, 4.6×250 mm$^2$) was employed with the mobile phase consisting of n-hexane (containing 0.1% trifluoroacetic acid) and ethanol (90/10, v/v) at 25 ˚C . The flow rate of the mobile phase was set at 1 mL/min, and the UV wavelength was set at 237 nm. The standard measurement errors are u(T) = 0.1 ˚C , u(pH) = 0.01, and u for amlodipine concentration measurement is 0.6%.

The adsorption amount of *(S)*-amlodipine ($q_t$) was calculated from the following equation:

$$q_t = (c_0 - c_t)\frac{V}{W} \quad (1)$$

where $c_0$ is the initial concentration of *(S)*-amlodipine and $c_t$ is the concentration of *(S)*-amlodipine at time *t*. *V* and *W* represent the volume of *(S)*-amlodipine solution and weight of SBA-IL(Glu) used for static adsorption, respectively.

**Equilibrium experiment**

A certain amount of *(S)*-amlodipine was dissolved into 0.3 mol·L$^{-1}$ NaAc/HAc buffer solution (pH = 6) to prepare *(S)*-amlodipine solutions with different concentrations. 0.1 g of SBA-IL(Glu) was added into 10 mL (*S*)-amlodipine solutions with different concentrations. All equilibrium experiments were carried out under the same condition with the kinetic experiment. After the adsorption equilibrium was established, the suspension was withdrawn and centrifuged. An aliquot of the supernatant was taken to determine the equilibrium concentration of amlodipine by HPLC. The equilibrium adsorption amount of *(S)*-amlodipine ($q_e$) was calculated from the equation as follows:

$$q_e = (c_0 - c_e)\frac{V}{W} \quad (2)$$

where $c_e$ is the equilibrium concentration of *(S)*-amlodipine.

**Influence of temperature on the adsorption of (*S*)-amlodipine**

Temperature is an important factor that affects the adsorption of (S)-amlodipine. The effect of temperature on adsorption amount was studied with the temperature varies from 5 °C to 45 °C. 0.1 g SBA-IL(Glu) were used to adsorb (*S*)-amlodipine (250 mg/L, 10ml) at different temperature for 2 h. The adsorption amount was calculated.

**Cycle ability**

The cycle ability of SBA-IL(Glu) was evaluated. After each absorption cycle, after static adsorption of 250 mg/L (S)-amlodipine, the used SBA-IL(Glu) (0.1 g) was immerged into 10 mL desorption solution (methanol/water, 50/50, v/v) for 60 min. After centrifugation, the concentration of amlodipine in the desorption solution was analyzed by HPLC. The SBA-IL(Glu) will be reused after desorption and drying for the next cycle.

## Separation of amlodipine racemate on SBA-IL(Glu) column

0.5 g of SBA-IL(Glu) was mixed with 5 mL of NaAc/HAc buffer solution (pH = 6), and then used as the absorbent to pack a glass chromatography column (length = 20 cm, i.d. = 1cm) via wet-packing method. This glass chromatography column was employed to separate 1 mL of racemic amlodipine with the concentration of 500 mg/L. A mixture of NaAc/HAc buffer solution (pH = 3) and methanol (50/50, v/v) was selected as the mobile phase. The elution rate was set at 0.05 mL/min and the solution are collected every 10 min, then analyzed with HPLC. The recycle ability of SBA-IL(Glu) was evaluated. After desorption and drying, the used column is used to the next cycle.

## Results and discussion

### Characterization of SBA-IL(Glu)

Figure 2a shows the FT-IR spectra of IL(Cl), SBA-15 and SBA-15 supported ionic liquid obtained at different steps. The typical Si-O-Si stretching vibration bands around 1081, 794, and 457 $cm^{-1}$ can be easily observed in all the tested samples except IL(Cl).[26]

Comparing to the FTIR spectra of SBA-15, the characteristic absorption peaks of stretching vibration of C=N and C=C bond can be found around 1630 $cm^{-1}$ and 1560 $cm^{-1}$ for products of each step,[27] which is the evidence of the immobilization of CILs. For the end product SBA-IL(Glu), the characteristic adsorption band at 1718 $cm^{-1}$ represents the stretching vibration C=O in carboxyl of chiral amino acid.[28] The bands located at 2952 and 2917 $cm^{-1}$ are corresponding to the vibration of unsaturated C-H and saturated C-H.[29] These characteristic absorption peaks show that chiral ionic liquid has been successfully grafted onto the surface of carrier. The typical broad peak at 3500 $cm^{-1}$ was assigned to the presence of OH group.[21]

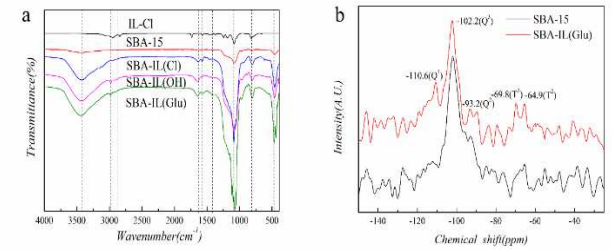



FIGURE 2 (a) FT-IR spectra of IL-Cl, SBA-15, SBA-IL(Cl), SBA-IL(OH), and SBA-IL(Glu); (b) $^{29}$Si-NMR of SBA-15 and SBA-IL(Glu).

Solid-state $^{29}$Si NMR was used to study the interaction between ionic liquid and SBA-15. The $^{29}$Si NMR spectra of SBA-15 and SBA-IL(Glu) were displayed in Figure 2b. The spectrum of SBA-15 has three characteristic peaks in the -110.6 ppm, $Q^4[Si(OSi)_4]$; -102.2 ppm, $Q^3[Si(OSi)_3(OH)]$ and -93.2 ppm, $Q^2[Si(OSi)_2(OH)_2]$. The spectra presents five peaks in Figure 2b. Compared to SBA-15, SBA-IL(Glu) shows a decrease in the -102.2 ppm, $Q^3[Si(OSi)_3(OH)]$; and -93.2 ppm, $Q^2[Si(OSi)_2(OH)_2]$; and an increase in the -110.6 ppm, $Q^4[Si(OSi)_4]$. New peaks are located at -69.8 ppm, $T^3[SiC(OSi)_3]$; and -64.9 ppm, $T^2[SiC(OSi)_2(OH)]$. The presence of $T^2$ and $T^3$ signal confirmed that SBA-15 was functionalized with ionic liquid by covalent bound. Triethoxysilane of IL(Cl) reacts with the alcohol hydroxyl group on the surface of SBA-15 by condensation reaction, then SBA-15 (pure silicon skeleton) turns to organosilicon material. There are some residual non-condensed silanol groups on the surface of SBA-15.[30,31]

The thermal stabilities of SBA-15 and SBA-15 supported ionic liquid obtained at different steps were evaluated by thermo gravimetric analysis (TGA). As shown in Figure 3a, SBA-15 shows no obvious weight loss with the temperature ranging from 120 °C to 700 °C, indicating the high thermal stability of SBA-15. The TGA curve of SBA-IL(Cl) exhibits two distinct weight loss stages in the same temperature range. The first weight loss stage between 200-400 °C (10.32%) can be attributed to the decomposition of organic moieties like amino acid and imidazole ring,[22] while the second weight loss (10.13%) occurred over 400 °C is mainly due to the break of the residual methoxy side group.[32]

For SBA-IL(Cl), the loading ratio is close to 20%, but because of the corrosion in the alkalization process, the total loading mass fraction of SBA-IL(OH) deceased to 8.22%. Compared with SBA-IL(OH), the mass loss rate of SBA-IL(Glu) from 400 °C to 700 °C is almost unchanged, while the mass loss in the first stage increased from 3.58% to 5.10%, due to the larger group weight of L-glutamate than hydroxyl. These behaviors indicate the process of ions exchange and the successful immobilization of the chiral ionic liquid and it was deduced that the

loading of ionic liquid is 86.4 mg/g.

Elemental analysis was carried out to support the above results of the chemical immobilization of ionic liquid. We found IL(Glu) [$C_{14}H_{28}N_4O_5Si$] in SBA-IL(Glu) was C, 1.48%; N, 5.54%; H, 1.89%. The element analysis result of C/N ratio (3.74) for SBA-IL(Glu) matches well with the calculated one (3.71), which indicates that the synthetic sample has high purity. The calculated load of amino ILs in SBA-IL(Glu) is 71.1 mg/g.

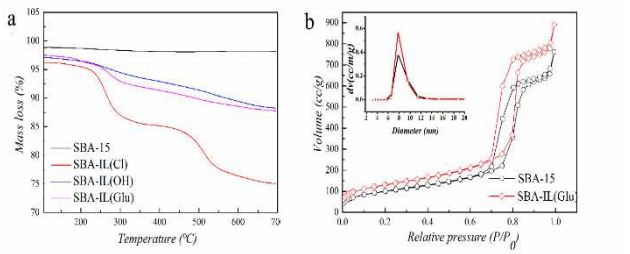



Figure 3 (a) TGA analysis of SBA-15 and modified SBA-15 samples; (b). Nitrogen adsorption desorption isotherms of SBA-15 and SBA-IL(Glu)

TABLE 1 The structure properties for SBA-15 and SBA-IL(Glu).

| Sample | $S_{BET}$ ($m^2$/g) | Pore volume (cc/g) | Pore diameter (nm) |
|---|---|---|---|
| SBA-15 | 599 | 0.76 | 7.80 |
| SBA-IL(Glu) | 435. | 0.73 | 7.83 |

Figure 3b presents the $N_2$ adsorption-desorption isotherms and pore size distribution of SBA-15 and SBA-IL(Glu). Both isotherms exhibit type IV isotherms with $H_1$ type hysteresis loop for the capillary condensation/evaporation of nitrogen in cylindrical open-ended mesopores of SBA-15.[33] This indicated that SBA-IL(Glu) preserved the mesoporous structure of SBA-15. Table 1 summarizes the specific surface area ($S_{BET}$) and pore properties of SBA-15 and SBA-IL(Glu). SBA-15 has a large specific surface area of 599 $m^2$/g, pore volume of 0.76 cc/g and pore diameter of 7.80 nm. After the immobilization of amino ionic liquid over SBA-15, the specific surface area and pore volume both decreased, while the pore diameter increased slightly. The decrease in specific surface area and pore volume may be attributed to the modification of CILs on the surface and in the channels of SBA-15. The slight increase of pore diameter may be resultant of the selective closing of small pores in the modification process.

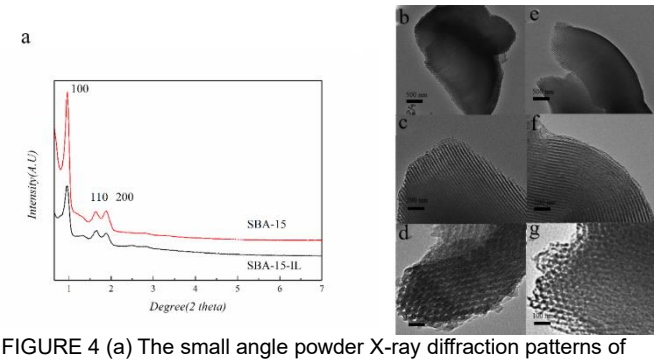



FIGURE 4 (a) The small angle powder X-ray diffraction patterns of SBA-15 and SBA-IL(Glu); (b, c, d) TEM images of SBA-15 and (e, f, g) SBA-IL(Glu).

Figure 4a shows the small-angel powder XRD patterns of SBA-15 and SBA-IL(Glu). The diffraction peaks at 2θ = 0.91°, 1.59°, 1.83° in the XRD pattern of SBA-15 can be assigned to (100), (110), and (200) reflections, respectively. SBA-IL(Glu) exhibits a similar XRD pattern to that of SBA-15, indicating the preservation of two-dimensional hexagonal mesostructure of SBA-15. This is in good accordance with the results of $N_2$ physisorption. Moreover, the intensities of diffraction peaks of SBA-IL(Glu) were much smaller than those of SBA-15, indicating the immobilization of ionic liquid inside the mesoporous channel of SBA-15.[34, 35]

The TEM images of SBA-15 and SBA-IL(Glu) are shown in Figure 4(b-g). SBA-15 and SBA-IL(Glu) possessed uniform and well-ordered mesopores with the diameter of 8-10 nm arranged in hexagonal structure. In TEM picture the structure of SBA-15 can still be observed, indicating that the modification of CILs onto the surface of SBA-15 didn't destroy the Si-O-Si network of the supporting materials.[21]

**Static adsorption of (*S*)-amlodipine over SBA-IL(Glu)**

The equilibration time is a key factor of the adsorption process. The influence of contact time in the adsorption process is investigated and the results are shown in Figure 5a. It is found that the initial adsorption rate is fast and most (*S*)-amlodipine was adsorbed within half an hour. More than 65% saturation adsorption could be obtained within 20 min because in the initial stage large amount of binding sites are empty. With the increase of adsorption time, the concentration of amlodipine in the solution deceased, which caused the decline of the adsorption rate. The adsorption amount increases gradually and the adsorption reaches equilibration at 80 min approximately.

Figure 5b depicts the influence of initial concentration of (*S*)-amlodipine on the equilibrium adsorption amount. The equilibrium adsorption amount increased dramatically from 0 to 12 mg/g as the initial concentration of (*S*)-amlodipine increased from 5 $mg \cdot L^{-1}$ to 250 $mg \cdot L^{-1}$. Then the equilibrium adsorption amount achieved

a plateau with the increase in initial concentration of (*S*)-amlodipine. Because the coordination sites of carrier are limited, the adsorption capacity tends to saturate while more amlodipine molecular are existing than the adsorption capacity of SBA-IL(Glu), thus the adsorption amount increases slowly and then the adsorption reaches a maximum. So, 250 mg·L$^{-1}$ is selected as the optimized concentration.

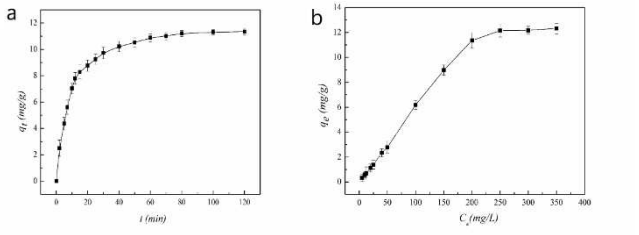



FIGURE 5 The adsorption experiment of SBA-IL(Glu) for (S)-amlodipine. (a) The kinetic experiments, (S)-amlodipine concentration: 250 mg/L, absorbent dose: 100 mg/10 mL.; (b) The equilibrium experiments, absorbent dose: 100 mg/10 mL, equilibrium time: 2 h.

**Influence of temperature on the adsorption of (*S*)-amlodipine**

**The influence of temperature on adsorption amount is shown in Figure 6. The absorbed amount of (*S*)-amlodipine decreased with the increase of temperature. Because interactions between SBA-IL(Glu) and (*S*)-amlodipine get weakened under higher temperature. Lower temperature is beneficial to improve the adsorption amount. Taking the energy saving and cost reducing into account, the room temperature (around 25 ℃) is the suitable adsorption temperature.**

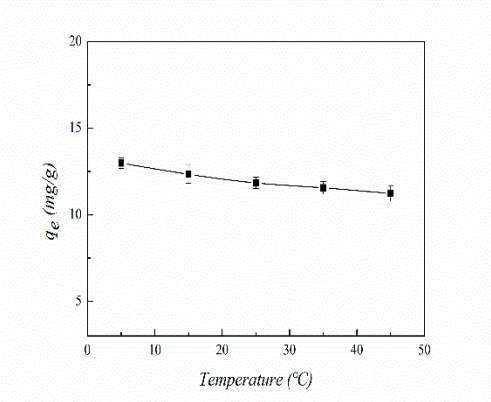



**FIGURE 6 The influence of temperature on adsorption of (*S*)-amlodipine**

**The correlation of static adsorption data**

Adsorption rate data are correlated by the pseudo-first-order kinetic model and pseudo-second-order equation, respectively.

$$ln\left(q_e - q_t\right) = lnq_e - k_1 t \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

$k_1$ is the pseudo-first-order rate constant for the adsorption process (min$^{-1}$), and $k_2$ is the pseudo-second-order rate constant for the adsorption (g·mg$^{-1}$·min$^{-1}$). The calculated parameters are listed in Table 2 and linear fitting curves are presented in Figure 7a and 7b. The correlation value $R^2$ of pseudo-second-order kinetic model is larger than that of the pseudo-first-order kinetic model, indicating that pseudo-second-order kinetic model is more suitable for the adsorption process of amlodipine.

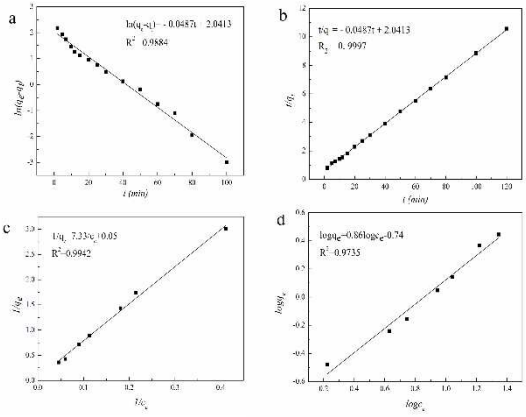



FIGURE 7 Line fitting of SBA-IL(Glu) towards (S)-amlodipine. (a) Pseudo-first-order kinetic models, (b) pseudo-second-order kinetic models, (c) Langmuir models, (d) Freundlich models.

TABLE 2 Parameters according to Pseudo-first-order kinetic model and Pseudo-second-order kinetic model for (*S*)-amlodipine adsorption by SBA-IL(Glu).

| Pseudo-first-order kinetic model | | | Pseudo-second-order kinetic model | | |
|---|---|---|---|---|---|
| $k_1$ (mg/L) | $q_e$ (mg/g) | $R^2$ | $k_2$ (mg/g) | $q_e$ (mg/g) | $R^2$ |
| 0.0487 | 7.7006 | 0.9884 | 0.0108 | 12.1654 | 0.9997 |

Langmuir adsorption isotherm model and Freundlich adsorption isotherm model are the two most widely used models in adsorption process. The corresponding equations can be expressed as follows:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{k_L q_m c_e} \quad (5)$$

$$\log q_e = \log k_F + \log \frac{c_e}{n} \quad (6)$$

where $q_m$ is the maximum adsorption amount, $k_L$ and $k_F$ represent the Langmuir adsorption constant and the Freundlich adsorption constant, respectively. $n$ is an empirical adsorption

constant.

TABLE 3. Parameters according to Langmuir and Freundlich equilibrium models for (*S*)-amlodipine adsorption by SBA-IL(Glu).

| Langmuir isotherm constants | | | Freundlich isotherm constants | | |
|---|---|---|---|---|---|
| $k_L$ (mg/L) | $q_m$ (mg/g) | $R^2$ | $k_F$ (mg/g) | n | $R^2$ |
| 0.0076 | 17.8571 | 0.9951 | 0.4767 | 1.1574 | 0.9779 |

Isothermal parameters are listed in Table 3 and isotherm constants are calculated through linear regression method (Figures 7c and 7d). The correlation value $R^2$ of Langmuir adsorption isotherm model is 0.9942, which shows a better fit with the experimental data. It can be deduced that the adsorption of amlodipine on immobilization chiral ionic liquid is accordance with a single molecule adsorption process.

**Amlodipine static desorption studied of SBA-IL(Glu) and cyclic performance**

In order to investigate the reusability and stability of the prepared SBA-IL(Glu), five cycles of amlodipine adsorption-desorption were studied. As a result, SBA-IL(Glu) can achieve three adsorption-desorption cycles with more than 90% of saturation adsorption capacity remained and decreased when reused for more times (Figure 8). This indicates SBA-IL(Glu) achieves good stability and reusability performance.

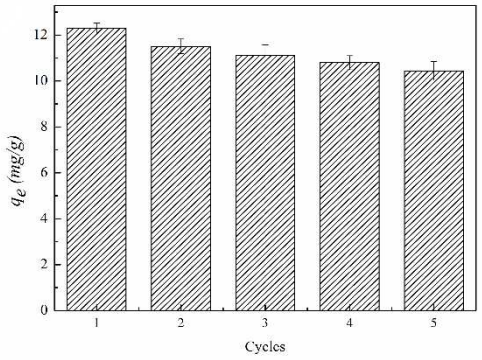



FIGURE 8 Adsorption performance of SBA-IL(Glu) for (S)-amlodipine for 5 cycles.

**Separation of amlodipine enantiomer on SBA-IL(Glu) column**

The separation results in column are shown in Figure 9(a). Two enantiomers of amlodipine were adsorbed selectively, and when the column was eluted with 2.5 mL elution, the enantiomeric excess (%ee) reached 24.67%. The SBA-IL-(Glu) interacts more strongly with (*R*)-amlodipine than (*S*)-amlodipine, the (R)-amlodipine adsorbed by SBA-IL(Glu) have longer retention time than (*S*)-amlodipine when the adsorbed amlodipine racemates were gradually eluted with eluent solution. Thus, the eluting solutions obtained %ee. At first %ee is low because the adsorbed amlodipine is limited compared with amlodipine in the solution. When more and more (*S*)-amlodipine is eluted, the proportion of adsorbed (*R*)-amlodipine increased, then %ee gets higher. At last, most racemates are eluted, the adsorbed (*R*)-amlodipine decreased, as well as %ee.

SBA-IL(Glu) shows appreciable enantiomeric enantioseparation of amlodipine, good repeatability and little waste discharged. The absorbent is used 5 times and the separation efficiency remains 18.24% (Figure 9b), without significant deterioration. The specific recognition ability of SBA-IL(Glu) is due to the ionic hydrophobic and the hydrogen-bonding interactions.

Enantiomeric excess (*%ee*) is of great importance for chiral pharmaceuticals separation. %ee can be expressed as the following equation:

$$\%ee = \frac{c_S - c_R}{c_S + c_R} \times 100 \quad (7)$$

$c_S$ and $c_R$ are the concentration of (*S*)-amlodipine and (*R*)-amlodipine in the eluent solution.

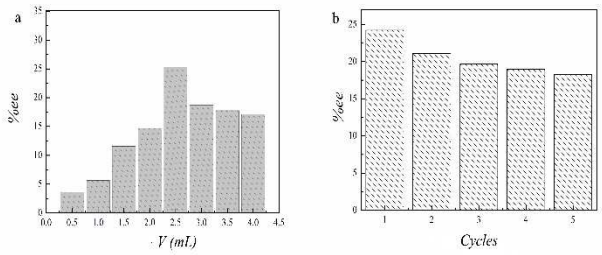



FIGURE 9 (a) Enantiomeric excess of (S)-amlodipine by chromatography column of SBA-IL(Glu); (b) Separation performance of SBA-IL(Glu) for 5 cycles.

## Conclusions

In this work, SBA-15 successfully modified with amino acid ionic liquid (SBA-IL(Glu)) by chemical bonding method is used as a novel chiral sorbent to separate amlodipine enantiomers. The adsorption for (*S*)-amlodipine reached complete equilibrium within 80 min. Under the optimal conditions (contacting time: 80 min, initial concentration: 250 mg·$L^{-1}$, temperature: room temperature, pH = 6), the adsorption capacity of SBA-IL(Glu) is 12 mg/g. The adsorption process could be better described by Langmuir isotherm and pseudo-second-order kinetics model. The adsorption rate of two enantiomers is different, thus SBA-IL(Glu) is used in column chromatography for the chiral separation of amlodipine racemate and good enantioselectivity is achieved. This work provides a probable reference for chiral separation of

amlodipine in industrial production.

## Acknowledgments

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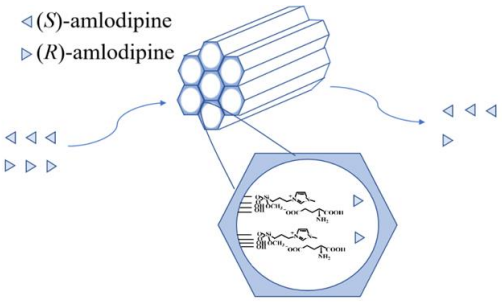
## Reference

1. Gummin, D; Mowry, J; Spyker, D; Brooks, D; Osterthaler, K; Banner, W, 2017 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 35th Annual Report. Clin Toxicol (Phila) **2018**;56(12):1213-1415.
2. Zhang, XP; Loke, KE; Mital, S; Chahwala, S; Hintze, TH, Paradoxical release of nitric oxide by an L-type calcium channel antagonist, the R+ enantiomer of amlodipine. J Cardiovasc Pharm **2002**;39(2):208-214.
3. Sun, YH; Fang, L; Zhu, M; Li, W; Meng, P; Li, L; He, ZG, A drug-in-adhesive transdermal patch for S-amlodipine free base: In vitro and in vivo characterization. Int J Pharmaceut **2009**;382(1-2):165-171.
4. Schuur, B; Verkuijl, BJ; Minnaard, AJ; de Vries, JG; Heeres, HJ; Feringa, BL, Chiral separation by enantioselective liquid-liquid extraction. Org Biomol Chem **2011**;9(1):36-51.
5. Zhang, WY; Cui, X; Jiang, SX; Song, HJ; Zhou, RF; Ye, XQ; He, CH, Enantiseparation of Ofloxacin Enantimers via Diethyl L-Tartrate Extraction. J Chem Eng Chin Univ **2017**;31(4):769-775.
6. Zhao, L; Yang, L; Wang, Q, Silica-based polypeptide-monolithic stationary phase for hydrophilic chromatography and chiral separation. J Chromatogr A **2016**;1446:125-33.
7. Cârje, AG; Balint, A; Muntean, DL; Hancu, G; Ion, V; Imre, S, Comparative Enantioseparation of Amlodipine by HPLC and Capillary Electrophoresis. Acta Medica Marisiensis **2018**;64(1):28-33.
8. Gotrane, DM; Deshmukh, RD; Ranade, PV; Sonawane, SP; Bhawal, BM; Gharpure, MM; Gurjar, MK, A Novel Method for Resolution of Amlodipine. Org Process Res Dev **2010**;14(3):640-643.
9. Gong, Z; Duan, L; Tang, A, Amino-functionalized silica nanoparticles for improved enantiomeric separation in capillary electrophoresis using carboxymethyl-β-cyclodextrin (CM-β-CD) as a chiral selector. Microchim Acta **2015**;182:1297-1304.
10. Sun, X; Du, Y; Zhao, S; Huang, Z; Feng, Z, Enantioseparation of propranolol, amlodipine and metoprolol by electrochromatography using an open tubular capillary modified with beta-cyclodextrin and poly(glycidyl methacrylate) nanoparticles. Mikrochim Acta **2019**;186(2):128.
11. Shan, RN; Cui, X; Ding, Q; He, CH, Racemic metoprolol separation by enantioselective liquid-liquid extraction with amino acid ionic liquids. J Chem Eng Chin Univ **2019**;33(6).
12. Lian, WP; Cui, X; Ding, Q; Shan, RN; He, CH, Enantioseparation of Ketoprofen Enantiomers with 1-Butyl-3-Methylimidazole L-Tryptophan Extraction. J Chem Eng Chin Univ **2018**;32(5):1147-1152.
13. Wu, H; Yao, S; Qian, G; Yao, T; Song, H, A resolution approach of racemic phenylalanine with aqueous two-phase systems of chiral tropine ionic liquids. J Chromatogr A **2015**;1418:150-157.
14. Tang, F; Zhang, Q; Ren, D; Nie, Z; Liu, Q; Yao, S, Functional amino acid ionic liquids as solvent and selector in chiral extraction. J Chromatogr A **2010**;1217(28):4669-74.
15. Cui, X; Ding, Q; Shan, RN; He, CH; Wu, KJ, Enantioseparation of flurbiprofen enantiomers using chiral ionic liquids by liquid-liquid extraction. Chirality **2019**;31(6):457-467.
16. Ding, Q; Cui, X; Xu, GH; He, CH; Wu, KJ, Quantum chemistry calculation aided design of chiral ionic liquid-based extraction system for amlodipine separation. AIChE J **2018**;64(11):4080-4088.
17. Wang, YH; Li, W; Luo, S; Liu, SX; Ma, CH; Li, J, Research Advances on the Applications of Immobilized Ionic Liquids Functional Materials. Acta Chim Sinica **2018**;76(2):85-94.
18. Qian, G; Song, H; Yao, S, Immobilized chiral tropine ionic liquid on silica gel as adsorbent for separation of metal ions and racemic amino acids. J Chromatogr A **2016**;1429:127-33.
19. Song, H; Yang, C; Yohannes, A; Yao, S, Acidic ionic liquid modified silica gel for adsorption and separation of bovine serum albumin (BSA). RSC Advances **2016**;6(109):107452-107462.
20. Zhong, CM; Sasaki, T; Tada, M; Iwasawa, Y, Ni ion-containing ionic liquid salt and Ni ion-containing immobilized ionic liquid on silica: Application to Suzuki cross-coupling reactions between chloroarenes and arylboronic acids. J Catal **2006**;242(2):357-364.
21. Hiremath, V; Jadhav, AH; Lee, H; Kwon, S; Seo, JG, Highly reversible CO2 capture using amino acid functionalized ionic liquids immobilized on mesoporous silica. Chem Eng J **2016**;287:602-617.
22. Zou, B; Hu, Y; Jiang, L; Jia, R; Huang, H, Mesoporous Material SBA-15 Modified by Amino Acid Ionic Liquid To Immobilize Lipase via Ionic Bonding and Cross-Linking Method. Ind Eng Chem Res **2013**;52(8):2844-2851.
23. Yue, CB; Mao, AQ; Wei, YY; Lu, MJ, Knoevenagel condensation reaction catalyzed by task-specific ionic liquid under solvent-free conditions. Catal Commun **2008**;9(7):1571-1574.
24. Ohno, H; Fukumoto, K, Amino acid ionic liquids. Accounts Chem Res **2007**;40(11):1122-1129.
25. Fukumoto, K; Yoshizawa, M; Ohno, H, Room temperature ionic liquids from 20 natural amino acids. J Am Chem Soc **2005**;127(8):2398-9.
26. Mitchell, S; Perez-Ramirez, J, Mesoporous zeolites as enzyme carriers: Synthesis, characterization, and application in biocatalysis. Catal Today **2011**;168(1):28-37.
27. Sahoo, S; Kumar, P; Lefebvre, F; Halligudi, SB, Oxidative kinetic resolution of alcohols using chiral Mn–salen complex immobilized onto ionic liquid modified silica. Appl Catal A-Gen **2009**;354(1-2):17-25.
28. Szewczyk, A; Prokopowicz, M; Sawicki, W; Majda, D; Walker, G, Aminopropyl-functionalized mesoporous silica SBA-15 as drug carrier for cefazolin: adsorption profiles, release studies, and mineralization potential. Micropor Mesopor Mat **2019**;274:113-126.
29. Zhao, H; Yu, N; Ding, Y; Tan, R; Liu, C; Yin, D; Qiu, H; Yin, D, Task-specific basic ionic liquid immobilized on mesoporous silicas: Efficient and reusable catalysts for Knoevenagel condensation in aqueous media. Micropor Mesopor Mat **2010**;136(1-3):10-17.
30. Klaus, A, Ernst, B, Characterization of bonded phases by solid-state NMR spectroscopy. J Chromatogr **1991**;544:345-370.
31. Bettina. P, KA, Ernst. B, Investigations by 29Si cross-polarization magic angle spinning NMR spectroscopy of reaction pathways of silica gel polyfunctional modification. J Chromatogr **1990**;1990(506):343-355.
32. Tian, ML; Yan, HY; Row, KH, Solid-phase extraction of tanshinones from Salvia Miltiorrhiza Bunge using ionic liquid-modified silica sorbents. J Chromatogr B **2009**;877(8-9):738-742.
33. Matsuura, S; Ishii, R; Itoh, T; Hamakawa, S; Tsunoda, T; Hanaoka, T; Mizukami, F, Immobilization of enzyme-encapsulated nanoporous material in a microreactor and reaction analysis. Chem Eng J **2011**;167(2-3):744-749.
34. Yiu, HHP; Wright, PA; Botting, NP, Enzyme immobilisation using SBA-15 mesoporous molecular sieves with functionalised surfaces. J Mol Catal **2001**;15:81-92.
35. Liu, AM; Hidajat, K; Kawi, S; Zhao, DY, A new class of hybrid mesoporous materials with functionalized organic monolayers for selective adsorption of heavy metal ions. Chem Commun **2000**;(13):1145-1146.

Graphical Abstract


(S)-amlodipine
(R)-amlodipine