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Amino-terminated ionic liquid modified graphene oxide coated silica composite stationary phase for hydrophilic interaction chromatography

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Abstract

In this study, an amino-terminated ionic liquid modified graphene oxide coated silica composite stationary phase (IL@GO@SiO_2) has been synthesized and applied for the separation of B vitamins, amino acids and aromatic acids in hydrophilic interaction chromatography (HILIC) mode. The synthesized novel material was characterized by elemental analysis, field emission scanning electron microscope and infrared spectroscopy. The chromatographic properties of IL@GO@ SiO_2 phase were compared with those of pure silica phase, amine-terminated ionic liquid modified silica phase, and graphene oxide modified silica phase. The results show that IL@GO@SiO_2 exhibits the best separation efficiency than the other three phases, which is ascribed to the existing of the multiple interactions, i.e., electrostatic interaction, π-π interaction and dipole-dipole interaction between the resulting stationary phase with the analytes. The differences between the four stationary phases illustrate that the combination of IL and GO plays an important role in improving the separation efficiency.

Keywords: Chromatographic stationary phase / graphene oxide / ionic liquid / hydrophilic interaction liquid chromatography

1. Introduction

"HILIC" is the acronym of hydrophilic interaction liquid chromatography, which was first proposed by Alpert in 1990.[1] HILIC stationary phases are polar like normal phase chromatography (NPLC), such as bare silica,[2,3] silica based-diol,[4] amino,[5] amide,[6] cyano,[7] β-cyclodextrin,[8] and other mix-mode,[9-12] and the mobile phase is similar to reversed phase chromatography (RPLC). The stationary phases of HILIC can have proper retention for strong polar compounds and the mobile phase containing water is suitable for dissolving hydrophilic compounds. Consequently, HILIC mode integrates the advantages of both NPLC and RPLC. Up to now, HILIC has made a great development and has been successfully applied for the analysis of peptides,[13-15] carbohydrates,[16,17] proteins,[18] oligosaccharides ,[19] and polar pharmaceuticals.[20,21]

Graphene (G), a monolayer of carbon atoms tightly packed into a two-dimensional honeycomb lattice, is a novel carbon material.[22] G has shown many outstanding features, such as large surface area, excellent optical transparency, unique mechanical and thermal properties.[23-25] Graphene oxide (GO) is an oxidized derivative of G, which contains a range of polar moieties (e. g. hydroxy, epoxy, and carboxy groups).[26] In general, G is regarded to be hydrophobic; whereas, GO is polar and hydrophilic due to the existence of abundant oxygen-containing groups.[27] Because of the existence of the active groups, it is easy for GO to be conducted further modification. So far, GO has been applied as stationary phase for high performance liquid chromatography,[28,29] capillary electrochromatography,[30] and as materials for solid phase extraction.[27,31,32]

The well-accepted identification of ionic liquid(IL) is any salt with melting point below 100℃ or close to room temperature (RTIL).[33] In most cases, RTILs consist of bulky asymmetric organic cations such as alkyl-imidazolium, pyridinium, pyrrolidinium or ammonium paired with inorganic anions (Cl^-, BF_4^-, PF_6^-, etc) or organic anions ($[(CF_3SO_2)_2N]^-$, $[(CF_3CO_2)_2N]^-$, $[CF_3SO_3]^-$, etc). Besides its low melting point due to the high asymmetry and effective charge delocalization of ions, RTILs have many other excellent properties, including good thermal stabilities, low volatilities, wide liquid ranges, good electrolytic conductivity, adjustable miscibility, nonflammability, and good extractability.[34] Based on the outstanding properties mentioned above, their applications of ILs have covered almost the entire chemistry fields, including organic synthesis, solvent extraction, chromatography, electrolytes in batteries, biocatalysis, enzyme reactions, sensors, etc.[35-41] 1-(2-aminopropyl)-3-methylimidazolium bromide, which is an amine-terminated ionic liquid (IL-NH_2), is hydrophilic and reactive. If we combine the amine-terminated ionic liquid with hydrophilic GO, the composite may exhibit amazing separation ability for strong polar and hydrophilic substances.

In this work, we first synthesized the IL-NH_2. SiO_2 particles were wrapped by GO sheets and then the synthesized IL-NH_2 was grafted onto the surface of GO through a nucleophilic ring-opening reaction between the epoxy groups of GO and the amine groups of IL-NH_2.[42] The novel stationary phase (IL@GO@SiO_2) was evaluated with aromatic acids, B vitamins and amino acids in HILIC mode. All of the chromatographic results of IL@GO@SiO_2 phase were compared with those of other

three stationary phases: pure silica phase, IL-NH_2 modified silica phase (IL@SiO_2), and GO modified silica phase (GO@SiO_2), achieving satisfactory results.

2. Experimental

2.1 Apparatus and reagents

In the paper, all chromatographic tests were performed on two Agilent 1100 Series modular HPLC systems (Agilent Technologies, Icn, USA), one with a UV–Vis detector and another with an evaporative light-scattering detector. Deionized water and acetonitrile (analytical grade) were both filtered through a 0.45 μm nylon membrane filter and were degassed ultrasonically prior to use. All samples used in chromatographic tests were analytical-grade reagents. Porous silica particles were synthesized using the polymerization-induced colloid aggregation method in our laboratory and the preparation process can be referred from the published paper; [43]their average diameter, pore size and surface area were 5μm, 15 nm and 150 m^2 g^{-1}, respectively. (3-aminopropyl)triethoxysilane, (3-chloropropyl)trimethoxysilane, VB6 and VB12 was purchased from Aladdin Chemical Reagent Co. Ltd. (Shanghai, China). Graphene oxide was purchased from Nanoon Co. Ltd. (Beijing, China). 2-bromoethylamine hydrobromide, 1-methylimidazole, N, N-diisopropylethylamine were purchased from Energy Chemical Reagent Co. Ltd. (Shanghai, China). Thymidine, cytosine and xanthine were purchased from Alfa Aesar Reagent Co. Ltd. (Tianjin, China). Nicotinamide, VB3, VB1, L- Leucine, DL- Valine, L- Proline, Glycine and L- Asparaginate were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). O-hydroxybenzoic, p-aminobenzene sulfonic acid,

o-iodobenzoic acid, p-hydroxybenzoic and 1, 5-naphthalenedisulfonic acid were purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China).

2.2 Process synthesis of the resulting stationary phase

2.2.1 Preparation of graphene oxide coated silica (GO@SiO_2).

Silica particles were first immersed in concentrated hydrochloric acid for 24 h for activation, which was in order to break more Si-O-Si bonds. Then the activated silica was repeatedly rinsed with deionized water until the water was neutral. The dried activated silicas (10.0 g) were suspended in 150 ml of dry toluene and then an excess of (3-aminopropyl)triethoxysilane (10.0 ml) was added. The suspension was mechanically stirred and refluxed for 24 h at 120 ℃. Subsequently, the modified silica was washed with toluene, ethanol and methanol in turn. Aminopropylated silica was dried at 60 °C for 12 h. The detailed description of GO@SiO_2 preparation can be found in previous paper.[44] Shortly, GO (0.10g) was added to 100ml deionized water under the lengthily ultrasonic treatment for 2 h. After the GO was well dispersed, 10.0 g of dried aminopropylated silica particles were added under ultrasonic treatment. Then the mixture was stirred at 80 °C for 24 h for the bonding of GO. During the reaction process, the amine group of aminopropylated silica reacted with the epoxy group and carboxyl group of GO. After the reaction, GO@SiO_2 was washed with deionized water and methanol in turn then dried under vacuum for 12 h at 60 °C.

2.2.2 Synthesis of IL-NH_2

IL-NH_2 was synthesized from 2-bromoethylamine hydrobromide (10.25g) and 1-methylimidazole (4.50g), acetonitrile as the solvent. The reaction was maintained for 30 h on 80 ℃。The resulting product needed further purification process.[45]

2.2.3 Assembly of IL-NH_2 to SiO_2 (IL@SiO_2)

The activated silicas (5g) were suspended in 100 ml of dry toluene and then an excess of (3-chloropropyl)trimethoxysilane (5 ml) was added. The suspension was mechanically stirred and refluxed for 24 h at 120 ℃. Subsequently, the chloropropylated silica was washed with toluene, ethanol and methanol in turn and then was dried at 60 °C for 12 h. The modified silica particles (3.0g), IL-NH_2 (2.5g) and N, N-diisopropylethylamine (1.2g) were added to 100 ml methanol and was continuously stirred for 24 h at 70 ℃. After the reaction, IL@SiO_2 was washed with methanol and then dried under vacuum for 12 h at 60 °C.

2.2.4 Assembly of IL-NH_2 to GO@SiO_2 (IL@ GO@SiO_2)

The covalent assembly process was as follows: GO@SiO_2 particles (3.5g), IL-NH_2 (2.7g) and N, N-diisopropylethylamine (1.2g) were added to 150 ml methanol and was continuously stirred for 24 h at 70 ℃ for the covalent assembly between the amine group of IL-NH_2 and epoxide group of GO. A schematic diagram of the synthetic approach for the preparation of IL@GO@SiO_2 is outlined in Fig. 1.

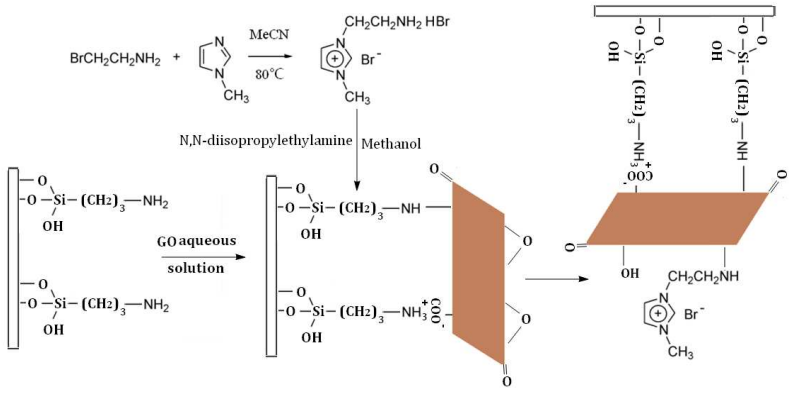

Fig. 1 Schematic diagram of preparation of IL@GO@SiO_2

2.3 Characterization of IL@GO@SiO_2 particles.

The elemental analysis of aminopropylated silica, GO@SiO_2, IL@SiO_2, and IL@GO@SiO_2 were performed on a Vario EL (Elementar, Germany). The surface morphologies of GO@SiO_2 were examined on a TF20 transmission electron microscopy (FEI, American). Infrared spectroscopy of GO@SiO_2 and IL@GO@SiO_2 were performed on IFS 66v/s infrared spectroscopy instrument (Bruker, Germany). ^{1}H NMR was conducted by INOVA-400M superconducting nuclear resonance spectrometer (INOVA).

2.4 Column packing

Columns (150×4.6 mm I.D.) made from stainless-steel tubing were packed in our laboratory and downward packed using a slurry method with tetrachloromethane as the solvent. A 40 MPa packing press (6752B-100, Beijing, China) was used and hexane was used as the propulsive solvent.

2.5 Conditions for chromatographic evaluation

The mixtures of benzoic acid compounds and water soluble vitamins were analyzed at room temperature at a flow rate of 1.0 ml min^{-1} with the ultraviolet (UV) detector at 254 nm and 260 nm, respectively. Amino acids were tested with evaporative light scattering detector (ELSD), with the tube temperature at 115.0 °C and gas flow at 2.0 L·min^{-1}. Each analyte was dissolved with the mixed solvent (acetonitrile : wate = 50 : 50).

3 Results and discussion

3.1 Characterization of GO@SiO_2 and IL@ GO@SiO_2 particles.

^{1}H NMR (400MHz, DMFO) chemical shifts of IL-NH2 were δ : 2.0 (m, 2H,

CH_2), 3.8 (s, 3H, CH_3), 4.7 (t, J＝5 Hz, 2H, CH_2), 7.5 (s, 1H, imH), 7.6 (s, 1H, imH), 8.9 (s, 1H, imH). Elemental analysis data of aminopropylated silica, IL@SiO_2, GO@SiO_2 and IL@GO@SiO_2 are listed in Table 1. Comparing the aminopropylated silica and GO@SiO_2, the increase of carbon content contributed to the assembly of GO to aminopropylated silica. From the nitrogen content increasing from 1.09 % to 1.57 %, the content of IL-NH_2 bonded to the surface of GO@SiO_2 was calculated to be 0.11 mmol g^{-1}. Comparing the chloropropylated silica and IL@SiO_2, the increase of nitrogen content contributed to the assembly of IL to chloropropylated silica. Similarly, the content of IL-NH_2 bonded to the surface of chloropropylated silica was calculated to be 0.16 mmol g^{-1}. The calculation formula of the content of IL-NH_2 is as follow: content of IL-NH_2 (mmol g^{-1}) = N% ×1000 / (3M). N% represents the percentage of the increased nitrogen content and M is the atomic mass of nitrogen. The scanning electron microscope of GO@SiO_2 shown in Fig. 2 can clearly illustrate that the aminopropylated silica was completely wrapped by GO sheets. In the infrared spectroscopy, the signal at 3500 cm^{-1} can be attributed to N-H stretches secondary amine. From fig. 3, it clearly showed that IL@GO@SiO_2 had higher content of secondary amine than that of GO@SiO_2, which also illustrated that IL-NH_2 was attached to GO@SiO_2. Because the results of the IR spectrum represent an average level, and a small number of silica was inevitably not completely wrapped by GO sheets so the IR spectrum of GO@SiO_2 exhibits a weak N-H absorption.

Table 1: Elemental analysis data of aminopropylated silica, GO@SiO_2 and IL@GO@SiO_2

Different particles	Elemental analysis data

	C (%)	N (%)	H (%)
Aminopropylated silica	3.13	1.14	1.18
chloropropylated silica	4.15	-	1.33
$GO@SiO_2$	4.01	1.09	1.15
$IL@SiO_2$	5.04	0.65	1.29
$IL@GO@SiO_2$	4.86	1.57	1.16

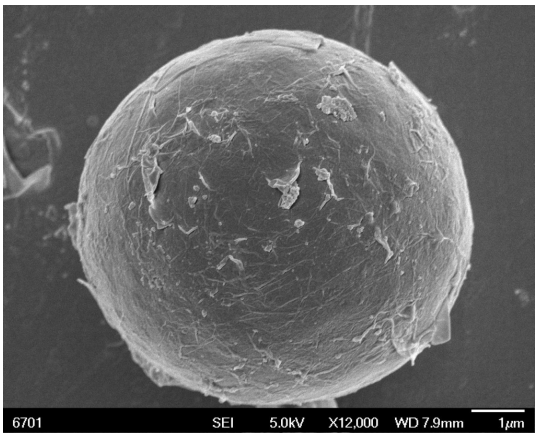

Fig. 2 Scanning electron microscope of GO@ SiO_2

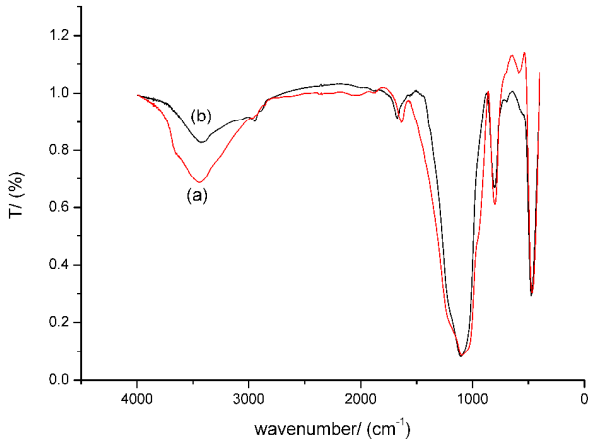

Fig. 3 Infrared spectroscopy of $IL@GO@SiO_2$ (a) and $GO@SiO_2$ (b)

3.2 HILIC characteristics of the pure silica, $GO@SiO_2$, $IL@SiO_2$, and $IL@GO@SiO_2$ columns

In order to investigate the HILIC characteristics of the pure silica, $GO@SiO_2$, $IL@SiO_2$, and $IL@GO@SiO_2$ columns, thymidine, cytosine and xanthine were selected as the probes. As shown in Fig. 4, the content of acetontrile was varied from 70 % to 90 % while keeping ammonium acetate concentration in aqueous phase constant at 50mmol L^{-1}. The retention factors were plotted against the acetonitrile content in the mobile phase, which showed that retention factors increased with the increase of acetonitrile content on the four columns, exhibiting the typical retention behavior of HILIC. Meanwhile, we can notice that the slopes of $GO@SiO_2$ and $IL@GO@SiO_2$ are bigger than those of the pure silica and $IL@SiO_2$, which illustrates that the former two columns have higher hydrophilicity than the latter two.

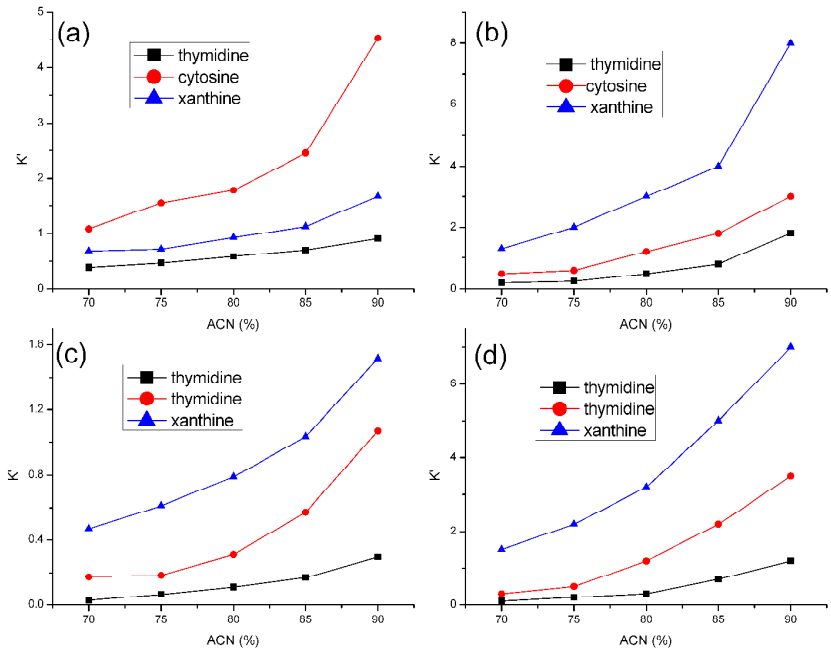

Fig. 4 The effect of acetontrile content on the retention of nucleosides and nucleobases on the pure silica column (a), $GO@SiO_2$ column (b), $IL@SiO_2$(c), and

IL@GO@SiO_2 column (d). Column temperature was room temperature and the aqueous phase contained 50mmol L^{-1} ammonium acetate. Flow rate: 1.0 ml min^{-1}. UV detection at 245 nm.

3.3 Chromatographic separation of vitamin B compounds.

The separation of vitamin B compounds was conducted on the four columns, and the results were all achieved under optimal conditions. A mixture of five B vitamins was baseline separated on IL@GO@SiO_2 column as shown in Fig. 5(d). Compared with the other three columns, IL@GO@SiO_2 column had better selectivity. From Fig. 5(a), we can notice that VB3 had a weak retention and VB1 had a strong retention. Beacause VB3 is electronegative, electrostatic repulsion exists between VB3 and silica, which resulted in the weak retention. On the contrary, VB1 is electropositive, so it had strong retention on silica column. Vitamin B6 and vitamin B1 were the two compounds which exhibited major differences on the two columns of GO@SiO_2 and IL@GO@SiO_2. The alkaline Vitamin B6 and vitamin B1 had stronger interaction with the weak acid surface of GO@SiO_2. At the same time, Vitamin B6 (intramolecular hydrogen bond forming) and vitamin B1 had stronger π-π interaction with GO@SiO_2. The two reasons mentioned above resulted in the longer retention and broad peaks. As for the IL@ SiO_2 column, due to the similar reason mentioned above, VB1 is electropositive, whereas, the surface of IL@ SiO_2 column is also electropositive. So, VB1 had a weak retention.

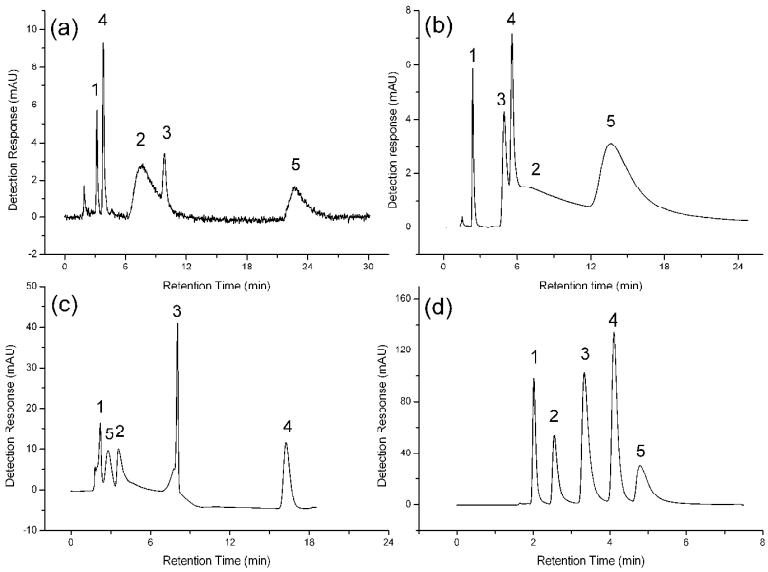

Fig.5. Separation of vitamin B compounds on: the pure silica column (a), GO@SiO_2 column (b), IL@SiO_2(c), and IL@GO@SiO_2 column (d). Mobile phases were: for (a) column: acetonitrile / water= (60/40, v/v) containing 50 mmol L^{-1} ammonium acetate; for (c) column, 0-4min 90% acetonitrile, 4-7min 90-60% acetonitrile; for (b) and (d) columns: acetonitrile / water= (63/37, v/v) containing 50 mmol L^{-1} ammonium acetate. Column temperature: room temperature. Flow rate: 1.0 ml min^{-1}. UV detection at 260 nm. Compounds: (1) nicotinamide, (2) vitamin B6, (3) vitamin B12, (4) vitamin B3, (5) vitamin B1

3.4 Chromatographic separation of amino acids.

Fig. 6 shows the separation of five amino acids including L- Leucine, DL- Valine, L- Proline, Glycine, L- Asparaginate on pure silica column (a), GO@SiO_2 column (b), IL@SiO_2 column (c), and IL@GO@SiO_2 column (d). The results were all achieved under optimal conditions. From the Fig. 6, we can see that five amino acids could be

completely separated on IL@GO@SiO_2 column, while not on the other three columns. Silica and IL@ SiO_2 columns had poor separation for the five amino acids. Compared with silica and IL@ SiO_2 columns, GO@SiO_2 column had a better performance, although it also can not achieve satisfactory results. The major difference of the two chromatograms between column (b) and column (d) was that peaks were tailing and broad on GO@SiO_2 column, especially for L- Asparaginate. It can be stated that the hydrophilcity of GO@SiO_2 column is weaker than IL@GO@SiO_2 column. So the peaks inevitably became wider when the retention was enhanced through decreasing the elution power of mobile phase.

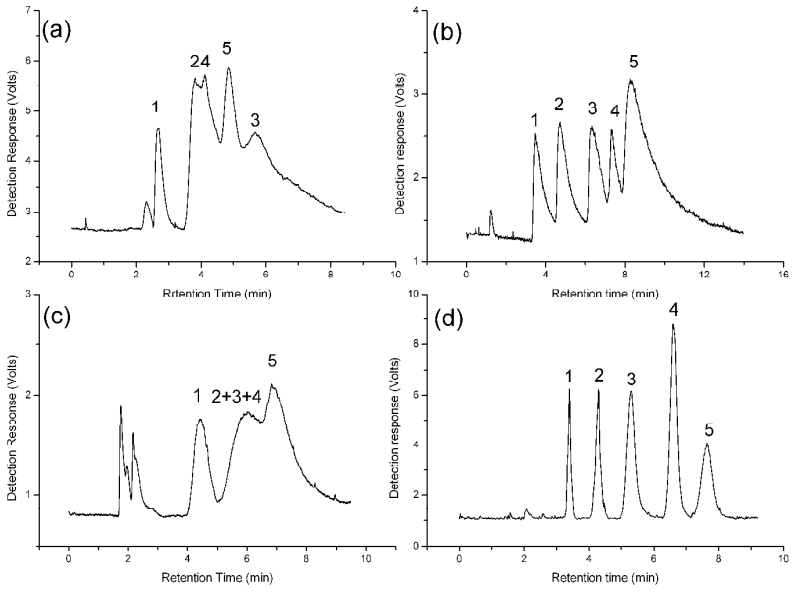

Fig.6. Separation of amino acids on: the pure silica column (a), GO@SiO_2 column (b), IL@SiO_2(c), and IL@GO@SiO_2 column (d). Mobile phases were: for (a) column: acetonitrile / water= (67/33, v/v) containing 50 mmol L^{-1} ammonium acetate; for (c) column, acetonitrile / water= (85/15, v/v) containing 50 mmol L^{-1} ammonium acetate

and the buffer was adjusted by 1% acetic acid; for (b) and (d) columns: acetonitrile / water = (75/25, v/v) containing 50 mmol L^{-1} ammonium acetate. Column temperature: room temperature. Flow rate: 1.0 ml min^{-1}. ELS detector: gas flow: 2L min^{-1}, tube temperature 115℃. Compounds: (1) L- Leucine, (2) DL- Valine, (3) L- Proline, (4) Glycine, (5) L- Asparaginate

3.5 Chromatographic separation of aromatic acid compounds.

The separation of five aromatic acid compounds was conducted on the four columns, and the results were all achieved under optimal conditions. For silica column, due to the electrostatic repulsion, 1, 5-naphthalenedisulfonic acid and p-aminobenzene sulfonic acid had weak retention. From the Fig. 7, it can be inferred that the attachment of GO or IL to silica all can increase the retention for the analytes. For GO@SiO_2 column, π-π interaction plays a major role in the separation process. Whereas, for IL@SiO_2 column, anion exchange interaction has the most impact. The attachment of mere GO or IL can not achieve good separation for the five aromatic acids. However, because of the coexistence of strong anion exchange and π-π interaction between analytes and IL@GO@SiO_2 column, the five aromatic acid compounds were got well separated on the IL@GO@SiO_2 column. The comparison of plates among the four columns for the five aromatic acid compounds is listed in table 2. From these data, we can confirm that the IL@GO@SiO_2 column has the best separation ability than the other three columns.

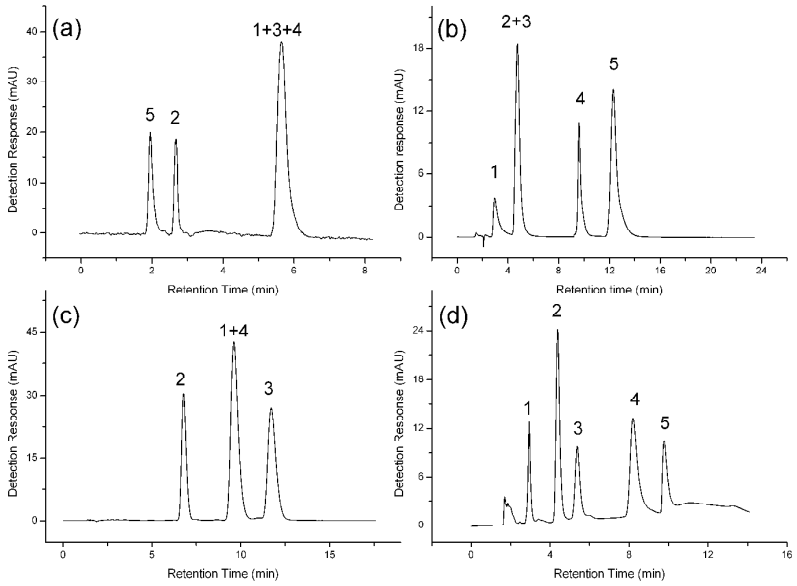

Fig.7. Separation of aromatic acid compounds on: the pure silica column (a), GO@SiO_2 column (b), IL@SiO_2(c), and IL@GO@SiO_2 column (d). Mobile phase: for column (a): acetonitrile / water= (90/10, v/v) containing 50 mmol L^{-1} ammonium acetate; for column (b): acetonitrile/water containing 50 mmol L^{-1} ammonium acetate (80/20, V/V); for column (c) : acetonitrile / water= (55/45, v/v) containing 50 mmol L^{-1} ammonium acetate; for column (d): acetonitrile / water containing 50 mmol L^{-1} ammonium acetate (0-3min 88/12; 3-11min 88-70/12-30, V/V). Column temperature: room temperature. Flow rate: 1.0 ml min^{-1}. UV detection at 254 nm. Compounds: (1) o-hydroxybenzoic, (2) p-aminobenzene sulfonic acid, (3) o-iodobenzoic acid, (4) p-hydroxybenzoic, (5) 1, 5-naphthalenedisulfonic acid

Table. 2 The comparison of plates among the four columns

	Plates

Stationary phase	o-HB	p-AB	o-IB	p-HB	1,5-NS
SiO_2	16313	23233	-	-	15706
GO@ SiO_2	2393	6100	-	11734	25766
IL@ SiO_2	17100	22140	21100	-	-
IL@IL@SiO_2	20426	18313	17040	16066	50433

o-HB : o-hydroxybenzoic, p-AB : p-aminobenzene sulfonic acid, o-IB : o-iodobenzoic acid, p-HB : p-hydroxybenzoic, 1,5-NS : 1, 5-naphthalenedisulfonic acid

4. Concluding remarks

A novel hydrophilic stationary phase of IL@GO@SiO_2 was successfully synthesized. The resulting stationary phase exhibited better separation ability for the separation of polar compounds in HILIC mode compared to the other three stationary phases of pure silica phase, GO@SiO_2 phase, and IL@SiO_2 phase. From the chromatographic results, we can confirm that GO changed the property of pure silica phase. The grafting of IL-NH_2 onto the surface of GO@SiO_2 not only changed the charge environment and acid-base property but also added strong anion exchange interaction for the IL@GO@SiO_2 phase and improved its selectivity ability and hydrophility. Consequently, the application of GO plus IL composite phase will have a bright future as a chromatographic stationary phase.

Acknowledgements

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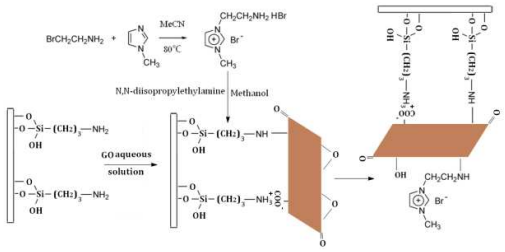

IL@GO@SiO_2 composite was synthesized and revealed good separation for three kinds of strong polar and hydrophilic compounds in HILIC mode.