Int. J. Electrochem. Sci., 9 **(2014)** 7948 - 7964

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Enantioselective Separation and Detection of D-Phenylalanine Based on Newly Developed Chiral Ionic Liquid Immobilized Silica Gel Surface

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Received: 26 August 2014 / *Accepted:* 9 October 2014 / *Published:* 28 October 2014

This study introduced a simple method developed for enantioselective extraction of D-phenylalanine prior to its determination by high performance liquid chromatography. The method was based on silica gel developed by surface immobilized new chiral ionic liquid (SG-L-PhAlaC_2NTf_2). Surface characteristics of the synthesized SG-L-PhAlaC_2NTf_2 phase were confirmed by both FT-IR spectroscopy and scanning electron microscope. Results of the selectivity study and effect of pH on SG-L-PhAlaC_2NTf_2 phase showed that SG-L-PhAlaC_2NTf_2 was the most enantioselective toward D-phenylalanine. Data of adsorption isotherm study revealed that the adsorption capacity of SG-L-PhAlaC_2NTf_2 for D-phenylalanine was the most by 97.35% as compared to activated silica gel at pH value of 3.0 and after one hour contact time. Results demonstrated that the adsorption of D-phenylalanine onto SG-L-PhAlaC_2NTf_2 phase well followed with Langmuir adsorption isotherm and pseudo second-order kinetic models. The efficiency of the methodology was ultimately validated by implementing it to real water samples with satisfactory results.

Keywords: D-phenylalanine; enantioselectivity**;** chiral ionic liquid**;** silica gel**;** adsorption capacity

1. INTRODUCTION

Chiral recognition has a significant influence on biological systems due to several naturally occurring chiral molecules and functions, based on chiral interactions. For instance, amino acids,

carbohydrates, DNA, enzymes, hormones and proteins are chiral compounds.[1-3] However, only one enantiomeric form is usually present in certain species and the other can reverse the effect of the target enantiomer. For example, in the human body carbohydrates exclusively exist as D enantiomers while amino acids exist only as L enantiomers. Even though they are equally stable as their respective counterparts, D-carbohydrates and L-amino acids are the naturally preferable enantiomers. Specifically, proteins composed of L-amino acids interact with a certain L-amino acid but not easily interact with the D form of the same amino acid. However, the existence of D-amino acids at low levels has been reported in humans; thus, the antagonistic biological functions may occur in this case.[4,5] Furthermore, many environmental pollutants, such as pesticides and herbicides, are chiral compounds that released into the environment as racemates containing multiple enantiomers.[6] Therefore, it has been continual interest to study the environmental behavior of the enantiomers of chiral pollutants.[7] It is also of great importance to improve enantioseparation and develop enantiomeric analysis methods for chiral compounds and explore the difference in environmental behaviors of the individual enantiomer to supply more accurate data for evaluating the biological or environmental risk.

Achiral analysis gives only partial information with traditional risk evaluations which are unreliable if enantioselective behaviors occur. Therefore, the use of a chiral selector for chiral recognition and enantioselectivity of enantiomers is essential.[8] In addition, several traditional chiral selectors have been utilized with the aim of achieving chiral recognition of enantiomers, such as, antibodies,[9,10] antibiotics,[11,12] crown ethers,[13,14] cyclodextrins[15,16] and molecular micelles.[17,18] However, the use of these chiral selectors is often limited because of their low solubility, instability at high temperature, difficult organic synthesis that involved time consuming, expensive reagents and multistep-processes with almost not optically pure.[19,20]

Recently, chiral ionic liquids (CILs) have been used as chiral selectors in various applications due to their chiral recognition abilities that may produce the required diastereomeric interactions with both enantiomeric forms of an analyte.[20-26] It has also been found that CILs can be effective alternatives than other chiral selectors. In general, room temperature ionic liquids (RTILs) have unique chemical and physical properties, including air and moisture stabilities, high solubility in water, high conductivities, high heat capacities, high viscosities and negligible vapor pressure.[27-29] Because of these properties, the applications of RTILs have been expanded in many fields of analytical chemistry, such as green solvents in liquid-liquid extraction,[30] electrolyte additives in capillary electrophoresis, mobile phase additives in liquid chromatography, stationary phases in gas-liquid chromatography[31,32] and supporting materials functionalized adsorbents for solid phase extraction.[33,34]

In accordance, the aim of the present study was to explore the potential utilization of L-PhAlaC_2NTf_2 ionic liquid as environmentally benign chiral selector, without the need for treatment by classical chiral selectors, based on immobilizing it on silica gel (SG) surface. The synthesis of the adsorbent was accomplished by a physical adsorption and hybrid combination of the chiral ionic liquid character and activated SG surface. Characterization of the newly synthesized adsorbent (SG-L-PhAlaC_2NTf_2) was achieved by use of Fourier transform infrared (FT-IR) spectroscopy and scanning electron microscope (SEM). The newly developed SG-L-PhAlaC_2NTf_2 phase was utilized for

enantioselective extraction and determination of D-phenylalanine (D-PhAla) prior to its determination by high performance liquid chromatography (HPLC) under the optimum conditions. The selectivity of SG-L-PhAlaC_2NTf_2 toward different racemic mixtures, including R-(+),S-(-)-1-(2-naphthyl)ethanol, D,L-tryptophan and D,L-PhAla was investigated. Based on the selectivity study and effect of pH, it was found that the selectivity of SG-L-PhAlaC_2NTf_2 toward D-PhAla was the most as compared to other compounds. In order to explore the analytical potential of SG-L-PhAlaC_2NTf_2 toward D-PhAla, different parameters controlling the maximum uptake of D-PhAla on the SG-L-PhAlaC_2NTf_2 phase were also studied under batch conditions. Adsorption isotherm data of D-PhAla were modeled using Langmuir classical adsorption isotherm. The kinetic analysis for adsorption process showed that the adsorption isotherm data obeyed the pseudo second-order kinetic model. The effect of matrix interferences form other compounds displayed that the extraction of D-PhAla was not affected by the medium composition containing mixed compounds. Finally, the efficiency of the proposed methodology was also confirmed by applying it to real water samples.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Bis(trifluoromethane)sulfonimide lithium (LiNTf_2), L-phenylalanine ethyl ester hydrochloride (L-PhAlaC_2Cl), R-(+),S-(-)-1-(2-naphthyl)ethanol, D,L-tryptophan, D,L-phenylalanine (D,L-PhAla), D-phenylalanine, L-phenylalanine, R-(+)-1-(2-naphthyl)ethanol, 9-fluorenone, toluene and HPLC grade acetonitrile (ACN) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). SG (SiO_2, particle size 10–20 nm) with purity of 99.5% was also obtained from Sigma-Aldrich. All reagents used were of analytical and spectral purity grade. Doubly distilled deionized water was used throughout experimental studies.

2.2. Preparation of the adsorbent

2.2.1. Preparation of L-PhAlaC_2NTf_2 ionic liquid

L-Phenylalanine ethyl ester bis(trifluoromethane)sulfonimide (L-PhAlaC_2NTf_2) ionic liquid was prepared according to procedures previously reported by Marwani.[35] Specifically**,** 1 g L-PhAlaC_2Cl was separately weighted and dissolved in 18.2 MΩ·cm distilled deionized water, and then mixed with an equimolar amount of LiNTf_2. The resultant reaction mixture was stirred for 2 h at room temperature. The result of reaction was obtained in two layers. The lower layer was separated and dried under vacuum overnight. The purification by vacuum drying is performed at a drying temperature of 353 K and vacuum of 0.4 kPa.

2.2.2. Activation of SG

The SG surface was activated by refluxing 25 g of SG with 200 mL of 50% (v/v) hydrochloric acid and stirred for 8 h in order to remove adsorbed impurities. The resultant activated SG was then

filtered and washed with 18.2 MΩ·cm distilled deionized water until acid free. Finally, the activated SG was dried in oven at 120 °C for 5 h.

2.2.3. Preparation of the SG-L-PhAlaC$_2$NTf$_2$ adsorbent

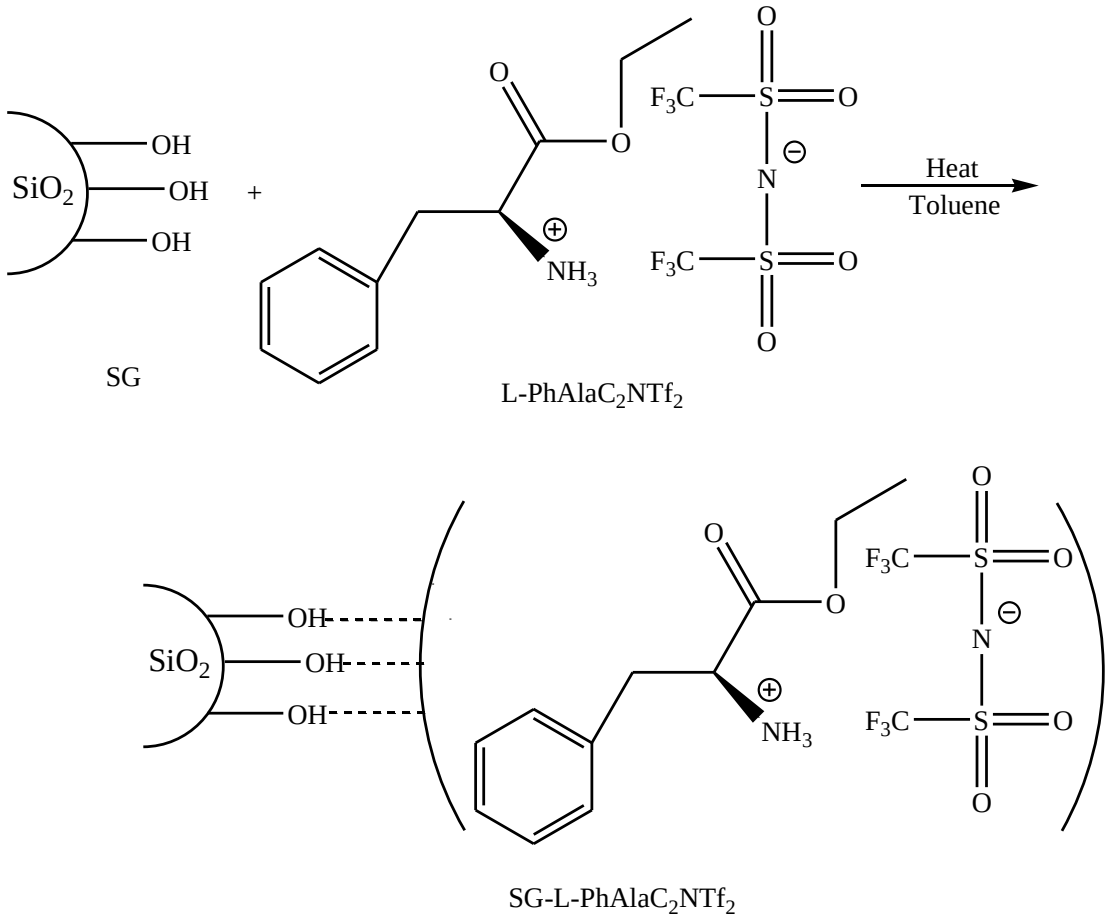

Figure 1. Synthetic route of the SG-L-PhAlaC$_2$NTf$_2$ phase.

The SG-L-PhAlaC$_2$NTf$_2$ phase was prepared based on the physical adsorption and hybrid combination of the chiral ionic liquid character (L-PhAlaC$_2$NTf$_2$) and activated SG surface (Fig. 1). An amount of 6 g activated SG was suspended in 100 mL toluene and 2 g L-PhAlaC$_2$NTf$_2$ was completely dissolved by warming in 50 mL toluene and added to the activated SG suspension. The reaction mixture was heated and stirred at 60 °C for 12 h. The modified SG-L-PhAlaC$_2$NTf$_2$ phase was filtered and washed with 50 mL toluene on three portions. Then, the SG-L-PhAlaC$_2$NTf$_2$ adsorbent was dried in an oven at 80 °C for 5 h and kept in a desiccator for further use.

2.2.4. Determination of the surface coverage value of the SG-L-PhAlaC$_2$NTf$_2$ phase

In thermal desorption method, 100 mg of the newly modified SG-L-PhAlaC$_2$NTf$_2$ adsorbent was weighed in a dry porcelain crucible and gradually heated into a furnace from 50 to 700 °C. Then,

the ignited phase was kept for 1 h at the same temperature. The remaining SG-L-PhAlaC_2NTf_2 phase was left to cool inside the furnace and then transferred to a desiccator. The weight loss of chiral ionic liquid was determined by the difference in sample masses before and after the process of thermal desorption. Based on thermal desorption method, the concentration of L-PhAlaC_2NTf_2 on the SG-L-PhAlaC_2NTf_2 surface was determined to be 0.54 mmolg^{-1}.

2.3. Samples preparation and procedure

All stock solutions were prepared in 18.2 MΩ·cm distilled deionized water and stored in the dark at 4 °C except 9-fluorenone, R-(+)-1-(2-naphthyl) ethanol and R-(+),S(-)-1-(2-naphthyl) ethanol were prepared in ACN (HPLC grade). For exploring the selectivity of SG-L-PhAlaC_2NTf_2 toward different racemic mixtures, standard solutions of 25 and 50 mgL^{-1} of R-(+),S-(-)-1-(2-naphthyl)ethanol, D,L-tryptophan and D,L-PhAla were prepared by adding appropriate amounts of each stock solution. An amount of 20 mg SG-L-PhAlaC_2NTf_2 was also mixed with each standard solution. For the effect of pH on the enantioselectivity of SG-L-PhAlaC_2NTf_2 toward D- and L-PhAla, standard solutions of 50 mgL^{-1} of each pure enantiomer were individually prepared and adjusted to pH values ranging from 1.0 to 9.0 with appropriate buffer solutions, (0.2 molL^{-1} HCl/KCl) for pH 1.0 and 2.0, (0.1 molL^{-1} CH_3COOH/CH_3COONa) for pH 3.0–6.0 and (0.1 molL^{-1} Na_2HPO_4/H_3PO_4) for pH 7.0–9.0. For estimation of the adsorption capacity of D-PhAla under batch conditions, standard solutions of 25, 30, 40, 50, 60, 80, 100, 125, 150 and 200 mgL^{-1} of D-PhAla were individually prepared as above, adjusted to the optimum pH value of 3.0 and individually mixed with 20 mg SG-L-PhAlaC_2NTf_2. All these mixtures were mechanically shaken for 1 h at room temperature. In addition, the effect of shaking time on the D-PhAla adsorption capacity was evaluated for 125 mgL^{-1} of D-PhAla at different equilibrium periods (2.5, 5, 10, 20, 30, 40, 50 and 60 minutes) under the same batch conditions. For the study of the effect of matrix interferences on the adsorption of D-PhAla on SG-L-PhAlaC_2NTf_2 phase, a standard solution of 100 mgL^{-1} D-PhAla, R-(+)-1-(2-naphthyl) ethanol and 9-fluorenone was prepared and mixed with 20 mg SG-L-PhAlaC_2NTf_2. The concentration of each analyte solution was monitored by HPLC and calculated from the calibration plot of the peak area as a function of the concentration of each compound.

2.4. Instrumental methods

FT-IR experiments were recorded before and after modification of the SG phase on a Perkin Elmer spectrum 100 series FT-IR spectrometer (Beaconsfield, Bucks, UK) in the range of 4000-600 cm^{-1}. SEM studies were carried out before and after modification of the SG phase by use of a field emission-scanning electron microscope (FE-SEM, QUANT FEG 450, Amsterdam, Netherlands). The microscope was operated at an accelerating voltage of 15 kV. The pH measurements were acquired by use of a Jenway model 3505 laboratory pH meter (CamLab, UK) with absolute accuracy limits being defined by NIST buffers. HPLC experiments were performed by use of an Agilent Technologies 1200 Series, USA. The chromatographic system consisted of a model G1361A pump and G1315B DAD UV

detector. All separations were accomplished on an analytical reversed phase column (ZORBAX ODS, 4.6×150 nm) with a flow rate of 1 mLmin^{-1}, and a volume of 20 μL was injected from each sample. An isocratic elution was established with a mobile phase composition of 60% ACN and 40% water at room temperature. The DAD UV detector was operated at wavelengths of 193 nm for phenylalanine, 218 nm for tryptophan, 219 nm for 1-(2-naphthyl)ethanol and 252 nm for 9-fluorenone.

3. RESULTS AND DISCUSSION

3.1. Characterization of SG-L-PhAlaC$_2$NTf$_2$

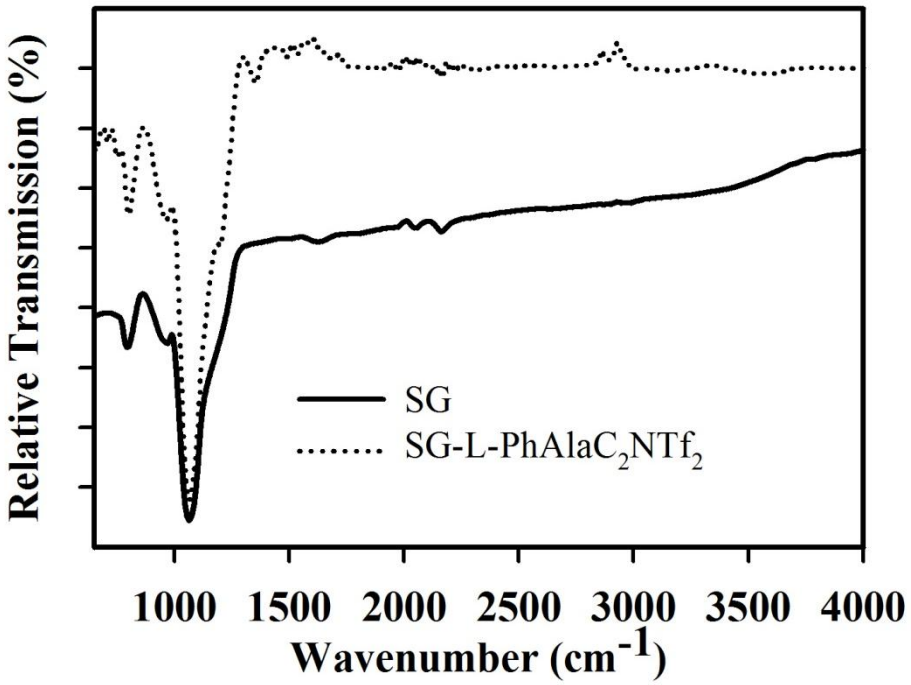

Figure 2. FT-IR spectra of activated SG and SG-L-PhAlaC$_2$NTf$_2$ phases.

FT-IR spectra of activated SG phase before and after immobilization of the L-PhAlaC$_2$NTf$_2$ ionic liquid was acquired in the region of 600–4000 cm^{-1} at room temperature by using KBr in order to observe the different functional groups present in L-PhAlaC$_2$NTf$_2$, activated SG and SG-L-PhAlaC$_2$NTf$_2$ and evaluate the physical adsorption process of L-PhAlaC$_2$NTf$_2$ on activated SG surface. The formation of the new physically modified SG-L-PhAlaC$_2$NTf$_2$ phase was confirmed by noticing the spectral features of both L-PhAlaC$_2$NTf$_2$ and SG matrix in FT-IR spectrum of the newly chiral modified adsorbent. The observed characteristic bands in this study were in similar range to those previously reported in other studies.[36-38] The Si–O absorptions were observed as two strong peaks at 981 and 1074 cm^{-1} in the FT-IR spectrum (Fig. 2). Other featured bands appeared in the FT-IR spectrum of SG-L-PhAlaC$_2$NTf$_2$ can be assigned by their characteristic absorbance v (cm^{-1}) as follows: 3501, 3161 (N–H); 1723 (CO); 1681, 1505 (C=C); 1373 (NSO$_2$); 798 (C–H).

In order to further confirm the formation of the physically modified SG-L-PhAlaC_2NTf_2 adsorbent, SEM graphs were taken for activated SG and SG-L-PhAlaC_2NTf_2 (Fig. 3a and b). As can be observed in Fig. 3b, surface morphologies of the modified activated SG with chiral ionic liquid (SG-L-PhAlaC_2NTf_2) resulted in a pronounced and characterized change with a particle size of 100 nm.[39] From Fig. 3b, it can be clearly noticed that L-PhAlaC_2NTf_2 ionic liquid completely covered activated SG particles and collected them in aggregate forms. Furthermore, Fig. 3b illustrates that the particles of SG-L-PhAlaC_2NTf_2 are individually distributed in uniform and homogeneous shapes as compared with that of activated SG (Fig. 3a).

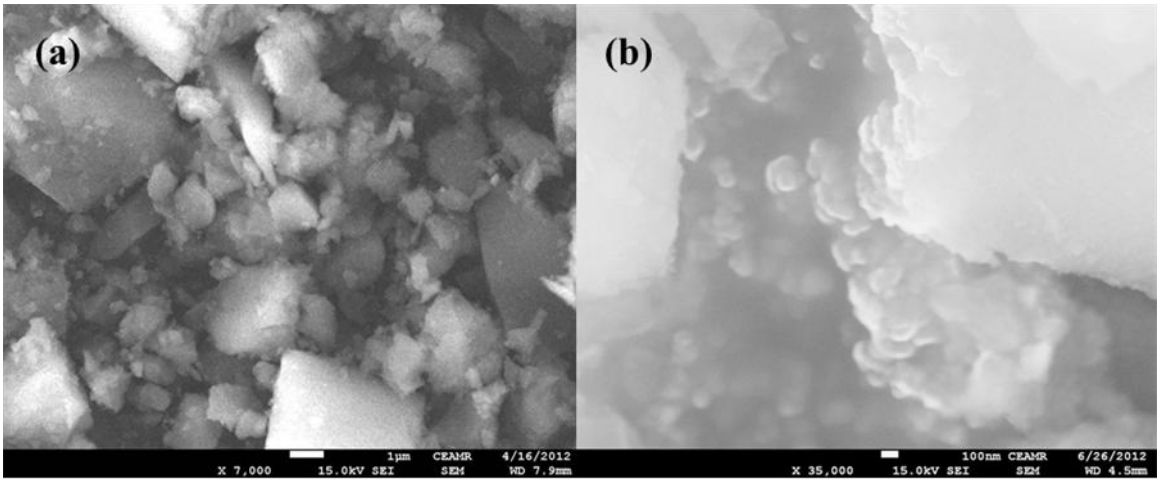

Figure 3. SEM images of (a) activated SG and (b) SG-L-PhAlaC_2NTf_2 phases.

3.2. Batch method

3.2.1. Selectivity and pH study

Selectivity of the newly synthesized SG-L-PhAlaC_2NTf_2 phase toward different racemic mixtures was investigated based on determination of the distribution coefficient (K_d, mLg^{-1}) value, which corresponds to the character of a compound adsorbed by an adsorbent.

Table 1. Selectivity study of 20 mg SG-L-PhAlaC_2NTf_2 toward different racemic mixtures.

Compound	Concentration (mgL^{-1})	q_e (mgg^{-1})	K_d (mLg^{-1})
R-(+), S-(-)-1-(2-Napthyl) ethanol	25	0.18	7.19
	50	3.44	72.84
D, L-PhAla	25	14.84	1130.56
	50	20.48	609.09
D, L-Tryptophan	25	4.24	196.01
	50	3.79	80.76

As can be illustrated in Table 1, the distribution coefficient value and amount of each compound adsorbed per unit mass of the SG-L-PhAlaC$_2$NTf$_2$ adsorbent at equilibrium (q_e, mgg^{-1}) were calculated from the following equations:[40,41]

$$K_d = \frac{(C_i - C_e)}{C_e} \times \frac{V}{m} \quad (1)$$

$$q_e = \frac{(C_i - C_e)V}{m} \quad (2)$$

where C_i and C_e are initial and final concentrations (mgL^{-1}), respectively, V refers to the volume of solution (mL), and m is the mass of adsorbent (g). From Table 1, it can be clearly noticed that D,L-PhAla has the greatest K_d value up to 1130.56 and 609.09 mLg^{-1} for 25 and 50 mgL^{-1}, respectively, on the SG-L-PhAlaC$_2$NTf$_2$ adsorbent as compared with other racemic mixtures examined on SG-L-PhAlaC$_2$NTf$_2$. Therefore, the results of selectivity study indicated that SG-L-PhAlaC$_2$NTf$_2$ was most selectivite for D,L-PhAla over all other racemic mixtures, including R-(+),S-(-)-1-(2-naphthyl)ethanol and D,L-tryptophan.

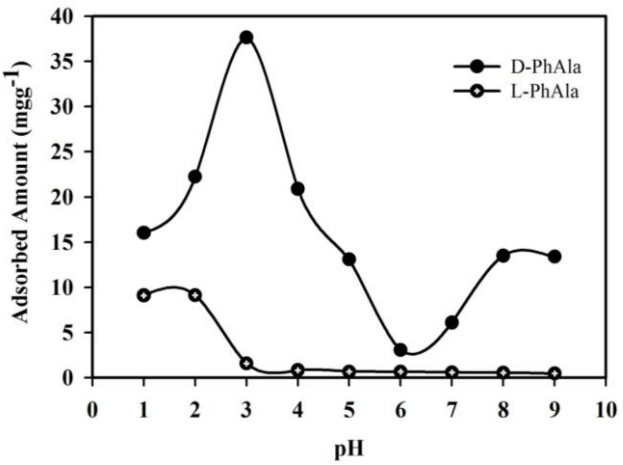

Figure 4. Effect of pH on the adsorption of 50 mgL^{-1} D-PhAla and L-PhAla on 20 mg SG-L-PhAlaC$_2$NTf$_2$ at pH 3.0 and 25 °C.

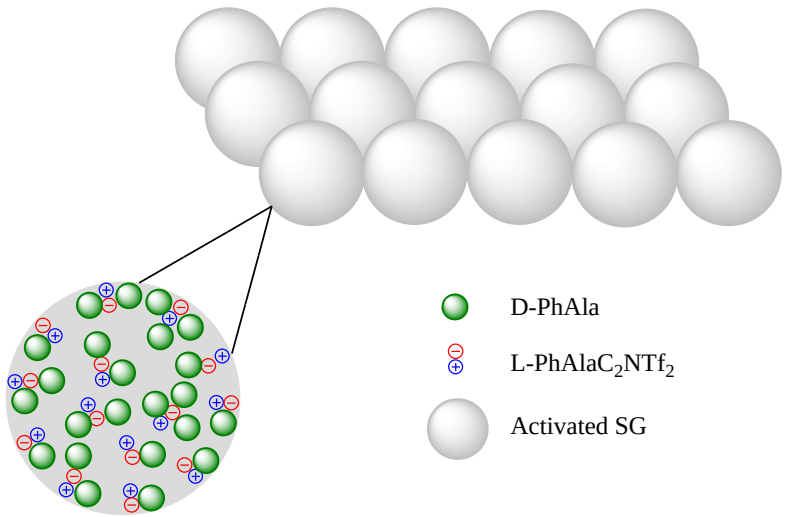

Figure 5. Illustration of the interaction between SG-L-PhAlaC$_2$NTf$_2$ and D-PhAla.

For a deeper mechanistic understanding of the analytical efficiency of the SG-L-PhAlaC_2NTf_2 phase, the enantioselectivity of SG-L-PhAlaC_2NTf_2 toward pure enantiomer of D- and L-PhAla under different pH values were also demonstrated since the pH value plays a critical role in regards to the adsorption of different species on the SG-L-PhAlaC_2NTf_2. Based on the results presented in Fig. 4, it was found that the adsorption capacity of SG-L-PhAlaC_2NTf_2 for D-PhAla was higher than that of L-PhAla. However, a close examination of Fig. 4 reveals that the adsorption capacity of D-PhAla was reached to the highest (37.64 mgg^{-1}) while minimum to no selectivity of SG-L-PhAlaC_2NTf_2 phase toward L-PhAla was observed at pH value of 3.0. Therefore, the enantioselectivity of the newly SG-L-PhAlaC_2NTf_2 phase toward D-PhAla was the most and pH 3.0 was chosen to be the optimum condition. The highest selectivity of SG-L-PhAlaC_2NTf_2 toward D-PhAla may be ascribed to the electrostatic interaction mechanism between SG-L-PhAlaC_2NTf_2 and D-PhAla (Fig. 5). In addition, the incorporated donor atoms (O, N and S) presented in L-PhAlaC_2NTf_2 were capable to bind the amine, carboxyl groups and aromatic ring presented in D-PhAla by hydrogen bonding. Generally, adsorption of amino acids is more favorable in acidic conditions, where the carboxylic group exists as the undissociated form and amine group is ionized. However, adsorption is lower in basic medium since the carboxylic group is ionized and amine group is unionized and in the intermediate pH where both carboxylic and amine groups are ionized.[42]

3.2.2. Adsorption capacity

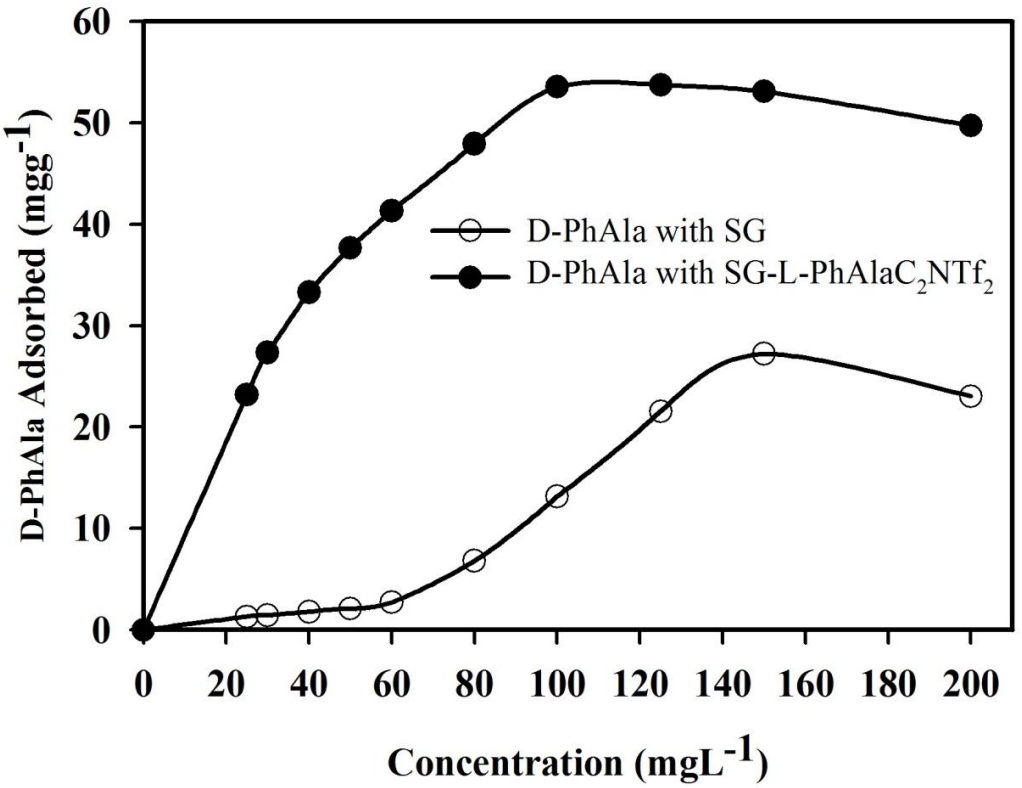

Figure 6. Adsorption isotherm of D-PhAla on 20 mg activated SG and SG-L-PhAlaC_2NTf_2 in relation to the concentration at pH 3.0 and 25 °C.

To evaluate the static adsorption capacity for D-PhAla on the SG-L-PhAlaC_2NTf_2 phase, 25 ml sample solutions of pure enantiomer of D-PhAla with different concentrations (0−200 mgL^{-1}) were adjusted to pH 3.0 with a buffered aqueous solution and mixed with 20 mg SG-L-PhAlaC_2NTf_2. These mixtures were mechanically shaken for 1 h at room temperature. The amount of D-PhAla adsorbed at each concentration level was determined from the supernatant after filtration. Fig. 6 illustrates the breakthrough curve for the uptake capacity of SG-L-PhAlaC_2NTf_2 for D-PhAla obtained by plotting the D-PhAla concentration (mgL^{-1}) versus milligrams of D-PhAla adsorbed per gram SG-L-PhAlaC_2NTf_2. As shown in Fig. 6, the adsorption capacity (53.72 mgg^{-1}) of SG-L-PhAlaC_2NTf_2 toward D-PhAla phase was high, further confirming the enantioselectivity preference of SG-L-PhAlaC_2NTf_2 for D-PhAla.

Table 2. Comparison of SG-L-PhAlaC_2NTf_2 adsorption capacity for D-PhAla reported in the present study with other approaches.

Adsorbent	pH	q_e (mgg^{-1})	Reference
LI [a] membrane	-	1.05	[43]
DIDP [b] bead	8	0.042	[44]
DIM [c]3 membrane	2	0.41	[45]
DIM [d] bead	4	3.83	[46]
SG-L-PhAlaC_2NTf_2	3	53.72	Current study

[a] L-Phe imprinted in-situ implanting
[b] D-Phe double imprinted P(AN-co-AA)
[c, d] D-Phe imprinted P(AN-AA-AAm)

In addition, the adsorption capacity of the activated SG was evaluated as well as SG-L-PhAlaC_2NTf_2 under the same batch conditions. The maximum adsorption capacity of D-PhAla with SG-L-PhAlaC_2NTf_2 (53.72 mgg^{-1}) was higher than that of activated SG (27.22 mgg^{-1}), providing that the adsorption capacity was improved by 97.35%. The maximum adsorption capacity value reached for D-PhAla with SG-L-PhAlaC_2NTf_2 in this study was also compared with the highest adsorption capacity values reached for D-PhAla with other materials previously reported in the literature, as shown in Table 2.[43-46] It can be noticed from Table 2 that the adsorption capacity of D-PhAla reported in the current study is the highest with SG-L-PhAlaC_2NTf_2 (53.72 mgg^{-1}) as compared with those previously reported for D-PhAla with other adsorbents.

3.2.3. Adsorption isotherm models

In order to develop an equation that accurately represents the results, the analysis of adsorption isotherm data is important. Therefore, different isotherm models were evaluated to interpret equilibrium isotherm data of D-PhAla with the SG-L-PhAlaC_2NTf_2 phase.[47,48] Langmuir equation,

which is based on an assumption of monolayer adsorption onto a completely homogeneous surface with a finite number of identical sites and a negligible interaction between the adsorbed molecules, is governed by the following relation:

$$C_e/q_e = (C_e/Q_o) + 1/Q_o b \quad (3)$$

where C_e is the equilibrium concentration of D-PhAla (mgL^{-1}), q_e and Q_o correspond to the equilibrium amount and maximum amount of D-PhAla adsorbed per unit mass of adsorbent (mgg^{-1}), respectively, and b denotes the Langmuir constant referred to the rate of adsorption. Adsorption isotherm data were well fit with the Langmuir model based on the least square fit (Fig. 7).

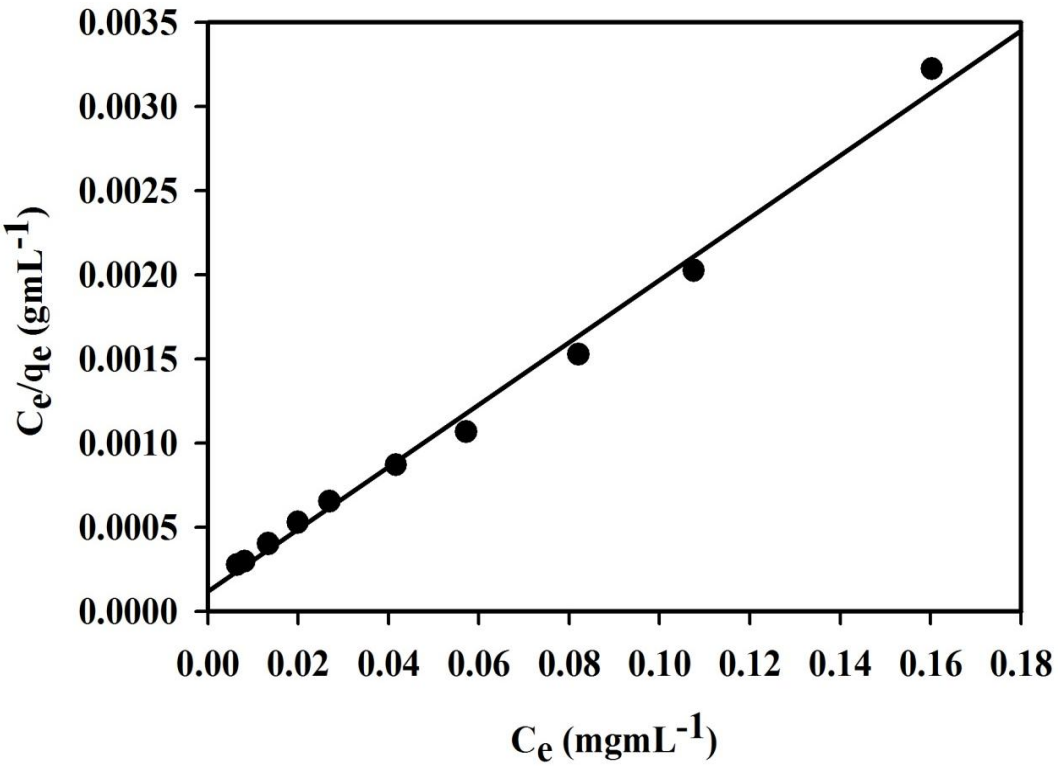

Figure 7. Langmuir adsorption isotherm model of D-PhAla adsorption on 20 mg SG-L-PhAlaC_2NTf_2 at pH 3.0 and 25 °C. Adsorption experiments were performed at different concentrations (0−200 mgL^{-1}) of D-PhAla under batch conditions.

As can be displayed in Fig. 7, a linear plot was obtained from Langmuir isotherm equation when plotting C_e/q_e against C_e with a slope and intercept equal to $1/Q_o$ and $1/Q_o b$, respectively, with correlation coefficient (R^2) value of 0.993. Thus, the adsorption isotherm data indicated that the adsorption process was mainly monolayer on a homogeneous adsorbent surface. The Langmuir constants Q_o and b were calculated to be 54.01 mgg^{-1} and 0.16 Lmg^{-1}, respectively. It is also interesting to note that the D-PhAla adsorption capacity (54.01 mgg^{-1}) calculated from Langmuir equation was closely in conformity with that (53.72 mgg^{-1}) experimentally carried out by the adsorption isotherm study.

To predict whether an adsorption system is favorable or unfavorable, it is important to evaluate the effect of the isotherm shape. The essential feature of the Langmuir isotherm can be represented in terms of a dimensionless constant separation factor or equilibrium parameter (R_L), calculated from the following relation:

$$R_L = 1/(1+bC_o) \quad (4)$$

where b is the Langmuir constant which indicates the nature of adsorption and the shape of the isotherm, and C_o corresponds to the initial D-PhAla concentration (mgL^{-1}). The R_L value indicates the type of the isotherm, and R_L values lying between 0 and 1 provided that the conditions were favorable for adsorption.[49] The R_L value of D-PhAla adsorption on SG-L-PhAlaC_2NTf_2 was determined to be 0.05, strongly supporting that the adsorption is favorable under conditions used in this study based on the Langmuir adsorption isotherm model.

3.2.4. Effect of contact time

For evaluating the possibility for applications of the newly modified SG-L-PhAlaC_2NTf_2 to selectively bind D-PhAla, the effect of contact time is also taken under account. In this study, different shaking times ranging from 2.5 to 60.0 min were investigated in order to study the behavior of adsorption kinetics for D-PhAla on the SG-L-PhAlaC_2NTf_2 phase. It was noticed from the results that equilibrium kinetics were very fast (Fig. 8). As illustrated in Fig. 8, an amount of 46.64 mgg^{-1} D-PhAla was adsorbed on SG-L-PhAlaC_2NTf_2 after only 10 min of the equilibrium period. The amount of D-PhAla adsorbed was also raised up to 49.18 mgg^{-1} after 30 min until the maximum adsorption of SG-L-PhAlaC_2NTf_2 for D-PhAla was reached to 53.72 mgg^{-1} after 60 min.

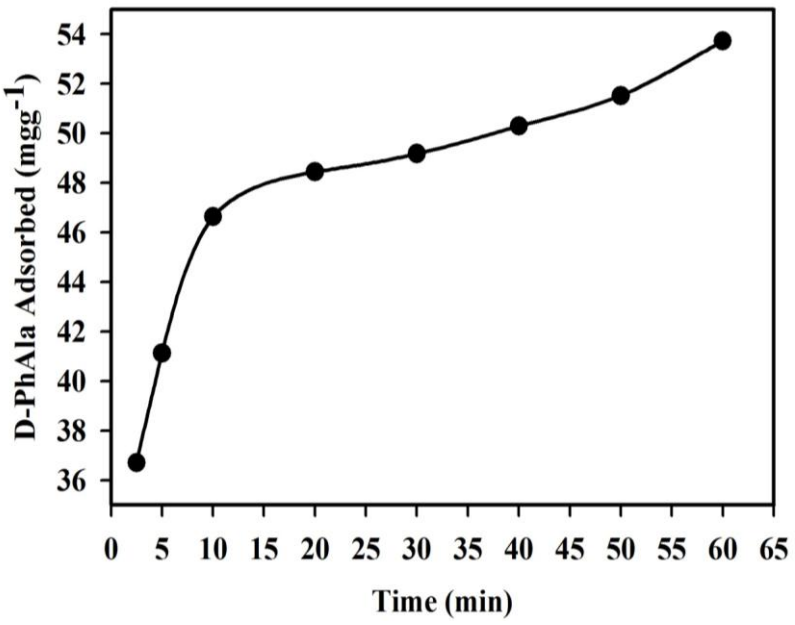

Figure 8. Effect of contact time on the adsorption of 125 mgL^{-1} D-PhAla using 20 mg SG-L-PhAlaC_2NTf_2 phase at pH 3.0 and 25 °C.

3.2.5. Kinetic models

The adsorption kinetic study is quite important since it describes the nature of compound uptake rate, which in turn controls the residence time of the adsorbent uptake at the solid solution interface. The adsorption kinetic data of D-PhAla on SG-L-PhAlaC_2NTf_2 phase were analyzed in terms of different kinetic models. The adsorption kinetic equation of a pseudo second-order adsorption is expressed as follows:[50]

$$t/q_t = 1/\upsilon_o + (1/q_e)t \quad (5)$$

where $\upsilon_o = k_2 q_e^2$ is the initial adsorption rate ($mgg^{-1}min^{-1}$) and k_2 ($gmg^{-1}min^{-1}$) refers to the rate constant of adsorption, q_e (mgg^{-1}) denotes the amount of compound adsorbed at equilibrium, and q_t (mgg^{-1}) corresponds to the amount of compound on the adsorbent surface at any time t (min). The kinetic parameters q_e and υ_o were calculated from the slope and intercept, respectively, of the linear plots of t/q_t versus t, as shown in Fig. 9. The correlation coefficient (R^2) factor was found to be 0.998, indicating the reliability and accuracy of the pseudo second-order adsorption.

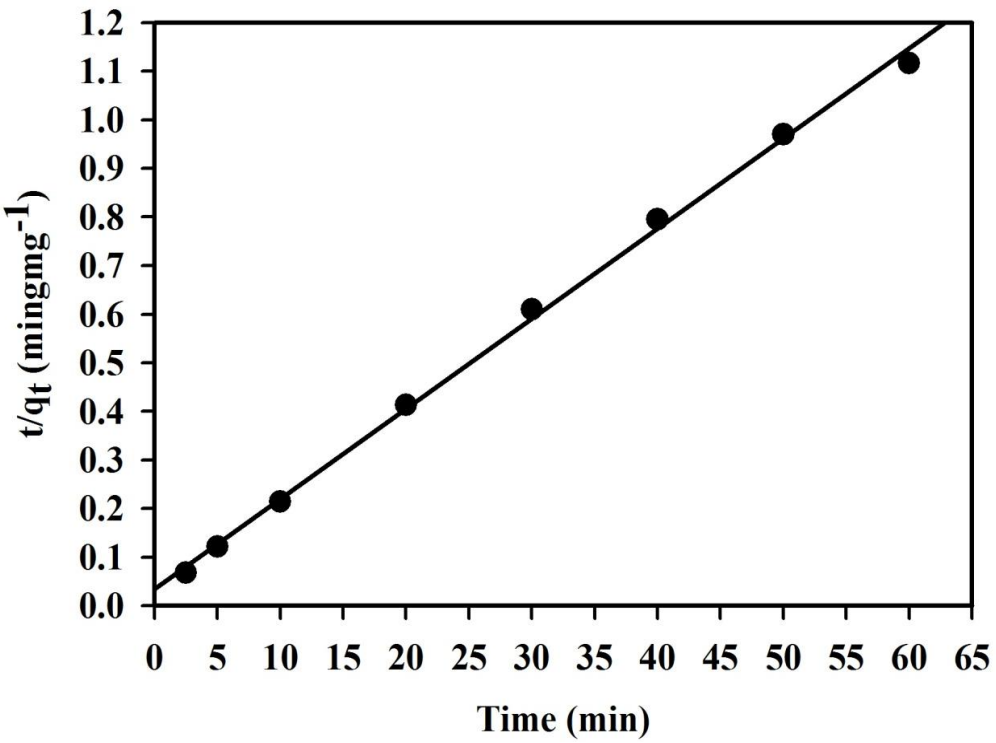

Figure 9. Pseudo second-order adsorption kinetic model of D-PhAla adsorption on 20 mg SG-L-PhAlaC_2NTf_2 at pH 3.0 and 25 °C.

The parameters q_e, υ_o and k_2 were determined to be 53.94 mgg^{-1}, 28.86 $mgg^{-1}min^{-1}$ and 0.01 $gmg^{-1}min^{-1}$. The D-PhAla adsorption capacity on SG-L-PhAlaC_2NTf_2 obtained from the pseudo second-order kinetic model (53.94 mgg^{-1}) was also in good agreement with adsorption capacities obtained from adsorption isotherm experiments (53.72 mgg^{-1}) and from the Langmuir isotherm model (54.01 mgg^{-1}), confirming the highest applicability of the pseudo second-order nature of the adsorption of D-PhAla on SG-L-PhAlaC_2NTf_2.

3.3. Performance of method in analytical applications

3.3.1. Effect of matrix interferences

For assessment of the possibility of the developed procedure in analyzing real samples, the effect of interfering compounds was investigated under optimized conditions. In this study, a model standard solution containing a fixed amount of 100 mgL^{-1} D-PhAla was mixed with 100 mgL^{-1} R-(+)-1-(2-naphthyl) ethanol and 9-fluorenone. Fig. 10a and b displays chromatograms obtained before and after batch method for the prepared standard solution sample. It can be clearly noticed that D-PhAla was adsorbed more than other compounds, indicating that the SG-L-PhAlaC_2NTf_2 adsorbent was most selective toward D-PhAla (Fig. 10b). Therefore, the effect of medium composition containing chiral or achiral mixed compounds has no significant influence on the extraction of D-PhAla. Thus, the selectivity of the proposed method is high enough to be implemented for analyzing D-PhAla in real samples.

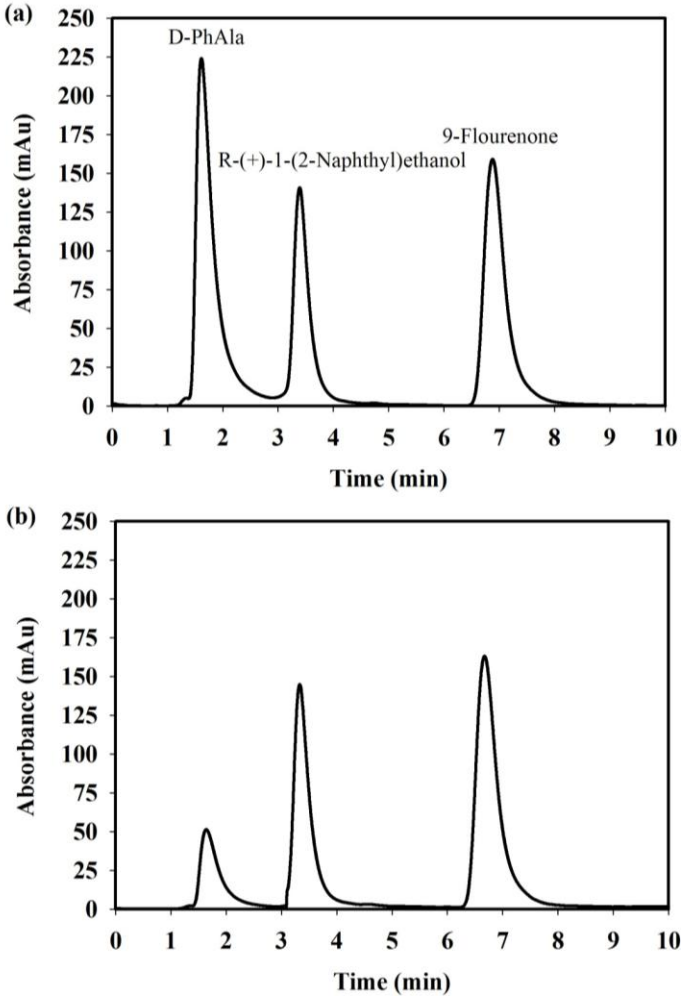

Figure 10. Effect of matrix interferences (a) before and (b) after batch experiment on the extraction of D-PhAla by 20 mg SG-L-PhAlaC_2NTf_2 at pH 3.0 and 25 °C. Adsorption experiments were performed with 100 mgL^{-1} D-PhAla, R-(+)-1-(2-naphthyl) ethanol and 9-fluorenone mixture under batch conditions.

3.3.2. Application of the proposed method

For validation of the applicability of the method to real samples, the method was implemented to the determination of D-PhAla in real environmental water samples via standard addition method. This procedure is an alternative to the use of a certified reference sample, which is unfortunately not in hand. An amount of 25, 50 and 75 mgL^{-1} D-PhAla was individually spiked in four different types of water samples, including ground water, lake water, seawater and waste water, collected from Jeddah in Saudi Arabia. The % extraction of each amount of D-PhAla in real water samples was calculated, as reported in Table 3. Results indicated that the extraction of D-PhAla in spiked water samples were satisfactory for trace analysis and apparently demonstrated that the method is reliable, feasible and suitable for analyzing real samples.

Table 3. Determination of D-PhAla at different concentrations (25, 50 and 75 mgL^{-1}) in real water samples using 20 mg SG-L-PhAlaC_2NTf_2 at pH 3.0 and 25 °C (*N*= 3).

Sample	Added (mgL^{-1})	Unadsorbed (mgL^{-1})	Extraction (%)
Ground water	25	0.95	96.19
	50	2.87	94.26
	75	6.64	91.15
Lake water	25	0.62	97.50
	50	2.86	94.28
	75	6.93	90.76
Seawater	25	0.90	96.38
	50	3.47	93.05
	75	7.20	90.41
Waste water	25	0.86	96.56
	50	3.79	92.41
	75	8.98	88.02

3.3.3. Characteristic performance of the method

The analytical performance of method was evaluated under optimal detection conditions by determination of the linearity, detection limit and reproducibility. The calibration curve for D-PhAla was obtained by using different concentration levels ranging from 0−200 mgL^{-1}. A good linearity was obtained over these concentration ranges with correlation coefficients value of 0.997. The reproducibility of the method was evaluated based on the average of three replicates of each water sample containing 25, 50 and 75 mgL^{-1} D-PhAla. The relative standard deviation (RSD) was estimated to be less than 2.02% for the target compound. Therefore, the accuracy obtained using the proposed method is satisfactory. The limit of detection (LOD) and limit of quantification (LOQ) of D-PhAla

were determined to be 3.64 mgL^{-1} and 12.12 mgL^{-1} by calculating signal/noise ratio (S/N = 3) and (S/N = 10), respectively.

4. CONCLUSIONS

The presented method proved the effectiveness of the newly synthesized SG-L-PhAlaC_2NTf_2 phase for the enantioselective adsorption and determination of D-PhAla in aqueous solution at short contact time. Adsorption isotherm data of D-PhAla adsorption on SG-L-PhAlaC_2NTf_2 were well fit with Langmuir adsorption isotherm model, indicating the adsorption process was mainly monolayer onto a homogeneous adsorbent surface. Results demonstrated that the adsorption of D-PhAla on the SG-L-PhAlaC_2NTf_2 phase obeyed the pseudo second-order kinetic model. In addition, the extraction of D-PhAla by SG-L-PhAlaC_2NTf_2 was not affected by the medium composition containing mixed chiral or achiral compounds. The proposed method was ultimately applied to real water samples, providing acceptable and reliable results for a selective extraction and determination of D-PhAla with complicated and variable matrices.

ACKNOWLEDGEMENT
This project was funded by the Center of Excellence for Advanced Materials Research (CEAMR), King Abdulaziz University, Jeddah, under Grant no. (CEAMR-SG-1-435).

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