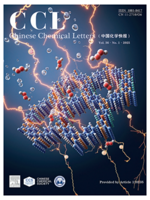
Chinese Chemical Letters 36 (2025) 109889

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet



An ionic liquid assisted hydrogel functionalized silica stationary phase for mixed-mode liquid chromatography

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ARTICLE INFO

Article history:
Received 4 December 2023
Revised 16 March 2024
Accepted 14 April 2024
Available online 15 April 2024

Keywords:
Ionic liquid
Hydrogel
Functionalized silica
Synergistic effect
Stationary phase

ABSTRACT

An ionic liquid assisted hydrogel modified silica was synthesized using a one-pot polymerization and physical coating technique and subsequently applied to mixed-mode liquid chromatography. Analytical techniques, including Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), and elemental analysis, *etc.*, confirmed the successful prepared of this innovative stationary phase. The unique combination of amide, long alkyl chain, and imidazole ring in the hydrogel coating enables the stationary phase to function effectively in hydrophilic/reversed-phase/ion exchange liquid chromatography. Notably, the stationary phase exhibited superior separation performance owing to the synergistic effect of the ionic liquid and hydrogel. This was particularly evident when analyzing various analytes such as organic acids, nucleosides/bases, polycyclic aromatic hydrocarbons (PAHs) and anions. Furthermore, under our operating conditions, an excellent column efficiency of 53,642.9 plates/m was achieved for theobromine. In summary, we have proposed a straightforward strategy to enhance the separation performance of hydrogel coatings in liquid chromatography, thereby broadening the potential applications of hydrogels in the field of separation.

Stationary phase is integral to high-performance liquid chromatography (HPLC), with its separation efficacy being intimately tied to its structural, chemical, and physical properties [1–3]. The advancement of HPLC technology, particularly in response to the growing demand for high selectivity, sensitivity, high-throughput separation and analysis of complex samples, has significantly propelled the development of new chromatographic stationary phases. A mixed-mode stationary phase, which offers a multiple retention mechanism during the separation process, has emerged as a highly sought-after solution [1]. Unlike single mode chromatography, which only offers one dominant type of interaction between the analytes and stationary phase, the mixed-mode stationary phase has demonstrated superior separation selectivity. This is particularly effective for the separation of polar and non-polar highly mixed organic compounds [4–6]. Nowadays, several materials have been prepared to be used as mixed-mode stationary phase due to possesses multiple interaction sites such as ionic liquid/COF/silica (IL-COF@SiO_2) [7], polymer brush–silica [8] and two ionic liquid bonded silica [9]. However, the range of mixed-mode chromatographic stationary phases with superior separation properties remains limited. Consequently, there is an imperative need for the design and synthesis of innovative mixed- mode stationary phases to address the separation needs of complex samples.

Hydrogels with a 3D network structure are hydrophilic polymers that can absorb water and swell to equilibrium [10]. It has been widely used in a widely fields such as tissue engineering [11], biomedicine [12], wearable sensors [13] and catalysis [14] benefit from its adjustable pore structure, functional designability, multiple interaction sites and good adhesion. Given their numerous advantages, hydrogels are particularly well-suited for use as liquid chromatographic stationary phases in separating intricate samples. However, the application of hydrogels in chromatography is hindered by their softness, which prevents them from being used as a stationary phase alone. To address this issue and harness the full potential of hydrogels in liquid chromatography, our research group has conducted a series of studies. In the initial stage of our research, we developed an organic gel-coated silica stationary phase [15]. By integrating organic gel with silica, the inherent weak mechanical strength of the gel was effectively addressed. Furthermore, our team developed a double-layer hydrogel-coated

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https://doi.org/10.1016/j.cclet.2024.109889

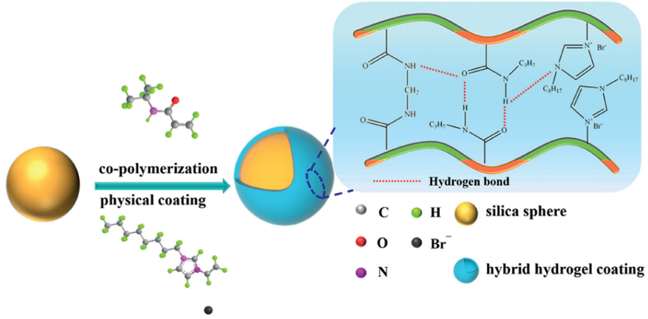

Fig. 1. The preparation route of the Sil@hybrid hydrogel.

silica stationary phase [16]. By incorporating the hydrophobic octadecene monomer into the secondary layer of the hydrogel network, we were able to overcome the poor retention capacity inherent in pure polyacrylamide hydrogel chromatographic columns. Additionally, we developed MOFs-assisted hydrogel co-modified silica stationary phases [17,18]. The incorporation of MOFs particles further augmented the mechanical properties of the hydrogel, providing more interaction sites. Consequently, the resulting stationary phase displayed effective separation performance.

In this study, we selected *N*-isopropylacrylamide and 1-octyl-3-vinylimidazolium bromide as the hydrogel monomers to create an ionic liquid assisted hydrogel functionalized silica stationary phase which possesses both hydrophilic groups and hydrophobic groups. Due to the coexistence of amide, long alky chain and imidazole ring in hydrogel coating, the obtained stationary phase can be used in hydrophilic/reversed-phase/ion exchange liquid chromatography with multiple retention mechanisms. In addition, the preliminary application of the obtained stationary phase in the analysis of traditional Chinese medicine formula was performed.

The novel hydrogel functionalized silica stationary was prepared *via* one-pot polymerization and physical coting technique, as shown in Fig. 1 (details can be found in the Supporting information). Furthermore, the successful synthesis of the ionic liquid assisted hydrogel functionalized silica was confirmed through a series of analyses, including Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), *etc*. As shown in Fig. S1a (Supporting information), the bare silica exhibited a weight loss about 4 wt%, which can be attributed to the removal of impurities. Following modification with hydrogel, ionic liquid, or hybrid hydrogel, there was a noticeable increase in weight loss. The Sil@hybrid hydrogel exhibited the most significant weight loss of approximately 12.4%. The increase in the contents of C, N, H indicated the successful prepared of hybrid hydrogel functionalized silica stationary phase (Table S1 in Supporting information). In addition, FT-IR spectrum of bare silica exhibited absorption peaks at 803 cm^{-1} and 1103 cm^{-1} which can be attributed to stretching vibrations of SiO−H and Si–O–Si bonds [19,20], respectively. After modification *via* ionic liquid assisted hydrogel, the FT-IR spectrum of Sil@hybrid hydrogel showed characteristic absorption peaks of -C=O and -C=N- at 1660 and 1552 cm^{-1} originated from the N-isopropylacrylamide and imidazole rings (Fig. S1b in Supporting information), which indicated the ionic liquid assisted hydrogel functionalized silica was successfully synthesized. Moreover, the bands at 2856 and 1393 cm^{-1} were assigned to the stretching vibration and deformation vibration of -C-H in long alkyl chain [21], respectively. Furthermore, the results of EDS mapping demonstrated that C and N were uniformly distributed on Sil@hybrid hydrogel (Fig. S2 in Supporting information). In addition, XPS results demonstrated that the existence of N element in Sil@hybrid hydrogel, show in Fig. S3 (Supporting information). The disparity in the specific surface area between bare silica and Sil@hybrid hydrogel, which caused by the pore blocking due to the functionalization of silica by hybrid hydrogel, underscores the successful synthesis of the composite materials (Table S2 in Supporting information).

Given that the hydrogel is a hydrophilic polymer with a three-dimensional structure, it has the ability to adsorb water. This process facilitates the formation of a water-rich layer on the surface of the Sil@hybrid hydrogel. Therefore, the hydrophilic retention mechanism of the Sil@hybrid hydrogel was examined by altering the volume fraction of water during the five saccharides separation process. A model incorporating the impact of partitioning and adsorption (Eq. 1) was chosen to delineate the relationship between retention factors (k) and the volume fraction of water [22,23].

$$\ln k = a_1 + a_2 \ln(\varphi) + a_3 \varphi \tag{1}$$

where, k is the retention factor, a_i is the regression coefficient and φ is the volume fraction of water in mobile phase. Fig. 2a and Table S3 (Supporting information) indicated that the plots of ln k *versus* φ (water) fitted very well with Eq. 1 at different eluent compositions ($\varphi = 0.15\text{-}0.35$, $R^2 = 0.9961\text{-}0.9996$). In addition, the retention time of five saccharides decreased with the increase volume fraction of water in mobile phase which was consistent with the typical characteristics of hydrophilic interaction liquid chromatography (HILIC) [24–26].

Over the past decades, various studies have underscored the pivotal role of buffer concentration in the separation process by modulating and optimizing the polarity. Consequently, the influence of buffer concentration on retention was explored by selecting five saccharides as analytes, which can disregard the electron static interaction. As shown in Fig. 2b, the retention factors of five analytes increased with increasing buffer concentration. It has been reported that increase of buffer concentration will increase the thickness of water-rich layer and/or its polarity [27,28]. As a result, the retention of hydrophilic substances will be enhanced in HILIC mode. Furthermore, the effect of temperature on retention was investigated by selected nucleosides/bases as analytes using Van't Hoff equation [29] (details can be found in Supporting information). Due to the viscosity reduction of the mobile phase and the mass transfer acceleration, the retention of eight nucleosides/bases decreased with increasing temperature from 15 °C to 35 °C (Fig. 2c). The good linear relationship of the van't Hoff plots except for thymine ($R^2 = 0.7492$, Table S4 in Supporting information), indicated that there may be multiple interaction (such as H-bond, π–π interaction and hydrophilic interaction) for the separation of eight nucleosides/bases.

The separation performance of the Sil@hybrid hydrogel for HILIC mode was investigated by separating various of hydrophilic analytes and comparing with bare silica and NH_2 column. As shown in Fig. 3a, eight nucleosides/bases were well separated by Sil@hybrid hydrogel with good peak shape and symmetry, which could not be separated by bare silica column or NH_2 column (Fig. 3b). It is worth noting that the retention order of eight nucleosides/bases did not follow the order of hydrophilicity (log $K_{o/w}$, Table S5 in Supporting information) of analytes, indicating the existence of multiple mechanism in Sil@hybrid hydrogel which was consistent with the results of the effect of temperature. In addition, the chromatographic performances of eight organic acids (Fig. S4 in Supporting information), five antibiotics (Fig. S5 in Supporting information) and six sulfonamides (Fig. S6 in Supporting information) further demonstrated the superiority of Sil@hybrid hydrogel compared with the bare silica column and NH_2 column.

In order to investigate the synergistic effect of ionic liquid and hydrogel, the separation performance of Sil@hybrid hydrogel was compared with that of Sil@hydrogel and Sil@PINL by selected amino acids and organic acids as model analytes. As shown in

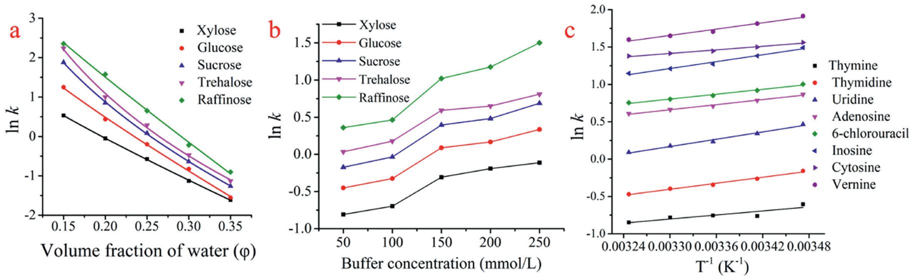

Fig. 2. Curves of (a) effect of the water content (b) buffer concentration on the retention factors for five saccharides and (c) effect of temperature on the retention factors of eight nucleosides/bases. Analytes in (a) and (b): (1) Xylose, (2) glucose, (3) sucrose, (4) trehalose, (5) raffinose. Mobile phase: (a) ACN and different content 150 mmol/L ammonium acetate aqueous solution; (b) 75% ACN and 15% H_2O with different ammonium acetate concentration; (c) 90% ACN and 10% 100 mmol/L NH_4OAc. (a) and (b) ELSD detection at evaporation temperature of 90 °C, gas flow: 2.5 L/min; (c) UV detection at 254 nm. Column temperature: 25 °C. Flow rate: 1.0 mL/min.

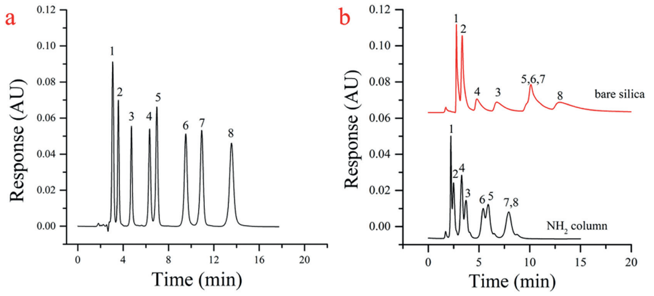

Fig. 3. Chromatograms for the separation of eight nucleosides/bases over (a) Sil@hybrid hydrogel, (b) bare silica and NH_2 column. Analytes: (1) Thymine, (2) thymidine, (3) uridine, (4) adenosine, (5) 6-chlorouracil, (6) inosine, (7) cytosine, (8) vernine. Mobile phase: 90% ACN and 10% 100 mmol/L NH_4OAc; UV detection at 254 nm. Column temperature: 25 °C. Flow rate: 1.0 mL/min.

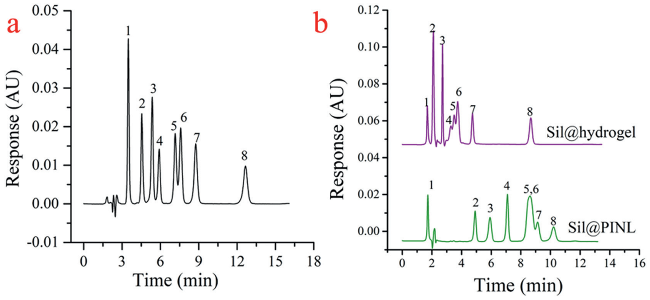

Fig. 4. Chromatograms for the separation of eight organic acids over (a) Sil@hybrid hydrogel, (b) Sil@hydrogel and Sil@PINL. Analytes: (1) 3,5-Dinitrobenzoic acid, (2) salicylic acid, (3) *p*-nitrobenzoic acid, (4) *α*-naphthylacetic acid, (5) benzoic acid, (6) cinnamic acid, (7) *p*-hydroxybenzoic acid, (8) nicotinic acid. Mobile phase: 86% ACN and 14% 100 mmol/L NH_4OAc; UV detection at 254 nm. Column temperature: 25 °C. Flow rate: 1.0 mL/min.

Fig. 4 and Fig. S7 (Supporting information), the ionic liquid assisted hydrogel functionalized silica column showed excellent separation performance with better selectivity and peak shapes than Sil@hydrogel and Sil@PINL both for amino acids and organic acids. This may be due to the multiple interaction sites provided by the co-existence of amide, long alkyl chain and imidazole ring in hybrid hydrogel coating. Thus, the selectivity of different analytes was improved. Furthermore, six alkaloids (Fig. S8a in Supporting information) and five saccharides (Fig. S8b in Supporting information) also were well separated by Sil@hybrid hydrogel.

The synthesized hybrid hydrogel, characterized by its long alkyl chains and imidazole rings, serves as hydrophobic and π-π interaction sites, thereby facilitating the separation of analytes in reversed-phase liquid chromatography (RPLC) mode. As shown in Fig. 5a, the retention factors of six alkylbenzenes exhibited a good linear correlation with their corresponding hydrophobicity (log $K_{o/w}$) which indicated that hydrophobic interactions play a key role in the separation process. It is well known that the retention time of non-polar analytes diminishes as the amount of organic phase content increases in RPLC mode [30,31]. As displayed in Fig. 5a, the retention factors of six alkylbenzenes were gradually decreased with increasing ACN content from 20% to 40%, distinctly exhibiting a characteristic of hydrophobic retention mechanism in RPLC. The following equation (Eq. 2) can be used to describe the hydrophobic interaction mechanism in RPLC mode [21]:

$$\ln k = c_0 + c_1 C_B + c_2 {C_B}^2 \qquad (2)$$

where c_i is the regression coefficient, k is the factors of six alkylbenzenes and C_B is the volume fraction of ACN in mobile phase. The fitting results ($R^2 = 0.9900-0.9980$) for six alkylbenzenes (Fig. 5b and Table S6 in Supporting information) demonstrating that the retention of alkylbenzene on Sil@hybrid hydrogel was principally rely on the hydrophobic interaction between the stationary phase and analytes. Furthermore, an excellent fitting results of van't

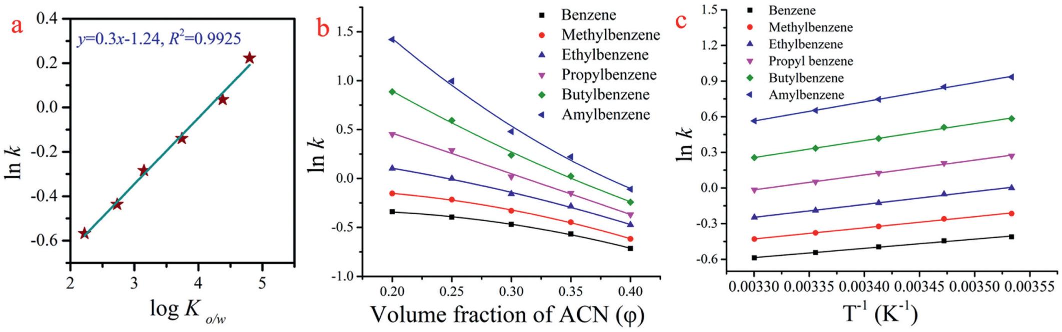

Fig. 5. (a) Plots of ln *k versus* log $K_{o/w}$ curves for six alkylbenzenes, (b) effect of ACN content and (c) temperature on retention factors of six alkylbenzenes. Mobile phase: (a) 35% ACN and 65% H_2O, (b) H_2O and different content ACN and (c) 26% ACN and 74% H_2O. UV detection at 207 nm. Column temperature: 10 °C. Flow rate: 0.8 mL/min.

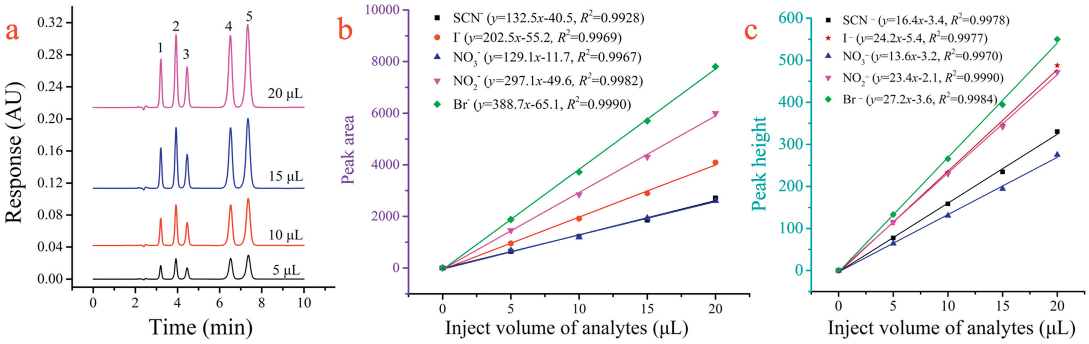

Fig. 6. (a) Chromatograms for the separation of five anions with different inject volume. Plots of peak area (b) and peak height (c) *versus* inject volume of analytes curves for five anions. Analytes: (1) SCN^-, (2) I^-, (3) $NO_3{}^-$, (4) $NO_2{}^-$, (5) Br^-. Mobile phase: 88% ACN and 12% 20 mmol/L KCl. UV detection at 211 nm. Column temperature: 25 °C. Flow rate: 1.0 mL/min.

Hoff plots for retention factors and temperature was obtained ($R^2 = 0.9927-0.9980$) which indicated the retention mechanism has not changed as the temperature varied (Fig. 5c and Table S7 in Supporting information). Six alkylbenzenes was separated by Sil@hybrid hydrogel (Fig. S9a in Supporting information) with the same elution order of C18 column (Fig. S10 in Supporting information) when the mobile phase was acetonitrile/water (26/74, v/v). The detection of pesticide toxicity and polycyclic aromatic hydrocarbons (PAHs) is crucial for public health. It has been observed that Sil@hybrid hydrogel effectively separated five PAHs in 12 min (Fig. S9b in Supporting information). Furthermore, the retention time of the analytes increased proportionally with the number of benzene units, suggesting that π-π interactions may be instrumental in the separation of PAHs. Additionally, baseline separation was achieved for four pesticides using Sil@hybrid hydrogel (Fig. S9c in Supporting information).

The separation of anions was performed to investigate the ion exchange interaction of Sil@hybrid hydrogel. It can be seen that the baseline separation was obtained for five anions with the mobile condition was 20 mmol/L KCl/ACN (12/88, v/v) (Fig. S11a in Supporting information). Furthermore, the correlation between flow rate and column plate height was examined using four selected anions as model analytes. The result demonstrated that the all the analytes exhibited the minimum column plate height when the flow rate was 0.4 mL/min (Fig. S11b in Supporting information). Among the analytes, SCN^- provided the minimum column plate height (3.55 µm) corresponding the biggest column efficiency 42,240.1 plate/m. Additionally, the quantitative capability of Sil@hybrid hydrogel was investigated by varying the inject volume of analytes. As shown in Figs. 6b and c, both peak area and peak height of five anions increased linearly with the increase of inject volume which indicated that stationary phases can be used for qualitative and quantitative analysis.

The good repeatability and stability of a chromatographic column are crucial for its practical application. Therefore, a combination of sulfonamides, nucleoside/bases, and organic acids was chosen as the model analytes to assess the repeatability and stability of Sil@hybrid hydrogel. Firstly, the repeatability of Sil@hybrid hydrogel was studied by ten successive injections of the mixture and the relative standard deviation (RSD) of retention time was between 0.14% and 0.32%, shown in Fig. S12a and Table S8 (Supporting information). Subsequently, the stability of the prepared stationary phase, Sil@hybrid hydrogel, was evaluated through continuous operation over a period of 80 h. The resulting RSD was found to be within the range of 0.38%-1.49% as indicated in Table S9 (Supporting information). These findings suggest that the Sil@hybrid hydrogel demonstrates both good repeatability and stability. Furthermore, we selected some representative analytes to calculate their column efficiency (Table S10 in Supporting information). It is can be seen that theobromine exhibited highest column efficiency 53,642.9 plate/m.

Traditional Chinese medicine, with its extensive clinical experience and rich history, has developed a unique theory that has significantly contributed to disease elimination. In particular, traditional Chinese medicine formula has played a crucial role in the prevention and treatment of COVID-19. However, the complexity and diversity of traditional Chinese medicine formulas pose significant challenges to the research on their characteristic active ingredients due to difficulties in separation. Furthermore, traditional Chinese medicine is typically administered *via* water decoction. Consequently, we selected a traditional Chinese medicine formula (additional details can be found in Supporting information) utilized for COVID-19 prophylaxis and treatment as the analyte for chromatographic separation in HILIC mode. It is well known that the greater the number of peaks obtained in chromatograms, the better the selectivity. As shown in Fig. 7, 18 kinds of compounds were detected by Sil@hybrid hydrogel when the mobile phase condition was acetonitrile/100 mmol/L NH_4AcO (80/20, v/v), which demonstrated that at least 18 kinds of compound were presented in the traditional Chinses medicine formula. However, under identical chromatographic conditions, the C18 column can only detect 3 chromatographic peaks, while the NH_2 column is limited to detecting only 5 peaks. Considering that the separation of substances in a C18 column is dependent on the hydrophobic interaction between the analytes and the chromatographic column, we modified the proportion of acetonitrile in the mobile phase from 80% to 20%. As shown in Fig. S13 (Supporting information), there are still 12 chromatographic peaks exhibited less than that of Sil@hybrid hydrogel, which further proves the superiority of Sil@hybrid hydrogel. The shape and selectivity of chromatographic peaks can be significantly enhanced by fine tuning the mobile phase conditions. These include the ratio of organic to aqueous phase, the type of buffer salts used, among others. While the characterization of each component remains a significant task requiring further investigation, the Sil@hybrid hydrogel chromatographic column has shown promise in facilitating the analysis of components in traditional Chinese medicine formula.

In conclusion, we leveraged the superior adhesion of hydrogel to produce an ionic liquid-assisted hydrogel functionalized silica stationary phase *via* a simplified physical coating technique. The synergistic interaction between hydrogel and ionic liquid was found to provide multiple retention mechanisms. The investigation into the chromatographic behavior of the Sil@hybrid hydrogel composite material revealed its potential as an effective stationary phase in mixed-mode liquid chromatography. The material exhibited superior separation performance for a wide range of hydrophilic, hydrophobic, and anionic analytes. Furthermore, the use of Sil@hybrid hydrogel in the analysis of traditional Chinese

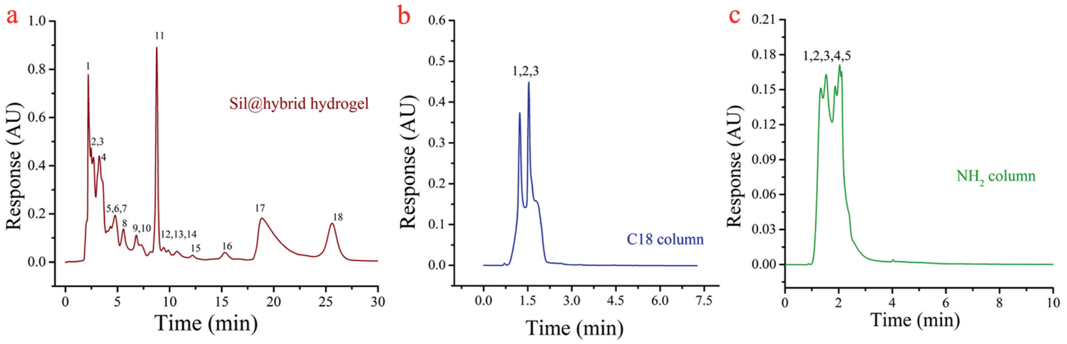

Fig. 7. Chromatograms for the analysis of a traditional Chinese medicine formula over (a) Sil@hybrid hydrogel, (b) C18 column and (c) NH_2 column. Mobile phase: 80% ACN and 20%100 mmol/L NH_4OAc. UV detection at 254 nm. Column temperature: 25 °C. Flow rate: 1.0 mL/min.

medicine has demonstrated that the stationary phase can potentially promote the analysis of traditional Chinese medicine components. In summary, this study provides a novel approach to broaden the application of hydrogel in liquid chromatography.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Tong Zhang: Conceptualization, Formal analysis, Investigation, Writing – original draft, Data curation, Validation. **Xiaojing Liang:** Conceptualization, Funding acquisition, Writing – review & editing, Data curation, Project administration, Visualization. **Licheng Wang:** Supervision, Validation. **Shuai Wang:** Validation, Supervision. **Xiaoxiao Liu:** Validation. **Yong Guo:** Methodology, Project administration, Resources, Data curation.

Acknowledgment

Innovation Groups of Basic Research in Gansu Province (No. 23JRRA570) is gratefully acknowledged.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2024.109889.

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