# Analytical Methods

## PAPER



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## 1 Introduction

In recent years, rising concern has been paid to mixed-mode chromatography (MMC) owing to its high selectivity and high loading capacity.<sup>1,2</sup> MMC is based on various retention mechanisms, and one MMC column can be employed to separate diverse samples under a single mode, thus avoiding the wastage of materials.<sup>3,4</sup> With the increasing development of MMC stationary phases, more and more mixed-mode columns have appeared.<sup>5-15</sup> Wang et al.<sup>10</sup> modified silica with diol and C18 groups for preparing an HILIC/RPLC mixed-mode stationary phase, and then carried out an assessment of its chromatographic separation performance by investigating twenty acids, bases, and neutrals. Bo and Wang et al.<sup>12</sup> prepared an HILIC/IEC mixed-mode material, and then applied the developed stationary phase in the separation of  $\beta$ -agonists and safflower injection. A novel benzimidazole modified silica for an RPLC/ IEC mixed-mode was introduced by Sun et al.14 A new HILIC/ RPLC/IEC mixed-mode stationary phase was successfully synthesized via a "thiol-ene" click chemistry method.15

# Preparation, characterization and evaluation of an imidazolium ionic liquid copolymer stationary phase for mixed-mode chromatography<sup>+</sup>

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A novel imidazolium ionic liquid mixed-mode stationary phase (named IBON) was successfully prepared by a controlled thiol-initiated radical polymerization method. The successful preparation of the IBON stationary phase was certified by Fourier transform infrared (FT-IR) spectrometry, elemental analysis, scanning electron microscopy (SEM) and thermogravimetric analysis (TGA). Meanwhile, the zeta potential showed the surface potential of the IBON stationary phase, while the results of gel permeation chromatography (GPC) demonstrated the successful control of the surface-initiated polymerization. The retention behaviour was investigated by various types of analytes, including acidic compounds, basic compounds and neutral compounds (containing polar nucleosides and bases, non-polar benzene series). Through investigating the influence of the acetonitrile content, pH of the mobile phase, buffer concentration on the retention and by employing the van't Hoff equation and linear solvation energy relationships model, it was found that the various test solutes could undergo multiple interactions with the IBON stationary phase, including hydrophobic, electrostatic, dipole-dipole, hydrogen bonding, ion exchange and  $\pi$ - $\pi$  interactions. The prepared stationary phase was employed to separate polar solutes, benzene series, and inorganic anions, and it displayed different separation characters when compared to the commercial Acclaim<sup>TM</sup> Mixed-mode WAX-1 column.

Ionic liquids (ILs) are widely used in the separation and analysis fields for their outstanding peculiarity of high thermal stability, low volatility and excellent solubility for chemicals.<sup>16,17</sup> To date, some papers<sup>18–21</sup> have reported IL bonded stationary phases. For example, Qiao *et al.*<sup>22</sup> introduced a glucaminiumbased IL separation material, and then separated nucleotides and flavonoids on the developed column. Liu *et al.*<sup>23</sup> used a novel imidazolium ionic liquid stationary phase for protein separation.

Generally, modified stationary phases can be classified into monolayer<sup>24-26</sup> and multilayer polymeric modified stationary phases.<sup>27-29</sup> However, conventional free radical polymerization lacks control of the polymerization, leading to a high polydispersity and long chains.<sup>30</sup> Controlled radical polymerization (CRP) technology may well control the chain length and polydispersity of polymers.<sup>31</sup> For instance, Shen and Yan *et al.*<sup>32</sup> successfully obtained a zwitterionic polymer bonded stationary phase using a controlled reversible addition fragmentation transfer (RAFT) polymerization method, and the developed material could be applied in the enrichment of glycopeptides.

In this work, an imidazolium ionic liquid copolymer stationary phase was obtained by controlling RAFT polymerization according to a previous report.<sup>33</sup> The obtained novel stationary phase was systematically characterized by FT-IR spectroscopy, elemental analysis, SEM, TGA, and zeta potential analysis. Meanwhile, the gel permeation chromatography



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(GPC) results proved the successful control of the RAFT polymerization. The retention behaviour was investigated under different chromatographic conditions (acetonitrile content, buffer concentration, buffer pH and column temperature). To further explore the retention mechanisms of the new stationary phase, the van't Hoff equation and linear solvation energy relationship model were applied under different chromatographic conditions. Furthermore, the separation of polar solutes (nucleosides and bases, carboxylic acid), benzene series and inorganic anions were successfully conducted on the prepared stationary phase, and the commercial Acclaim<sup>™</sup> Mixed-mode WAX-1 column was used for comparison.

## 2 Experimental section

## 2.1 Reagents and materials

1-Vinylimidazole, 4-bromomethylbenzoic acid, tetrahydrofuran (THF), tetradecylamine, acryloyl chloride, triethylamine, trichloromethane, toluene, *N*,*N*-dimethylformamide (DMF) and absolute ethanol were obtained from Macklin (Shanghai, China).  $5 \mu m$  Spherical silica particles (pore size: 120 Å, surface area: 170 m<sup>2</sup> g<sup>-1</sup>) were provided by Thermo Fisher Scientific Corporation (Shanghai China), 2,2'-azobis(2-methylpropionitrile) (AIBN) and (3-mercaptopropyl)trimethoxysilane (MPS) were obtained from Aladdin (Shanghai, China). 4-Cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPA-DB) was provided by Laajoo (Suzhou, China). Acetonitrile (ACN) and methanol (CH<sub>3</sub>OH) of HPLC grade were obtained from Aladdin (Shanghai, China). All the other reagents were analytically pure, except for special labels. All the probe solutes used in the chromatographic experiments were purchased from different commercial sources.

#### 2.2 Instruments and apparatus

The chromatographic evaluation experiments were conducted with an Agilent 1100 series system, a G1311A quaternary pump, and a G1316A column oven using a G1315B DAD detector. FT-IR spectra were recorded on a spectrometer Tensor 27 (Bruker, Germany). Elemental analysis was performed on a Vario micro elements analyzer (Elementar, Germany). TGA was performed on an SDT-Q600 thermal gravity analyzer (TA Instruments, USA). The zeta-potentials were measured on a Nanobrook Omni (Brookhaven Instruments Corporation, USA). GPC was performed on a Waters 1515 HPLC pump and a Waters 2414 refractive index detector using a PSS column in DMF as an eluent at a flow rate of 1 mL min<sup>-1</sup>.

The commercial columns used for comparison included the XB-C18 column (5  $\mu$ m, 120 Å, 150  $\times$  4.6 mm) provided by Shanghai Welch Materials Technology Co., Ltd, APS-2 Hypersil<sup>TM</sup> column (5  $\mu$ m, 120 Å, 150  $\times$  4.6 mm) and Acclaim<sup>TM</sup> Mixed-mode WAX-1 (5  $\mu$ m, 120 Å, 150  $\times$  4.6 mm), both purchased from Shanghai Thermo Fisher Scientific Technology Co., Ltd.

## 2.3 Preparation of the IBON stationary phase

1-Vinylimidazole (4.040 g, 42.9 mmol) dissolved in 30 mL THF was added to a three-necked flask, and 4-bromomethylbenzoic

acid (4.327 g, 20.1 mmol) dissolved in 40 mL of THF was added drop by drop while stirring at room temperature. After stirring for 12 h, the mixture was filtered and washed with THF three times and dried at 50 °C for 24 h; the <sup>1</sup>H NMR of the obtained product is shown in Fig. S1.† Then, the *N*-tetradecyl acrylamide was successfully synthesized according to previous reports;<sup>34,35</sup> the <sup>1</sup>H NMR spectrum of *N*-tetradecyl acrylamide is shown in Fig. S2.†

Silica (4.0 g) was suspended in 30 mL anhydrous toluene, and MPS (4.0 g) was added with stirring. The mixture was refluxed under nitrogen atmosphere for 24 h. Then, the resulting product was filtered and respectively washed with toluene, ethanol, water, and methanol, and then dried under vacuum at 50 °C for 48 h.

The SH-silica (3.7 g), imidazolium IL (3.092 g, 10.0 mmol) and *N*-tetradecyl acrylamide (2.620 g, 9.8 mmol) were added to 100 mL methanol, and the polymerization was operated with the initiator AIBN (0.122 g, 0.74 mmol) and CPA-DB (0.084 g, 0.3 mmol) under nitrogen at 60  $^{\circ}$ C for 48 h; the whole process is shown in Fig. 1. The final product was centrifuged, washed with methanol, and then dried under vacuum at 60  $^{\circ}$ C overnight to give a novel bonded stationary phase called IBON material.

## 2.4 Column packing

The IBON material was packed as an HPLC column (150  $\times$  4.6 mm i.d.) by a slurry packing method. The process of column packing was performed on an RPL-ZD10 packing machine (Dalian Replete Science and Technology Co., Ltd) by using isopropanol as the slurry solvent and methanol as the propulsion solvent under 25 MPa pressure.

## 3 Results and discussion

## 3.1 Characterization of the IBON stationary phase

The IBON material was characterized by FT-IR spectroscopy. As shown in Fig. S3,† the peaks at 3437 and 1101 cm<sup>-1</sup> of the silica, SH-silica and bond-silica represent the stretching vibrations of O-H and Si-O-Si, respectively. Moreover, the characteristic signals of the C-H group around 2932 and 2859 cm<sup>-1</sup>, the absorption band of the C=O group at 1650 cm<sup>-1</sup> and the



Fig. 1 Preparation of the IBON stationary phase.

Table 1 Molecular weight of the copolymer grafted on the silica

<i>M</i> <sub>n</sub> (Daltons)	$M_{ m w}$ (Daltons)	Polydispersity M <sub>w</sub> /M <sub>n</sub>	Graft density μmol m <sup>-2</sup>		
41 897	47 339	1.13	0.023		

imidazole group at 1551  $\text{cm}^{-1}$  proved that the *N*-tetradecyl acrylamide and imidazolium IL had been bonded.

To verify the bonding amounts of the IBON stationary phase, elemental analysis was carried out. As shown in Table S1,† the carbon content and nitrogen content were 1.54% and 0.00% for S–H silica, and 11.12% and 0.92% for the IBON material, respectively. The remarkable increase in C and N content proved that the imidazolium IL and the *N*-tetradecyl acrylamide were successfully immobilized on the surface of silica. The bonded density of imidazolium IL was 1.06 µmol m<sup>-2</sup> and the bonded density of *N*-tetradecyl acrylamide was 2.46 µmol m<sup>-2</sup> after calculation. The results of SEM are shown in Fig. S4.†

TGA was performed and the results are shown in Fig. S5.† The weight loss below 100  $^{\circ}$ C was ascribed to the absorbed water on the surface of the silica. The bare silica lost nearly 3% weight, thus it could be interpreted that the bare silica absorbed much water on its surface. The weight loss of 13.25% between 250–600  $^{\circ}$ C was attributed to the loss of the bonded copolymer.

The zeta-potential of the stationary phase was measured with 90% ACN ( $_{w}^{w}pH: 3.0-7.0$ , as measured from buffer solution). As shown in Fig. 4B, the zeta potential of the IBON stationary phase gradually changed from positive to negative with the increasing pH, and the isoelectric point was located near  $_{w}^{w}pH: 4.9$ .

To verify the successful control of the surface-initiated polymerization, GPC experiments were conducted. The procedure for the grafted copolymer on the modified silica was reported in a previous study.<sup>36</sup> The polydispersity was calculated by the weight averaged molecular weight ( $M_w$ ) and the number averaged molecular weight ( $M_n$ ) of the copolymer. As shown in Table 1, the small value of polydispersity indicated a good control of the RAFT polymerization. The graft density of the copolymer on the prepared material was estimated using:

Graft density = 
$$\frac{m_{\rm p}}{M_{\rm n}S}$$
 (1)

where  $m_p$  is the weight of the grafted copolymer and *S* is the specific surface area of the prepared silica (146 m<sup>2</sup> g<sup>-1</sup>).

## 3.2 Chromatographic evaluation

3.2.1 Effect of ACN content on the mobile phase. The influence of ACN contents is regarded as the main factor affecting the retention of the probe solutes. Here, 11 kinds of solutes, including acidic solutes, basic solutes, non-polar benzene series, polar nucleosides and bases, were used for chromatographic evaluation. As shown in Fig. 2A, the retention of non-polar benzene series increased with the decrease in the ACN content and it reflected a typical RPLC mode, while the retention of polar nucleosides and bases presented a contrary tendency and it reflected a typical HILIC mode.37 However, the retention behaviour of carboxylic acids reflected the HILIC mode with the ACN content above 80%, while when the ACN content was less than 80%, the retention behaviour of carboxylic acids reflected the RPLC mode. So, U-shaped curves of carboxylic acids were observed in Fig. 2B, which indicated the dual retention behaviours (RPLC and HILIC) of the IBON column.38

**3.2.2 Effect of the buffer concentration.** The influence of the buffer concentration was investigated for acidic solutes, basic solutes, polar nucleosides and bases. As illustrated in Fig. 3, the retention factors for the polar and hydrophilic nucleosides and bases increased with the increase in the buffer concentration. It could be interpreted<sup>39</sup> that the increasing buffer concentration resulted in a thickened water-rich layer on the surface of the stationary phase, strengthening the HILIC interaction between the stationary phase and solutes. However, the retention of carboxylic acids increased initially and then decreased with the increase in the ammonium formate concentration. This phenomenon could be interpreted by the electrostatic repulsion–hydrophilic interaction chromatography (ERLIC).<sup>40</sup> The three



Fig. 2 The effect of ACN contents in the mobile phase for neutral compounds (A), acidic and basic compounds (B). Conditions: ACN/H<sub>2</sub>O (v/v) containing 5 mM NH<sub>4</sub>FA;  $_{w}^{w}$ pH : 4.0; column temperature: 25 °C; flow rate: 0.8 mL min<sup>-1</sup>.

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Fig. 3 Effect of the buffer concentration on the retention of acids, basic compounds, polar nucleosides and bases. Conditions: ACN/NH<sub>4</sub>FA (2, 5, 10, 15, 20 mM) = 90/10 (v/v);  $^{w}_{w}$ pH : 6.02; column temperature: 25 °C; flow rate: 0.8 mL min<sup>-1</sup>.

carboxylic acids were negatively charged (-) under the operating condition. Thus, the carboxylic acids could undergo attractive electrostatic interaction with the positively charged (+) imidazolium ring. Meanwhile, repulsive electrostatic interaction could exist between the ionized carboxylic acids (-) and negatively charged (-) carboxylic group of the IBON material. With the increase in the salt concentration, more and more buffer salt gathers around the charged groups, and weakens the electrostatic interaction, including the attractive and repulsive forces.<sup>40</sup>

**3.2.3 Effect of the buffer pH.** The pH of the mobile phase also plays an important role in the retention of the probe solutes. As shown in Fig. 4A, the retention time of acidic solutes increased first and then decreased with an increase in the pH. At a low pH, the surface potential of the stationary phase was positive (+), as illustrated in Fig. 4B, meanwhile, the acidic solutes  $(3.5 < pK_a < 4.2)$  were initially dissociated and had some negative charge (-), so the retention time of the solutes

increased with an increase in the pH. When the pH exceeded 5.0, the surface potential of the stationary phase was negative (-), and the solutes still had some negative charge (-), so the retention was weakened due to electrostatic repulsion. The retention time of the other test solutes did not change significantly with the varying pH.

**3.2.4 Effect of the column temperature.** To further comprehend the influence of the chromatographic conditions, a different column temperature was investigated. The chromatographic conditions were maintained at 95% ACN and 5 mM ammonium formate in the mobile phase ( $^{w}_{W}$ pH : 4.0), while the column temperature was changed from 10 °C to 50 °C. As shown in Fig. 5, the retention factors of most solutes decreased with an increase in the temperature. The link between the column temperature and retention factor is described by the van't Hoff equation, as follow:

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \Phi$$
 (2)



Fig. 5 Effect of column temperature for neutral, acidic and basic compounds. Conditions:  $ACN/H_2O = 95/5$  (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0; column temperature varied from 10 °C to 50 °C; flow rate: 0.8 mL min<sup>-1</sup>.



Fig. 4 (A) The effect of the buffer pH for neutral, acidic and basic compounds. Conditions:  $ACN/H_2O = 90/10$  (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>v</sub>pH : kept at 3.0, 4.0, 4.8, 5.0, 5.2, 6.0, 7.0, respectively; column temperature: 25 °C; flow rate: 0.8 mL min<sup>-1</sup>. (B) The surface potential of the IBON stationary phase.

where k' is the retention factor, R is the universal gas constant,  $\Delta H$  and  $\Delta S$  are regarded as the enthalpy and the entropy of solute transfer, T is the absolute column temperature and  $\Phi$  is defined as the volume ratio of the stationary phase to the mobile phase, respectively.

The van't Hoff equation is usually used in RPLC,<sup>41</sup> and it could be applied in HILIC while the retention conforms to a partition mechanism.<sup>42</sup> The retention factors of hydrophilic solutes (benzoic acid, melamine, cytidine, adenosine, uracil) decreased with an increase in the temperature and the correlation coefficient of the hydrophilic solutes was greater than 0.95, indicating no obvious change in the interaction mechanism. Further,  $\Delta H$  with a large negative value (as illustrated in Table S2<sup>†</sup>) indicated a change in the interaction mechanism, and  $\Delta H$  with a large negative value (as illustrated in Table S2<sup>†</sup>) indicated an exothermic, partitioning retention process. However, as shown in Fig. 5, the van't Hoff plots for 3-nitrobenzoic acid and p-nitrobenzoic acid were for the most retained analytes by the IBON column and were the least sensitive to temperature changes. This phenomenon might be due to the small  $\Delta S$  value, even reaching a small positive value as seen in Table S2,† and the small  $\Delta H$  value (as illustrated in Table S2,† compared to the  $\Delta H$  values of other compounds) in eqn (2) cancelled the temperature dependence of the retention factors.42 In other words, the nonlinear van't Hoff plot of 3nitrobenzoic acid and p-nitrobenzoic acid on IBON proved that the retention of 3-nitrobenzoic acid and p-nitrobenzoic acid was influenced by mixed retention mechanisms (ion-exchange and hydrophilic interaction).43

#### 3.3 Linear solvation energy relationships model

**3.3.1 Theory.** To further understand the interactions between analytes and a chromatographic system, and which one dominates during the separation process, the linear solvation energy relationship (LSER) model can be used. This model is frequently used in RPLC with five Abraham descriptors,<sup>44,45</sup> even for HILIC.<sup>46</sup> For better exploring the interaction between a chromatographic system and ionizable compounds in the HILIC mode, another two descriptors ( $D^-$  and  $D^+$ ) are used.<sup>47</sup>

$$\log k = c + eE + sS + aA + bB + vV + d^{-}D^{-} + d^{+}D^{+}$$
(3)

$$D^{-} = \frac{10^{(\mathrm{pH}^{*} - \mathrm{p}K^{*})}}{1 + 10^{(\mathrm{pH}^{*} - \mathrm{p}K^{*})}}$$
(4)

$$D^{+} = \frac{10^{(pK^{*}-pH^{*})}}{1+10^{(pK^{*}-pH^{*})}}$$
(5)

where *k* defines the retention factor of the solutes. The capital letters in eqn (3) are the solute descriptors, *E* is the excess molar refraction, *S* is the dipolarity or polarizability of solutes, *A* and *B* respectively represent the effective or overall hydrogen bond acidity and basicity, *V* refers to the McGowan characteristic volume,  $D^-$  refers to the negative charge produced from anionic or zwitterionic species and  $D^+$  refers to the positive charge produced from cationic or zwitterionic species. Meanwhile, the lower-case letters (*e*, *s*, *a*, *b*, *v*,  $d^-$ ,  $d^+$ ) are system constants reflecting the interactions with the chromatographic system.

The coefficients are measured by a multiple linear regression of log k and known solute parameters; the intercept c is an independent system constant, e is related to the n and  $\pi$  electrons, s shows the dipole–dipole interactions, a and b measure the ability of the HB-acceptor and HB-donor, v is a measure of the cavity effect (hydrophobic interaction) and  $d^-$  and  $d^+$  are the interactions with charged solutes. Positive coefficients represent the contribution to the retention of the probe solutes towards the stationary phase, while negative coefficients are considered to represent the solute dispersion into the mobile phase.<sup>48</sup> In eqn (4) and (5), pH\* is measured from the buffer–organic mixing solvents,<sup>48</sup> while pK is the dissociation constant of the solutes.

3.3.2 Comparison of the retention behaviours with different columns. To further comprehend the retention characters of the IBON column, the XB-C18 column and APS-2 Hypersil<sup>™</sup> column were used for comparison in HILIC, using 36 kinds of probe solutes. Plots of the logarithm of the retention factors  $(\log k)$  on the one column *versus* another are interesting to compare the elution orders that would be obtained on the two columns. The interrupted line is the bisector, indicating iso-elution. As shown in Fig. 6A, most benzene series (hydrophobic solutes) fell below the bisector, indicating that the retention of benzene series on the IBON material was stronger than that on APS-2 Hypersil™, but most basic solutes, nucleosides and bases fell above the bisector, indicating that the retention of these compounds on the IBON material was weaker than that on APS-2 Hypersil<sup>™</sup>. As shown in Fig. 6B, most acidic solutes, nucleosides and bases fell below the bisector, indicating that the retention of these compounds on the IBON material was stronger than that on XB-C18, but the benzene series (hydrophobic solutes) and most basic solutes fell above the bisector, indicating that the retention of these compounds on the IBON material was weaker than that on XB-C18. The linear solvation results, as shown in Table 2, showed that there were significant differences for a, b, v,  $d^-$  and  $d^+$  among the three columns. IBON material had a bigger value of a than the other two columns, probably because the distal carboxyl group of the IBON material was in the dissociated state with a negative charge. The coefficient b of the IBON material was less than that



Fig. 6 Comparison of the retention factors with different columns. Conditions: mobile phase:  $ACN/H_2O = 95/5$  (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0; column temperature: 25 °C; flow rate: 0.8 mL min<sup>-1</sup>; DAD: 254 nm. (A) Columns: IBON and APS-2 Hypersil<sup>TM</sup>; (B) columns: IBON and XB-C18.

**Table 2** System constants and statistics for three columns,  $R_{adj}^2$  is the adjusted correlation coefficient, *n* is the number of solutes considered in the regression, SE is the standard error of the fit

Stationary phase	с	е	\$	а	b	ν	$d^{-}$	$d^+$	$R_{\rm adj}^2$	n	SE
IBON	-1.139	0.413	-0.326	1.004	0.349	-0.121	0.790	-0.627	0.745	36	0.265
APS-2 Hypersil™	-0.525	0.182	0.333	0.503	1.100	-1.738	1.566	-0.491	0.919	36	0.234
XB-C18	-0.779	0.271	-0.383	-0.253	-0.457	0.616	-0.419	0.063	0.752	36	0.179

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for APS-2 Hypersil<sup>™</sup>, indicating that the hydrogen bond acidity of the IBON material was less than that of APS-2 Hypersil™. As expected, XB-C18 had a larger value of v than the other columns. The  $d^-$  value of APS-2 Hypersil<sup>TM</sup> was larger than that of the IBON column, but the  $d^+$  values of both these columns were negative; here, it may be because the surfaces of the two columns were positively charged, but the positive charge of APS-2 Hypersil<sup>™</sup> was more than that on the surface of the IBON column. on log k under one condition versus another can be used to compare the elution orders that could be obtained under the two conditions. As shown in Fig. 7A, most nucleosides and bases fell below the bisector, indicating that the retention of these solutes on the IBON material with 95% ACN was stronger than with 65% ACN, but most basic solutes and benzene series fell above the bisector, indicating that the retention of these compounds on the IBON material with 95% ACN was weaker than with 65% ACN. As shown in Fig. 7B, most acidic solutes, nucleosides and bases fell below the bisector, indicating that the retention of these compounds on the IBON

material with 95% ACN was stronger than with 95% CH<sub>3</sub>OH, but the benzene series (hydrophobic solutes) and most basic solutes fell above the bisector, indicating that the retention of these compounds on IBON material with 95% ACN was weaker than with 95% CH<sub>3</sub>OH. As shown in Table 3, there were significant differences in  $a, b, v, d^-$  and  $d^+$  with different mobile phase conditions. The value of a with 95% ACN was positive and stronger than with 65% ACN and 95% CH<sub>3</sub>OH, which could be interpreted to indicate that the interaction of solutes with the stationary phase was weakened with the increase in the water content or CH<sub>3</sub>OH used. The values of *b* with 65% ACN and 95% CH<sub>3</sub>OH were negative, which may be interpreted to indicate that the mobile phases of water and methanol were protonated solvents (with hydrogen bond acidity).49,50 As expected, the value of v with 65% ACN was larger than that with 95% ACN, and the value of v under the CH<sub>3</sub>OH mobile phase was larger than under the same percentage of ACN, indicating that the RPLC mode was reflected when using CH<sub>3</sub>OH as the organic mobile phase. The value of  $d^-$  increased and  $d^+$  decreased maybe because of



**Fig. 7** Comparison of the retention factors with different mobile phases. Column: IBON; column temperature: 25 °C; flow rate: 0.8 mL min<sup>-1</sup>; DAD: 254 nm. (A) Mobile phase:  $ACN/H_2O = 95/5$  (v/v) containing 5 mM NH<sub>4</sub>FA; wpH : 4.0, and  $ACN/H_2O = 65/35$  (v/v) containing 5 mM NH<sub>4</sub>FA; wpH : 4.0; (B) mobile phase:  $ACN/H_2O = 95/5$  (v/v) containing 5 mM NH<sub>4</sub>FA; wpH : 4.0, and  $CH_3OH/H_2O = 95/5$  (v/v) containing 5 mM NH<sub>4</sub>FA; wpH : 4.0, and  $CH_3OH/H_2O = 95/5$  (v/v) containing 5 mM NH<sub>4</sub>FA; wpH : 4.0.

**Table 3** System constants and statistics for IBON columns with different mobile phases,  $R_{adj}^2$  is the adjusted correlation coefficient, *n* is the number of solutes considered in the regression, SE is the standard error of the fit

Mobile phase	с	е	s	а	b	ν	$d^{-}$	$d^+$	$R_{\rm adj}^2$	n	SE
95% ACN	-1.139	0.413	-0.326	1.004	0.349	-0.121	0.790	-0.627	0.745	36	0.265
65% ACN	-0.833	0.367	-0.548	0.261	-0.926	0.885	0.699	0.067	0.860	36	0.185
95% CH <sub>3</sub> OH	-1.495	0.521	-0.396	0.190	-0.344	0.557	0.837	-0.110	0.689	36	0.209

the different solubilities between methanol and ACN to the probe solutes.

**3.3.3 Comparison of the interactions under varied chromatographic conditions.** The effect of the mobile phase plays an important role in retention, so the retention characteristics of different modes (HILIC mode: 95% ACN and RPLC mode: 65% ACN) and the linearization parameters of different organic solvents (ACN and CH<sub>3</sub>OH) were compared to further explore the influence of the mobile phase.

#### 3.4 Applications

**3.4.1 Applications under HILIC mode.** The separation of nucleosides, including uracil, adenosine, adenine, cytosine and cytidine, was achieved on the IBON with 95% ACN and the result is illustrated in Fig. 8. For comparison, the Acclaim<sup>™</sup> Mixed-mode WAX-1 was used for the separation of the same analytes, and adenosine and adenine have different retention times compare to those on the IBON column.

**3.4.2 Separation of benzene series.** The separation of five alkylbenzenes (namely, benzene, toluene, ethylbenzene, propylbenzene and butylbenzene) were achieved in the RPLC mode (65% ACN, 5 mM ammonium formate buffer solution). As illustrated in Fig. 9A, the Acclaim<sup>™</sup> Mixed-mode WAX-1 was used for comparison.

Four hydrophobic solutes (namely, benzene, naphthalene, phenanthrene, pyrene) were successfully separated with 80% ACN as illustrated in Fig. 9B. For comparison, the Acclaim<sup>™</sup> Mixed-mode WAX-1 was used for the separation under conventional RPLC mode, and it was found that some benzene series had different retention times compared to those on the IBON material.

**3.4.3 Applications under the IEC mode.** Anion-exchange separation features of the IBON stationary phase were investigated with five carboxylic acids with 90% ACN and 5 mM ammonium formate buffer solution. As illustrated in Fig. 10A, the retention on the IBON phase for the carboxylic acids had a different peak order compared to that on the Acclaim<sup>™</sup> Mixed-mode WAX-1.

As shown in Fig. 10B, the potential ability of the IBON material for the IEC mode was measured by separating four inorganic anions. These anions, including  $NO_2^-$ ,  $NO_3^-$ ,  $I^-$  and



**Fig. 8** Separation of nucleosides on two columns. Analytes: (1) uracil, (2) adenosine, (3) adenine, (4) cytosine, (5) cytidine. Conditions on IBON: ACN/H<sub>2</sub>O = 95/5 (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0. Conditions on Acclaim<sup>TM</sup> Mixed-mode WAX-1: ACN/H<sub>2</sub>O = 85/15 (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0. The same conditions: column temperature: 25 °C; flow rate: 0.8 mL min<sup>-1</sup>; DAD: 254 nm.



Fig. 9 (A) Separation of five alkylbenzenes on the IBON and Acclaim<sup>™</sup> Mixed-mode WAX-1. Analytes: (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5) butylbenzene. Conditions: ACN/H<sub>2</sub>O = 65/35 (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0; column temperature: 25 °C; flow rate: 0.6 mL min<sup>-1</sup>; DAD: 254 nm. (B) Separation of benzene series on two columns. Analytes: (1) benzene, (2) naphthalene, (3) phenanthrene, (4) pyrene. Conditions on IBON: ACN/H<sub>2</sub>O = 80/20 (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0. Conditions on Acclaim<sup>™</sup> Mixed-mode WAX-1: ACN/H<sub>2</sub>O = 65/35 (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0. The same conditions: column temperature: 25 °C; flow rate: 0.6 mL min<sup>-1</sup>; DAD: 240 nm.



Fig. 10 (A) Separation of carboxylic acids on two columns. Analytes: (1) *p*-aminobenzoic acid, (2) *trans*-cinnamic acid, (3) 3-nitrobenzoic acid, (4) *p*-nitrobenzoic acid, (5) salicylic acid. Conditions on IBON: ACN/H<sub>2</sub>O = 90/10 (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0. Conditions on Acclaim<sup>TM</sup> Mixed-mode WAX-1: ACN/H<sub>2</sub>O = 85/15 (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0. The same conditions: column temperature: 25 °C; flow rate: 0.8 mL min<sup>-1</sup>; DAD: 240 nm. (B) Separation of anions on two columns. Analytes: (1) NO<sub>2</sub><sup>-</sup>, (2) NO<sub>3</sub><sup>-</sup>, (3) I<sup>-</sup>, (4) SCN<sup>-</sup>. Conditions on IBON: ACN/H<sub>2</sub>O = 30/70 (v/v) containing 5 mM Na<sub>2</sub>SO<sub>4</sub>; <sup>w</sup><sub>w</sub>pH : 6.0; column temperature: 25 °C; flow rate: 0.8 mL min<sup>-1</sup>; DAD: 210 nm. Conditions on Acclaim<sup>TM</sup> Mixed-mode WAX-1: ACN/H<sub>2</sub>O = 50/50 (v/v) containing 50 mM Na<sub>2</sub>PO<sub>3</sub>; <sup>w</sup><sub>w</sub>pH : 6.0; column temperature: 30 °C; flow rate: 0.6 mL min<sup>-1</sup>; DAD: 210 nm.

SCN<sup>−</sup>, were successfully separated and the Acclaim<sup>™</sup> Mixedmode WAX-1 was used for comparison.

3.4.4 Practical applications in liquid milk. According to a previous report,<sup>51</sup> 1 mL of liquid milk was dissolved in 10 mL of 1%(v/v) formic acid solution and subjected to ultrasonication for 15 min, followed by the addition of 40 mL of ACN. Then, the mixture was centrifuged at 10 000 rpm for 10 min. Finally, the supernatant was collected and filtered through a 0.2  $\mu$ m membrane for chromatographic analysis.



Fig. 11 Application in the separation of melamine in milk. Conditions:  $ACN/H_2O = 95/5$  (v/v) containing 5 mM NH<sub>4</sub>FA; <sup>w</sup><sub>w</sub>pH : 4.0; column temperature: 25 °C; 0.8 mL min<sup>-1</sup>; DAD: 240 nm.

Melamine, also known as protein concentrate, is a triazine nitrogen-containing heterocyclic compound. It has a certain toxic effect on the human body, especially for young children, so the relevant regulations clearly stipulate that melamine cannot be used in foods and their additives.<sup>52</sup> Therefore, the detection of melamine is of practical significance.

The chromatograms of melamine, milk extract sample and melamine-spiked milk extract sample are shown in Fig. 11. From the chromatograms, no obvious melamine peak appeared in the milk sample; when melamine was spiked into the milk sample, the melamine peak clearly appeared. Thus, the IBON stationary phase could be used to detect melamine in dairy products.

## 3.5 Retention repeatability and efficiencies

Satisfactory retention repeatability and efficiencies are important for both qualitative and quantitative work. A mixture of toluene, uridine and cytidine was injected and recorded, which was repeated over 15 times in this study. The relative standard deviation (RSD) for the retention time, symmetry factors and plate numbers for each peak are shown in Fig. S6.† The IBON stationary phases were prepared in three batches and packed into three columns. The results for the RSD are shown in Table S3,† where it can be seen that both columns presented satisfactory retention repeatability.

## 4 Conclusions

A novel imidazolium IL stationary phase (named IBON) was successfully synthesized by copolymerizing imidazolium IL and *N*-tetradecyl acrylamide onto the surface of SH-silica through a controlled surface-initiated RAFT polymerization method. The retention features of the prepared stationary phase were investigated as a function of changes to the chromatographic conditions. Furthermore, the retention mechanisms were investigated through the van't Hoff equation and linear solvation energy relationship model. In the application of the IBON column, it showed good separation performance for polar solutes, benzene series and inorganic anions under the RPLC, HILIC and IEC modes, respectively. Additionally, the practical application for the separation of melamine in liquid milk showed a potential prospect for the modified stationary phase. All the results proved that the retention of test solutes on the IBON material was based on more than one retention mechanism, including a partitioning mechanism, surface adsorption, electrostatic interactions and ion-exchange interactions. Thus, the obtained stationary phase has potential as a new type of stationary phase for MMC.

## Conflicts of interest

There are no conflicts of interest to declare.

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