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Received March 20, 2008
Revised June 30, 2008
Accepted August 13, 2008

Research Article

Successful establishment of MEKC with electrochemiluminescence detection based on one functionalized ionic liquid

Herein, one water-soluble functionalized ionic liquid, 1-butyl-3-methylimidazolium dodecyl sulfate ([$BMIm^+$][$C_{12}H_{25}SO_4^-$]), was designed and its superiorities either used as supporting electrolytes or as additives for successful establishment of MEKC with electrochemiluminescence (ECL) detection (MEKC-ECL) method were investigated. Compared with the common supporting electrolytes such as phosphate solution, 1-butyl-3-methylimidazolium dodecyl sulfate solution used as running buffers led to greatly enhanced ECL intensities and column efficiencies for negative targets, a little increase for neutral-charge ones while maintained nearly unchanged for positive ones due to the electrostatic forces between the large cation $BMIm^+$ and the solutes and the hydrophobic interactions resulting from the large anion $C_{12}H_{25}SO_4^-$. Moreover, resolution efficiency between analytes was significantly improved. As the existence of ionic liquid moiety, $BMIm^+$, accelerated the electron transfer at the electrode surface, the poisoning effect of long chain $C_{12}H_{25}SO_4^-$ on the electrode surface was eliminated and reproducible ECL intensities with relative standard derivation of 1.02% ($n = 6$) were obtained. The proposed novel MEKC-ECL system was again validated by the baseline separated two similar amino acids of proline and hydroxyproline with lower detection limits of 0.5 and 0.8 μM ($S/N = 3$), respectively.

Keywords:
Electrochemiluminescence / Functionalized / Ionic liquid / MEKC
DOI 10.1002/elps.200800187

1 Introduction

MEKC is a separation mode based on micellar solubilization employing the instrumental technique of capillary zone electrophoresis. In MEKC, a charged surfactant such as SDS is added to the CE buffer, forming micelles, which work as a pseudo-stationary phase of chromatography [1]. Its separation mechanism is based on the different partition between the aqueous phase and the micellar pseudophase [2]. It is nowadays a widely used analytical separation technique for a vast number of charged and uncharged species such as nonsteroidal, mutagenic compounds, carbohydrates, amino acids, proteins, *etc.* [1–8] due to high separation efficiency, short analysis times and compatibility with small sample volumes. $Ru(bpy)_3^{2+}$ electrochemiluminescence (ECL) has been proved to be useful for analytical applications because of its unique advantages such as simple and inexpensive instrumentation, low background noise, high detection sensitivity and a wide dynamic linear range. It has attracted much attention and reflected in the growing number of recent reports [9–12]. MEKC has the potential to be a powerful analytical tool that combines the inherent properties with the sensitivity and selectivity of $Ru(bpy)_3^{2+}$ ECL detection. The development of ECL detection for MEKC has been preliminarily established by Wang *et al.* [13, 14]. In both the reports, SDS was applied as the additives in the supporting electrolytes and certain purposes were achieved; however, the compatibility between surfactant and electrode have not been investigated. Surfactants may potentially foul or poison the electrode surfaces [15].

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Abbreviations: BDS, 1-butyl-3-methylimidazolium dodecyl sulfate**; DBAE,** 2-(dibutylamino)ethanol**; DMBA,** 4-(dimethylamino)butyric acid**; ECL,** electrochemiluminescence**; IL,** ionic liquid**; MEKC-ECL,** MEKC with ECL detection**; N,** plate number**; Pro,** proline**; TPA,** Tri-*n*-propylamine

Whereupon, it is urgent to introduce a simple but effective separation medium into MEKC meanwhile without poisoning the effect on the electrode surface, whose capability is crucial for ensuring the veracity of the proposed method and increasing the functionalities of MEKC-ECL.

Ionic liquids (ILs) are new advanced materials and have arised growing attention in various fields of modern chemistry and technology [16]. They have relatively large potential windows and high conductivity and can be used both as supporting electrolytes [17–20] and as additives to running buffers [21–24] in CE applications. Among all these studies, short-chain ILs were applied and IL-assisted MEKC technique has not been reported. Meanwhile, due to their facile tunability of the physicochemical properties, functionalized ILs have been developed surgingly for specific purposes such as catalysis, organic synthesis, separation technology, ion conductive, *etc*. [25–30]. Subsequently, ILs with significant and specific value for MEKC-ECL could be designed and may exhibit unexpected and innovative characteristics; however, to the best of our knowledge, it was never reported.

In the experiment, inspired by the facile tunability of ILs, one functionalized IL was designed and its solution could be used as both supporting electrolyte and additive to construct MEKC-ECL systems. Furthermore, it has more excellent and novel performances (such as good resolutions between analytes, higher ECL intensities and column efficiencies for negatively charged analytes, no fouling effect on the electrode surface, *etc*.) than other common salts or surfactants. Detailed experiments were conducted and reasons were exploited. The proposed novel MEKC-ECL system was again validated by the baseline separated two similar amino acids of proline (Pro) and hydroxyproline with lower detection limits.

2 Materials and methods

2.1 Chemicals and materials

Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Tri-*n*-propylamine (TPA), 2-(dibutylamino)ethanol (DBAE) and 4-(dimethylamino)butyric acid (DMBA) were purchased from Aldrich Chemical Co. Pro, hydroxyproline, arginine, phenylalanine and histidine were provided by Sigma–Aldrich (Steinheim, Germany). SDS was obtained from Beijing chemical plant (Beijing, China). The IL 1-butyl-3-methylimidazolium dodecyl sulfate ($[BMIm^+][C_{12}H_{25}SO_4^-]$, BDS) were synthesized by the Centre for Green Chemistry and catalysis, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Beijing, China) according to our design idea, and the starting materials were obtained from Sigma–Aldrich. All chemicals were analytical reagent grade and used as received without further purification. All stock solutions were prepared with deionized water, which was processed with Milli-Q ultra-high purity water system (Millipore, Bedford, MA, USA). They were stored in the refrigerator at 4°C and filtered through 0.45 μm pore disposable filter membranes (Jiangsu Qilin Medical Instrument Factory, China) just before use. A series of Pro or hydroxyproline standard solutions for calibration curve, reproducibility, detection limit and other parameters at different electrophoresis conditions were prepared by diluting 2 mM Pro or hydroxyproline stock solution in appropriate electrophoresis buffers.

2.2 Apparatus and equipment

All ECL experiments were carried out with a computer controlled CE-ECL system (Xi'an Remex Electronics Co., Xi'an, China), including a high voltage power supply for electrophoretic separation and electrokinetic injection, an electrochemical potentiostat, a multifunctional chemiluminescence detector and a multichannel data processor. A three-electrode configuration was used in the detection cell consisting of a 500 μm Pt disk as a working electrode, Ag/AgCl as a reference electrode and Pt wire as a counter electrode. The axes of working electrode and separation capillary were aligned setting the distance 100 μm between each other with the aid of an optical microscope (40 × magnification). ECL detection reservoir used herein is the same as the one reported previously [31] and its schematic diagram is shown in Supporting Information Fig. 1. All separations were performed in a 40 cm-long fused-silica capillary with 50 μm id and 360 μm od (Yongnian Optical Conductive Fiber Plant, Hebei, China). The capillary was rinsed with 0.1 M NaOH overnight, washed for 5 min with 0.1 M NaOH, followed by double-distilled water and equilibrated with the running buffer for 5 min before use so as to maintain an active and reproducible inner surface. The voltage of the photomultiplier tube for collecting the ECL signal was set at 850 V in the process of detection. Electrokinetic injections were performed at 12.5 kV for 8 s. The inlet end of the capillary was held at a positive potential and the outlet end was maintained at ground. $Ru(bpy)_3^{2+}$ (5 mM) with 50 mM PBS was added in the detection cell.

3 Results and discussion

The functionalized IL BDS designed specially for the CE modifications belongs to one kind of anionic surfactant; its critical micelle concentration was about 3.2 mM at room temperature by employing pyrene as a fluorescent probe for the micellization of BDS system [32, 33]. It also belongs to an ionic compound and its melting point was below 100°C; it can be defined as a type of ILs, named surfactant IL or IL surfactant. In addition, it has good solubility and miscibility in water. Thus, the effects of BDS solution used in MEKC-ECL establishment were investigated in a detailed manner.

3.1 Effects of BDS solution used as supporting electrolyte

The background electrolyte affects the ECL intensities, migration times and the separation resolution between compounds. To investigate the effects of BDS used as a supporting electrolyte in CE process, contrast experiments between BDS and the most popular buffer for CE, PBS, were conducted. As the pH value of BDS solutions were measured as ~7.4, equivalent concentrations and pH values (pH 7.4) of BDS and PBS have been applied, ensuring the same ionic strength and direct comparison of experiment results. Similar trends were observed for contrast experiments between equivalent concentrations of BDS and PBS. In addition, according to the investigations of the dependences of the ECL peaks and migration times on the electrolyte concentrations, 10 mM for either PBS or BDS is sufficient for the electrophoresis. TPA and Pro are two common co-reactants that generate strong ECL emission on a platinum electrode at pH ~7.4 in the presence of $Ru(bpy)_3^{2+}$; thus the two targets were chosen as the representatives of the analytes. As shown in Fig. 1A, an obvious increase (*ca.* 280%) in ECL intensity (peak height) of Pro was obtained for 10 mM BDS compared with 10 mM PBS (pH 7.4) used as running buffer, while that of TPA was nearly kept unchanged. Similar trends were also presented when a mixture of DBAE and DMBA was investigated, as shown in Fig. 1B. Owing to the application of 10 mM BDS, 220% enhancement in peak intensity of DMBA was observed, while that of DBAE maintained nearly constant.

To test the generality of BDS impact on CE separation, other analytes with a different polarity and net charge such as arginine, phenylalanine and histidine were also applied in the contrast experiments. The sequence of ECL intensity-enhancement magnitude is phenylalanine (*ca.* 235%) > histidine (*ca.* 115%) > arginine (*ca.* 95%). All the results are summarized in Table 1; it was noticed that TPA, DBAE and arginine were positively charged; Pro, DMBA and phenylalanine were with negative charge; histidine were neutral in the pH 7.4 media, respectively. Accordingly, a general rule for BDS effect on the CE analysis of various targets could be summarized: compared with the common buffer PBS, BDS used as supporting electrolytes in CE led to greatly enhanced ECL intensity for negative targets, a little increase for neutral-charge ones whereas no obvious change for that of those with positive net charge. In addition, the increase in ECL intensity had no proportional relationship to the polarity of the analytes.

To further confirm our conclusion, 100 μM Pro (pI = 6.3) was prepared in phosphate media of pHs 12.3, 6.3 and 2.1, respectively, and was used for sample analysis. In the three cases, Pro was negative, neutral and a little positively charged, respectively. Thus their ECL intensities should be affected in different degrees when PBS replaced by BDS was used as running buffers. Compared with PBS used as running buffers, when BDS was applied, corresponding increases of 105, 137 and 216% in ECL intensity were presented for Pro in pHs 2.1, 6.3 and 12.3, respectively (Fig. 2), indicating that the increase was directly dependent on the negative net charge of the analytes when the other conditions were kept the same.

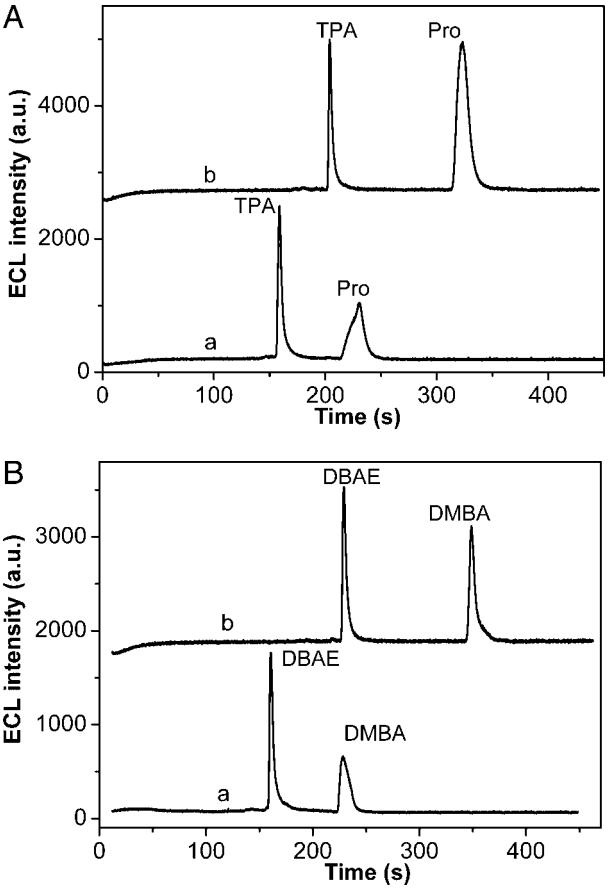

Figure 1. Comparison results of CE with ECL detection using (a) 10 mM PBS (pH 7.4) and (b) 10 mM BDS as running buffers. Mixtures of (A) 0.2 μM TPA and 50 μM Pro and (B) 2 μM DBAE and 10 μM DMBA. Conditions: detection potential, 1.2 V; 5 mM $Ru(bpy)_3^{2+}$ with 50 mM PBS (pH 7.4) in the detection reservoir; electrokinetic injection, 8 s at 12.5 kV; separation voltage, 15 kV.

The resolution between two targets was greatly improved by using BDS as running buffer, although the migration times were prolonged in a certain extent. As shown in Table 1, while using PBS as running buffer, the migration times of TPA and DBAE were 158 and 161 s, respectively. Thus their mixture could not be separated by PBS (see Fig. 3A). However, when equivalent concentration of BDS was applied, baseline separation was achieved (see Fig. 3B). It is again indicative of the enhanced resolution between two same charged species.

In addition, the column efficiency was evaluated in terms of plate number (N). It was calculated using the following equation: $N = 5.54\ (t_m/W_{1/2})^2$, in which t_m is the migration time of the analyte and $W_{1/2}$ is its half peak height width. Accordingly, it was found that the column efficiencies for TPA, DBAE, Pro and DMBA have been increased from 11963 to 23756, from 11080 to 21036, from

Table 1. Comparison results of various analytes with different polarities or charges using BDS and PBS as the running buffer in CE-ECL, respectively

Analytes	Polarity	Charge (in water)	Migration time (s)		ECL intensity (a.u.)	
			10 mM PBS	10 mM BDS	10 mM PBS	10 mM BDS
50 μM Pro	Nonpolar	Negative	229	322	852	2127
0.2 μM TPA	Nonpolar	Positive	158	203	2258	2168
2 μM DBAE	Polar	Positive	161	228	1679	1647
10 μM DMBA	Polar	Negative	227	348	593	1209
1 mM Arginine	Polar	Positive	210	236	280	260
1 mM Phenylalanine	Nonpolar	Negative	325	362	300	705
300 μM Histidine	Polar	Neutral	298	370	352	406

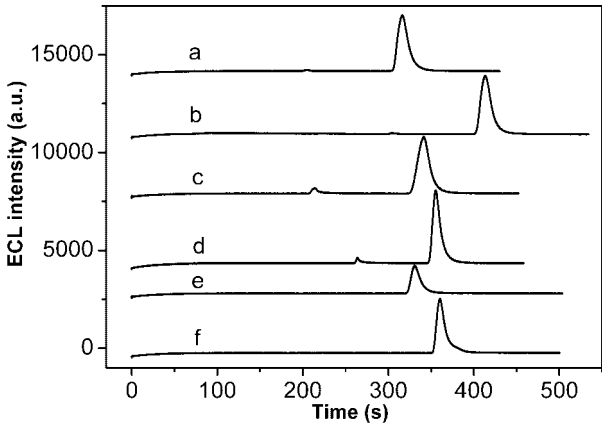

Figure 2. Electropherograms of 100 μM Pro dissolved in 10 mM PBS with different pH values of (a and b) pH 2.1, (c and d) pH 6.3 and (e and f) pH 12.3 using (a, c and e) 10 mM PBS and (b, d and f) 10 mM BDS as running buffers, respectively. Conditions are the same as in Fig. 1.

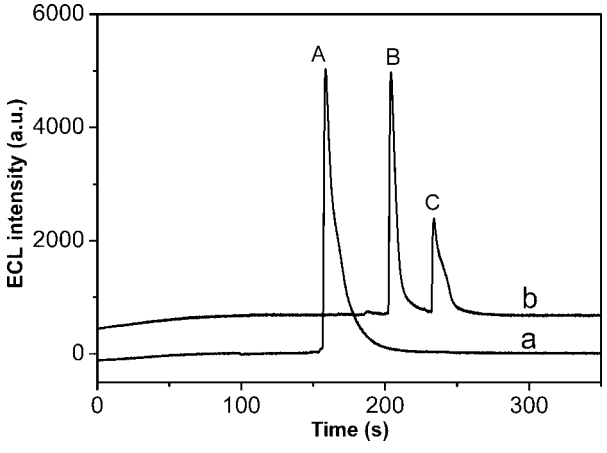

Figure 3. Electropherograms of mixtures of 2 μM TPA and 5 μM DBAE when (a) 10 mM PBS and (b) 10 mM BDS were used as running buffers, respectively. ECL intensities of (A) unseparated of TPA and DBAE, (B) TPA, (C) DBAE. Conditions are the same as in Fig. 1.

1617 to 4579 and from 2743 to 24811, respectively, by varying PBS to BDS as running buffers. It clearly showed that BDS solution used as running buffer rendered much higher column efficiencies, especially for Pro and DMBA. This is consistent with the more symmetrical and sharper peak shapes of Pro and DMBA than those of PBS, and maintained the well-shaped TPA and DBAE in Fig. 1.

All these above-mentioned comparisons illuminated the superiorities of BDS over PBS used as background electrolyte in CE applications:

As BDS comprises a cation and an anion, its solution belonged to one kind of electrolyte solution and could play the role of supporting electrolytes in CE applications. Furthermore, the large anion combining with the large cation in BDS solution completed the task of capillary surface modification together. On the one hand, due to the large anion dodecyl sulfate ($C_{12}H_{25}SO_4^-$), it possessed the properties of anionic surfactant; neutral or charged compounds incorporated into the micelles and interacted with micelles *via* hydrophobic mechanism depending on the different hydrophobicities of the solutes. On the other hand, the distribution of the solutes between micelles can also be influenced by the electrostatic forces between the large cation 1-butyl-3-methylimidazolium ($BMIm^+$) and the solutes. For negatively analytes, electrostatic attraction existed between negative ones and $BMIm^+$, leading them more convenient to solublize into the micelles. Thus, both the hydrophobic and electrostatic forces worked mutually favoring the sample solubility, narrowing the sample zone as well as facilitating the rapid end-column ECL reaction on the electrode surfaces. As a result, the greatly enhanced ECL intensities, higher column efficiencies and more symmetrical were achieved by using BDS solution as running buffers than those of PBS. Meanwhile, the hydrophobic interaction was attenuated by the electrostatic repulsion existed between positive ones and $BMIm^+$, which makes them more difficult to approach the micelles. Therefore, the influences of BDS on the ECL intensities and column efficiencies of positive analytes lay on the balance between the two interaction mechanisms. Thus the properties of the positive solutes such as TPA and DBAE were maintained little changed compared with those using PBS as buffers. As

no electrostatic force occurred for neutral ones, thus only a little enhanced ECL intensity was observed for them such as histidine.

As one functionalized IL, BDS should has the general properties of ILs such as high viscosity [16]; thus its aqueous solution is more viscous than equivalent concentration of PBS. Subsequently, a little longer migration times were observed for the same analytes. Usually, appropriate prolonged migration time can lead to improved resolution between analytes. Combining with the above-mentioned differences in the distribution of charged and hydrophobic sites of the solutes in BDS modified capillary surface, separation efficiencies can be greatly increased by using BDS solution as running buffers. Coincidently, these effects of pure BDS solution on CE analysis nicely met one of the requirements for establishment of the MEKC separation mode [1–3].

3.2 Effects of BDS solution used as an additive

When 10 mM pure BDS solution was applied as the running buffer, both the ECL intensities and migration times of the analytes had excellent reproducibility. It indicated that no negative effects of the IL surfactant existed for the electrodes. To further confirm our proposal, the effects of BDS used as an additive in supporting electrolytes on the end-column ECL process was investigated, taking the common anionic surfactant contrast SDS as the contrast. A sample of 0.1 μM TPA was injected consecutively three times to determine the repeatability of ECL intensity based on peak height and migration time. When 10 mM PBS containing 10 mM SDS was applied, in the first cycle, high ECL intensity (426 a.u.) and sharp ECL peak was presented (Fig. 4A-a). However, in the subsequent cycle, the ECL intensity dropped significantly (152 a.u.) (Fig. 4A-b). Not to mention in the third cycle, the ECL nearly disappeared due to the high background noise. This result clearly indicated the obvious poisonous effect of SDS on the electrode. During the electrophoresis process, the buffer flow of effluent from the capillary spurted at the electrode. The surfactant SDS would adsorb at the electrode surface [34] and could not be desorbed by the chemical reaction itself. Moreover, the adsorbed SDS was with low conductivity due to its long chain alkyl group, and thus the electron transfer at the electrode surface was blocked cycle by cycle, finally resulting in disappeared ECL response. Meanwhile, when the solution of functionalized IL BDS was used as additives, due to the previously reported weak binding between the IL moiety, $BMIm^+$, and $Ru(bpy)_3^{2+}$[35], the existence of $BMIm^+$ would accelerate the electron transfer at the electrode surface; thus the fouling effect of long chain $C_{12}H_{25}SO_4^-$ to the electrode surface was eliminated. As a result, upon addition of BDS in the running buffer, sharp, symmetrical and reproducible peaks (relative standard derivation = 1.02%, $n = 6$) as well as higher ECL intensities were obtained.

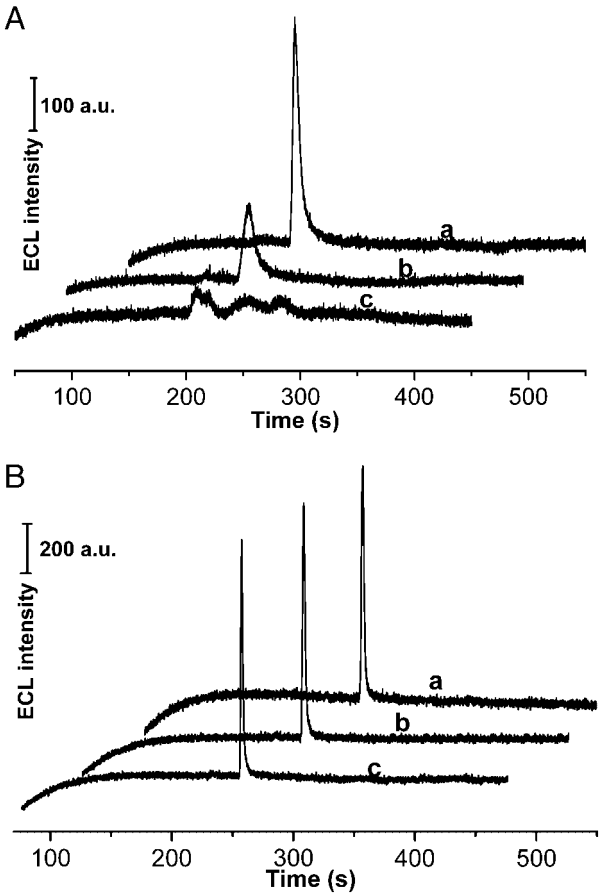

Figure 4. Different effects of (A) 10 mM SDS or (B) 10 mM BDS used as additives in 10 mM PBS running buffer on the repeatability of ECL intensity of TPA by CE with ECL detection. (a–c) Electropherograms of 0.1 μM TPA, which was injected consecutively three times, respectively. Conditions are the same as in Fig. 1.

3.3 Comparison between BDS solution and the hybrid system of IL and surfactant

Using IL 1-butyl-3-methylimidazolium tetrafluoroborate as the supporting electrolyte, SDS as an additive, the hybrid system was previously proposed as the buffer of MEKC-ECL to achieve narcotic drugs on a microchip [36]. Thus contrast experiment between 10 mM BDS solution and the hybrid system 10 mM (IL+SDS) used as running buffers for MEKC-ECL system was conducted. Take mixtures of 0.2 μM TPA and 50 μM Pro as an example, for the hybrid system, as shown in Supporting Information Fig. 2, well-separated and reproducible peaks can also be obtained as those of the BDS solution. However, the hybrid system resulted in longer migration times, broader peaks and lower ECL intensities of both TPA and Pro, especially for Pro. For the hybrid system, it contained not only the ions of $BMIm^+$ and $C_{12}H_{25}SO_4^-$ that guaranteed the separation efficiencies and reproducible peaks but also chloride and sodium ions. BDS was synthesized according to the ion exchange reaction between

the IL 1-butyl-3-methylimidazolium chloride and SDS. Both chloride and sodium ions were removed from the final product BDS; thus compared with that of equivalent concentrations of BDS, the ionic strength of the hybrid system was twice as much as that of BDS, and then it would lead to longer migration times as well as diffusion effects in the capillary channel. Correspondingly, broader and lower ECL peaks were obtained. This contrast experiment showed the superiority of BDS solution over the hybrid system as running buffers and the advantages to synthesize the functionalized IL for MEKC-ECL establishment. In other words, using BDS as running buffers, not only stable pseudo-stationary phase was formed in the capillary and the MEKC separation mode was established, but also the activity of the electrode surface could be assured. Either as supporting electrolytes or as additives, MEKC combined with ECL detection could be successfully constructed.

3.4 Applications

Pro and hydroxyproline are rich in collagen; their changes in body fluid are related to many diseases such as cancer [37], bone disease [38], *etc*. Thus simultaneous determination of them has great value in clinical applications. However, the only difference in molecular structure of them is the presence of an additional hydroxyl in hydroxyproline. Separation of them using CE-ECL detection was only reported by Xue *et al*. [39], in which acetonitrile was used as additives and prolonged migration times and greatly decreased ECL intensities could not be avoided. Herein, Pro and hydroxyproline were chosen as analytes to verify the enhanced separation capability of the established MEKC-ECL method. While testing various concentrations of SDS contained in 10 mM PBS, the mixtures of Pro and hydroxyproline could not be separated at all (Fig. 5a). Fortunately, while using the various concentrations of BDS included in 10 mM PBS, signs of separated Pro and hydroxyproline peaks were presented. When 30 mM BDS as additives in 10 mM PBS was optimized, successfully baseline resolution between Pro and hydroxyproline was obtained (Fig. 5b). Confirmation of the analytes in the mixture samples was operated by comparing the electropherograms among samples with different concentrations, where the increase in peak area at equivalent migration time was directly proportional to the amount spiked with Pro and hydroxyproline. Compared with pure 10 mM PBS with or without 30 mM SDS used as running buffers, although the migration times were a little prolonged, symmetrical and reproducible peaks as well as enhanced ECL intensities were insured upon addition of 30 mM BDS in 10 mM PBS. To evaluate the linearity of the established method, standard curves were prepared by analyzing different concentrations of mixture of Pro and hydroxyproline both ranging from 10 nM to 1 mM. The standard curves were linear in the range of 3.00×10^{-6}–8.00×10^{-3} M for Pro and 3.0×10^{-6}–1.0×10^{-3} M for hydroxyproline, respectively.

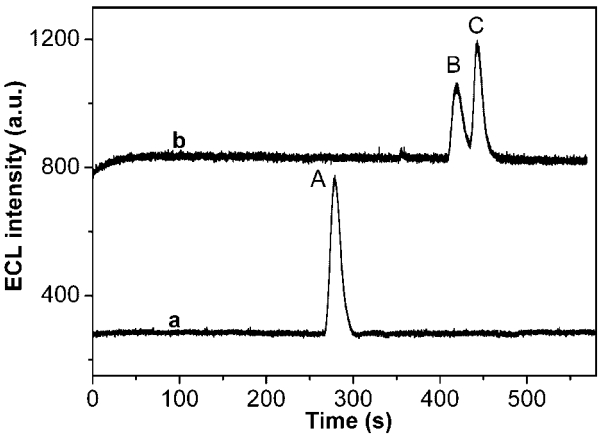

Figure 5. Electropherograms of mixtures of 10 μM Pro and 10 μM hydroxyproline when (a) 10 mM PBS (pH 8.5) containing 30 mM SDS and (b) 10 mM PBS (pH 8.5) containing 30 mM BDS was used as running buffers, respectively. ECL intensities of (A) unseparated of Pro and hydroxyproline, (B) Pro, (C) hydroxyproline. Conditions are the same as in Fig. 1.

The calibration equations and regression coefficients were $Y = 34.04 + 5.66X$ ($R = 0.997$; $n = 11$) for Pro and $Y = 100.11 + 6.38X$ ($R = 0.998$; $n = 12$) for hydroxyproline, respectively. Detection limits of 0.5 μM for Pro and 0.8 μM for hydroxyproline were achieved ($S/N = 3$), respectively. Compared with the previously reported detection limits of 2 μM for Pro and 4 μM for hydroxyproline [37], the improved separation efficiency, higher sensitivity and lower detection limits of Pro and hydroxyproline again confirmed the superiority of BDS used as buffer additive over other common additives in the establishment of MEKC-ECL method.

4 Concluding remarks

In the experiment, one functionalized IL, BDS, which belongs to a type of surfactant IL, was designed according to the requirements of MEKC-ECL. Its superiorities used as either supporting electrolytes or additives for successful establishment of MEKC-ECL method were investigated. Different from the common supporting electrolytes such as PBS, BDS solution used as running buffers led to greatly enhanced ECL intensities and column efficiencies for negative targets, a little increase for neutral-charge ones whereas no obvious change for that of those with positive net charge. Moreover, resolution efficiency between analytes was significantly improved. As the existence of IL moiety, $BMIm^+$ accelerated the electron transfer at the electrode surface, the fouling effect of long chain $C_{12}H_{25}SO_4^-$ to the electrode surface was eliminated and reproducible ECL intensities with relative standard derivation of 1.02% ($n = 6$) were obtained. The proposed novel buffer system in MEKC-ECL application was again validated by the successfully separated two similar amino acids of Pro and hydroxyproline. That is to say, the large anion combining with the large

cation in BDS solution completed the task of MEKC-ECL construction together. Not only stable pseudo-stationary phase was formed in the capillary and the MEKC separation mode was established, but also the activity of the electrode surface for ECL reaction could be assured. These findings will surely exploit novel scopes for MEKC-ECL and functionalized ILs applications in the future.

This work is supported by the National Natural Science Foundation of China with the grant nos 20675078 and 20735003, "863" project no. 2006AA020701 and "973" project no. 2007CB714500.

The authors have declared no conflict of interest.

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