

Nonaqueous capillary electrophoresis using a titania-coated capillary

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Abstract

In this work, an ordered mesoporous titania film was introduced to coat a capillary by means of the sol–gel technique. Its electroosmotic flow (EOF) property was investigated in a variety of nonaqueous media (methanol, formamide and *N,N'*-dimethylformamide and mixtures of methanol and acetonitrile). The titania-coated capillary exhibited a distinctive EOF behavior, the direction and magnitude of which were strongly dependent on various parameters such as the solvent composition, apparent pH (pH*) and the electrolytes. The nonaqueous capillary electrophoresis separation of several alkaloids was investigated in the positively charged titania-coated capillary. Comparison of separation between coated and uncoated capillaries under optimal nonaqueous conditions was also carried out.

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1. Introduction

The use of titania as a sorbent in normal-phase, reversed-phase and ion-exchange liquid chromatography has been reported [1–4]. It possesses some advantages that other sorbents cannot provide, such as the desirable mechanical and physical properties of silica and a chemical stability superior to those of silica [5,6]. Furthermore, titania is virtually insoluble in acids and bases, which enables separations to be performed at extreme pH values. Moreover, it is an amphoteric metal oxide, which has anion-exchange properties at acidic pH and cation-exchange properties at alkaline pH [7].

Though its applications in HPLC have been widely investigated, little attention has been paid to the applicability of titania in CE and capillary electrochromatography (CEC). Tsai et al. [8] produced a titania coating in a capillary by the sol–gel method for the CE separation of proteins. Fujimoto [9] focused on the switchable electroosmotic flow (EOF) character and CEC separation of proteins in aqueous media.

Compared to nonaqueous capillary electrophoresis, NACE has a variety of merits, such as reducing Joule heating effect and wall adsorption effect, and improving selectivity.

In addition, it is especially beneficial for the separation of hydrophobic compounds that cannot be dissolved in aqueous background electrolytes (BGEs). Therefore, it has attracted considerable attention recently [10]. In order to suppress analyte-wall interaction or to control the EOF, polymer coatings are most commonly used in NACE [11–21].

Due to the amphoteric character, a titania-coated capillary generates the switchable EOF in aqueous media [8,9] and it is predicted to show different behaviors of the EOF in varieties of organic media. In this work, to expand the application of inorganic oxide-coated capillary electrophoresis and obtain an efficient method to control the EOF in nonaqueous media, we introduced a titania-coated capillary into NACE and concentrated on its EOF behavior in nonaqueous media. In order to improve the surface area and pore structure of titania, an ordered mesoporous titania film was for the first time used to coat the capillary. Separation of some alkaloids with the titania-coated capillary in NACE was achieved.

2. Experimental

2.1. Reagents and material

Fused-silica capillaries with 50 μm i.d. \times 365 μm o.d. were obtained from Hebei Yongnian Optical Fiber Factory (Hebei, China). Titanium chloride, tris(hydroxymethyl)

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aminomethane (Tris), ammonium acetate (AcONH_4), methanesulphonic acid (CH_3SO_3), methanol (MeOH), ethanol, acetonitrile (ACN), thiourea, formamide (FA) and *N,N'*-dimethylformamide (DMF) were purchased from Shanghai General Chemical Reagent Factory (Shanghai, China). Jateorrhizine, pilocarpine, berberine and atropine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (NICPBP, Beijing, China). Poly(alkylene oxide) block polymer (abbreviated as $\text{EO}_{106}\text{PO}_{70}\text{EO}_{106}$, F127) was a gift from BASF (USA). Distilled water was from a quartz apparatus.

2.2. Instrumentation

CE experiments were carried out at room temperature with NT1229 HPCE from Beijing Institute of New Technology Application (Beijing, China), which consisted of a ± 30 kV high voltage power supply and a UV detector. Data collection and manipulation were carried out on TL9900 chromatographic workstation from Teleh Electronics Tech Co. (Beijing, China). The effective length of the uncoated capillary was 40.2 cm and the total length 55.6 cm. The effective length of the coated capillary was 35.8 cm and the total length 51.1 cm. Thiourea was used as the EOF marker. Apparent pH values (pH^*) of the BGE solutions were measured with a Delta 320-S pH Meter from Mettler Toledo Instruments (Shanghai, China).

To perform the Brunauer–Emmett–Teller (BET) and transmission electron microscopy (TEM) measurements, the titania sol was dropped onto the glass surface. The film thus formed experienced the same disposal as the titania-coated capillary and was peeled for the following measurements. The TEM experiment was performed on a JEOL JEM-2010 electron microscope (200 K) using copper grids. The Nitrogen sorption experiment was carried out at 77 K using a Coulter (FL, USA) SA 3100 Plus surface area and pore size analyzer. The sample was activated by evacuating in vacuum and heating to 473 K for 10 h to remove any physically adsorbed materials before the analysis. The specific surface area value was calculated according to the BET method at P/P_0 between 0.05 and 0.2. The pore parameters (pore volume and pore diameter) were evaluated from the desorption branches of isotherms based on Barrett–Joyner–Halenda (BJH) model.

2.3. Preparation of a titania-coated capillary

The fused-silica capillary was activated at ambient temperature by rinsing sequentially with 1 mol l^{-1} sodium hydroxide for 2 h, water for 30 min, 1 mol l^{-1} hydrochloric acid for 2 h and water for 30 min. After being connected to the nitrogen regulator, the capillary was placed in a GC oven at 433 K and purged with nitrogen for 4 h.

Titania sol was used to accomplish the coating process, and it was prepared according to previous work [22]. Briefly, 1.0 g F127 was dissolved in 10 g ethanol. To the solution,

0.01 mol titanium chloride was added with vigorous stirring for 30 min. The resulting sol solution was aged in an open flask at 40°C in air for 5 days. The titania sol thus formed passed through the fused-silica capillary at a flow rate of $1 \mu\text{l min}^{-1}$ for 1 h. Afterwards, the solvent was removed by passing nitrogen through the capillary under 1 bar for 10 min and then under 0.2 bar for 60 min. Subsequently, the above coating process was repeated. Finally, the titania-coated capillary was calcined by heating at a rate of 1°C/min to 400°C and holding for 8 h to remove the block copolymer species and increase cross-linking of the inorganic framework, leading to the formation of titania coating.

3. Results and discussion

3.1. Modification and characterization of the titania-coated capillary

In the present work, we employed a method of producing ordered mesoporous film by the sol–gel method [22] to coat the capillary. The sol–gel process provides an alternative way to modify the capillary and may be helpful for the formation of a well-distributed film of appropriate thickness with a larger surface area and ordered mesopores on the capillary wall. Therefore, it is beneficial to show titania's properties, to most great extent, in NACE. On the other hand, the ordered mesoporous titania film with large surface area might be helpful for CEC due to the increase in the stationary phase surface area and phase ratio. The CEC study of the titania-coated capillary will be reported elsewhere.

According to the result of BET measurements, the surface area of the employed film is found to be $236.8 \text{ m}^2/\text{g}$, while it is only $25\text{--}36 \text{ m}^2/\text{g}$ [23] for conventional titania sorbent. From the curve of nitrogen adsorption and desorption

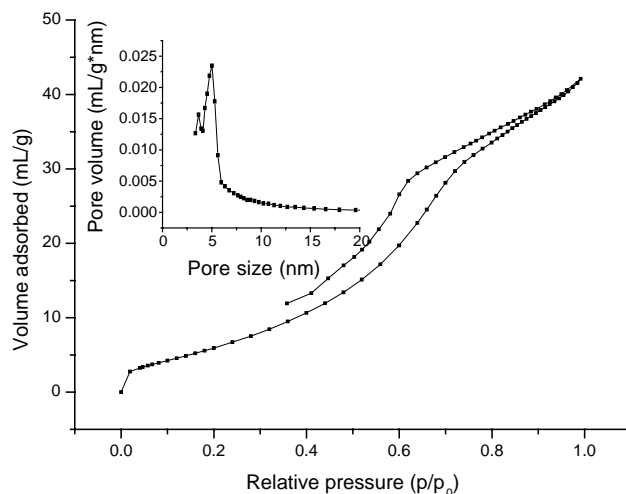


Fig. 1. Nitrogen adsorption–desorption isotherm for the titania coating. Inset: the pore size distribution curve from the desorption branch of the titania coating.

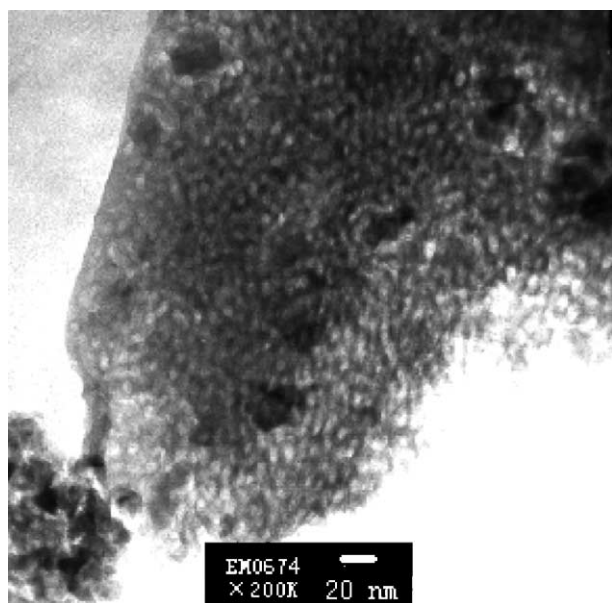


Fig. 2. Transmission electron micrograph of the titania coating.

isotherm in Fig. 1, it can be found that the pores of the titania are mainly distributed in the mesoporous range. The isotherm is of type IV of the IUPAC classification with a H2 hysteresis loop that is characteristic for capillary condensation in the mesopore structures. The initial part of the isotherms is attributed to monolayer–multilayer adsorption. Distinct limiting adsorption at high relative pressures indicates the absence of macropores. The inset shows the pore size distribution curve of the titania. Obviously, the pore size distribution (PSD) for titania is narrow. The mean pore size and pore volume for titania are found to be 5.02 nm and 0.0677 ml g⁻¹, respectively.

Table 1
Physicochemical data of the solvents applied [25]

Solvent	Polarity	η (cP)	ϵ	pK_{auto}	Solvent type
Water	10.2	0.89	80	14.0	Amphiprotic
FA	9.6	3.3	111	16.8	Basic amphiprotic
DMF	6.4	0.8	36.7	36.7	Basic amphiprotic
CAN	5.8	0.34	37.5	–	Aprotic
MeOH	5.1	0.54	32.7	17.2	Amphiprotic

Polarity index, viscosity η , relative permittivity ϵ , autoprotolysis exponent pK_{auto} .

From the large surface area, one obvious character of the ordered mesoporous materials, the coating should possess the mesoscopic order, which was confirmed by TEM image, as shown in Fig. 2.

3.2. The EOF

3.2.1. The EOF in simple organic systems

One advantage of using organic media is that the direction and magnitude of the EOF can be controlled by only employing varieties of solvents with different physical and chemical characteristics [10,24]. The solvents used in this study were chosen in order to cover as many groups as practically possible. ACN, MeOH, DMF and FA are the commonly used solvents in NACE. The properties of these solvents and water are listed in Table 1 [25]. Because of the limited solubility of the electrolytes, pure ACN was not employed in the study. In order to obtain a lower pH*, methanesulphonic acid, instead of the conventional acetic acid, was chosen to adjust the BGE pH*. The highest pH* in different solvent systems was obtained by dissolving AcONH₄ in the solvent directly and fixing the concentration at 25 mM in the absence of methanesulphonic acid. To get the BGE at

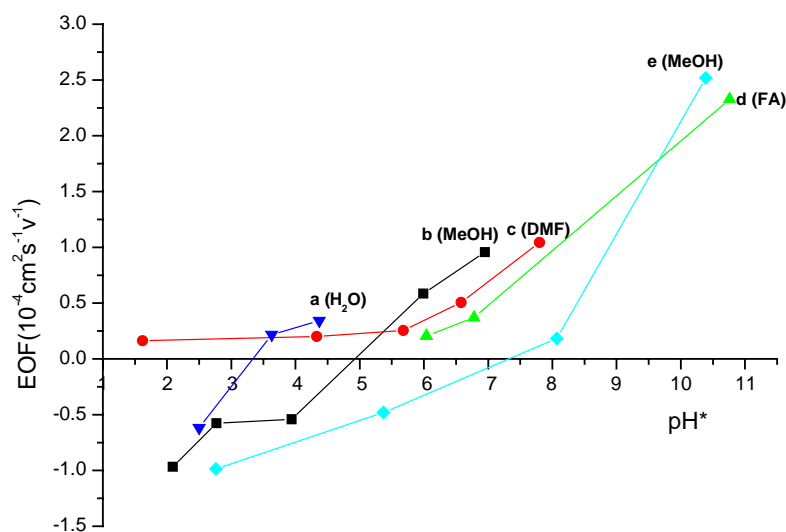


Fig. 3. Comparison of EOF profiles obtained on the titania-coated capillary in different simple organic solvents and electrolytes. Solvents: (a) H₂O; (b) MeOH; (c) DMF; (d) FA; electrolyte: 25 mM AcONH₄ + CH₄SO₃, applied voltage: 25 kV. Solvent: (b, e) MeOH; electrolyte: 25 mM Tris + CH₄SO₃, applied voltage: 25 kV.

a lower pH^* , a different content of methanesulphonic acid was added to the above AcONH_4 solution for control of pH^* value.

Fig. 3a–d shows the pH^* –EOF curves in different simple organic systems with AcONH_4 electrolyte. It can be found that the capillary exhibited a switchable EOF in the methanolic BGE (Fig. 3b) like in an aqueous one (Fig. 3a) [9]. The result indicates that the surface hydroxyl groups of titania in methanolic media could also deprotonate or protonate in solutions of different pH (pH^*). At pH (pH^*) above the isoelectric point (pI), the Brønsted acid character of surface hydroxyl groups enabled them to deprotonate and the degree of deprotonation increased with the increasing of pH (pH^*), which was responsible for the increase in EOF with increasing pH (pH^*); At pH (pH^*) below pI the Brønsted base sites made the hydroxyl groups protonate and the degree of protonation increased with the decreasing pH (pH^*), which was responsible for the increase in EOF with decreasing pH (pH^*).

However, only a cathodic flow was observed in DMF (Fig. 3c) and FA (Fig. 3d) systems. The disappearance of the anodic flow in DMF and FA systems may be the result from two factors: the adsorption of AcO^- and the restraint of titania protonation. As shown in previous papers [4,26,27], due to the Lewis acid–base interaction, AcO^- may be adsorbed onto the titania to some extent, which would produce an additional negative charge on the surface. This adsorption may be increased in the more polar organic solvent [11]. Similar behavior was also observed in DMF and FA systems. DMF and FA are two more polar solvents than MeOH, which facilitates the adsorption of AcO^- onto the capillary wall. If the adsorption exceeded a certain amount at low pH^* the anodic flow disappeared. When pH^* was lower than 6.0 in the FA system, the signal could not be observed in 90 min. It may result from the much more adsorbed AcO^- because it is the most polar one among the selected solvents. On the other hand, the restraint of titania protonation in DMF and FA may also contribute to the result. Because DMF and FA are both basic amphiprotic solvents, the protonation of DMF and FA may be stronger than that of titania. Therefore, only the cathodic flow was observed in DMF and FA systems.

The fastest cathodic flow was obtained in a methanolic BGE, while the lowest was obtained in the FA system as indicated in Fig. 3b–d (e.g. pH^* 6.5). This result implies that the magnitude of the EOF is dependent on the nonaqueous solvents used. As shown in Table 1, the ratio of dielectric constant to the viscosity was reduced from MeOH to DMF and then to FA, which was in agreement with the change of the magnitude of the cathodic flow. Although the adsorption of AcO^- onto the capillary wall may be increased in DMF and FA as discussed above, which produced an additional negative charge which increased the cathodic EOF, the effect of ratio of dielectric constant to viscosity on the EOF is expected to be dominant in the case. Thus, the fastest flow was observed in the MeOH system, while the lowest was in the FA system.

3.2.2. Effect of electrolyte on the EOF

Tris is another commonly used electrolyte in NACE. The EOF behavior in the Tris system may be different from that in the AcONH_4 system, because of the weak or nonadsorption interaction with the surface. As shown in Fig. 3b and e, the EOFs in methanolic media with Tris and AcONH_4 electrolytes were both switchable. The cathodic flow in the AcONH_4 system was faster than that in the Tris system from the trend of the curve (e.g. pH^* 7.5), while it was the opposite as for the anodic flow (e.g. pH^* 4). However, the pI value obtained in MeOH– AcONH_4 was lower than that in MeOH–Tris. These results were in accordance to those in aqueous systems [28–30]. They can be mainly attributed to the adsorption of AcO^- to the surface of titania by Lewis acid–base interaction.

At pH^* above pI , deprotonation of titania resulted in net negative charges on the surface of capillary. When adsorbed onto the wall, AcO^- increased negative charge on the surface, leading to the increase of the cathodic flow. At pH^* below pI , protonation of titania resulted in the net positive charges on the surface of capillary. Adsorption of AcO^- on the surface would decrease the positive charge, leading to the decreased anodic flow. As a result, pI values shifted to lower pH^* .

3.2.3. The EOF in binary systems

A MeOH–ACN mixture may show different characteristics from both MeOH and ACN. Here, we focus our study to MeOH–ACN binary solvents both in Tris and AcONH_4 electrolytes.

Fig. 4a shows the effect of pH^* on the EOF behavior in Tris systems containing 25 and 50% ACN, respectively. Whether the anodic flow or the cathodic flow in the case of 25% ACN was faster than that in the case of 50% ACN (e.g. pH^* 4, pH^* 10). But as for AcONH_4 systems, as shown in Fig. 4b, the cathodic flow in 50% content of ACN was faster (e.g. pH^* 6.5) while the anodic flow in 25% ACN (e.g. pH^* 2.2) was faster. These may be explained from three factors: (i) viscosity decreased as the content of ACN increased. However, the dielectric constant of the binary system was almost constant [31] because of the close dielectric constant values of MeOH and ACN, so the ratio of the dielectric constant to viscosity increased. According to the Smoluchowski equation, it may result in an increased EOF (positive effect); (ii) variation of the ACN proportion induced a change in the surface charge. The degree of deprotonation or protonation of surface hydroxyl groups on titania-coated capillary was further suppressed with the increasing proportion of ACN [17]. So it led to a decrease in the ζ potential as well as in the electroosmotic mobility. It was an important reason for the decreased EOF (negative effect); (iii) the adsorption of electrolyte always played an important role in affecting the EOF. The titania-coated capillary exhibited a much different EOF behavior in the same organic solvent with different electrolytes (AcONH_4 and Tris), because the adsorption of Tris was very small, while the adsorption of AcO^- was rel-

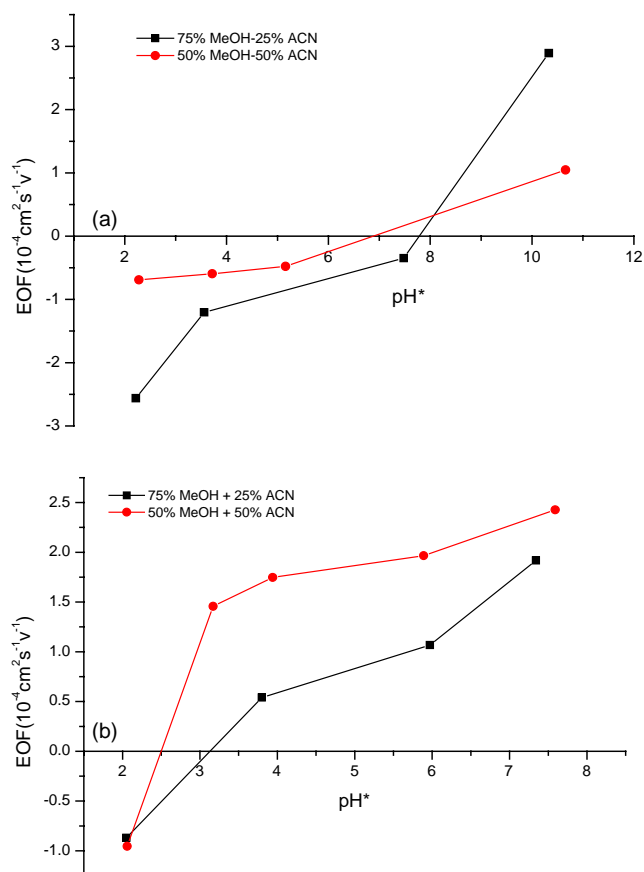


Fig. 4. Comparison of EOF profiles obtained on the titania-coated capillary in binary systems with (a) Tris system and (b) AcONH₄ system. Applied voltage: 25 kV.

atively greater. The adsorption of AcO⁻ would result in the increased negative charge on the titania surface, leading to a positive effect on the cathodic flow and a negative effect on the anodic flow.

All the observed distinctive phenomena are related to the complication of nonaqueous systems, in which many factors affect the EOF, respectively, or mutually. When the positive effects are larger than the negative ones, the faster EOF is produced and vice versa. It is not easy to determine which one of the factors would be dominant under different conditions. So it is hard to predict the EOF behavior under a given set of conditions.

3.3. Application: separation of alkaloids

To our knowledge, titania adsorbent exhibited some advantages when applied to separate basic compounds. Four alkaloids were used to investigate the effectiveness of the positively charged titania-coated capillary with the anodic flow in nonaqueous media in counter-electroosmosis mode. When alkaloids are analyzed, they are protonated and pos-

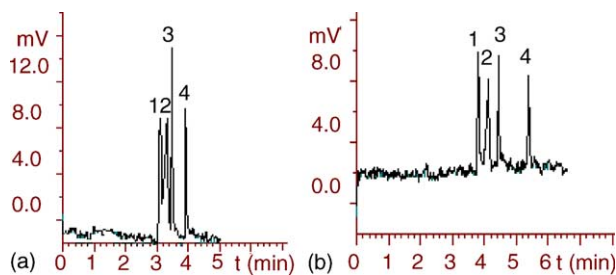


Fig. 5. Separation of alkaloids on uncoated (a) and titania-coated (b) capillaries. Buffer: MeOH-ACN (50:50, v/v), 25 mM Tris + CH₄SO₃, pH* 2.28. Applied voltage: 25 kV. UV detection at 214 nm. Samples: (1) berberine; (2) jateorrhizine; (3) pilocarpine; (4) atropine.

itively charged. The direction of the analytes' migration and the EOF are opposite. In this mode analytes are able to enter the capillary and migrate to the detector, as long as the electrophoretic mobilities of analytes are larger than the electroosmosis mobilities.

Fig. 5 showed the separation electropherograms of four alkaloids on the uncoated (a) and the titania-coated (b) capillaries under optimal conditions. On the two capillaries, berberine, jateorrhizine, pilocarpine and atropine migrated out in order. Compared to the separation on the uncoated capillary under the same condition, all analytes on the titania-coated capillary were baseline separated and tailing of analytes was almost eliminated, but migration time was longer. Obviously, the titania-coated capillary with the reversed EOF has the potential to enlarge the peak capacity for positively charged analytes. Furthermore, the existence of a positively charged wall eliminates tailing of identical charged analytes. However, the decreased mobilities on the coated capillaries give rise to longer diffusion times, which might partly contribute to band broadening.

3.4. Reproducibility

The reproducibility of the capillary preparation was investigated. Determination of EOF is an effective method for this evaluation. Capillaries from column-to-column and batch-to-batch were underwent EOF measurement using 25 mM AcONH₄ of pH 2.76 as buffer solution. The variation of the EOF mobility altered between 5.6×10^{-5} and $6.5 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ with a 7.8% R.S.D. ($n = 3$). It may be due to the difficulty of the sol-gel preparation, uneasy control of the coating process, instability of the nonaqueous system and the CE equipment. However, in consideration of the tiny structure of the capillary, it is reasonable to accept the result and the anodic flow is observed in any case.

4. Conclusion

The inner surface of a fused-silica capillary has been modified using a sol-gel method to give an ordered mesoporous titania-coated surface. The electroosmotic properties of the

titania-modified capillary in nonaqueous media have been studied under various conditions such as different type of the electrolytes, pH* and solvent composition. The direction and magnitude of the EOF can be easily controlled by adjusting all these operating parameters in nonaqueous media. The potential use of nonaqueous inorganic oxide-coated capillary electrophoresis for the analysis of alkaloids has been demonstrated. The example clearly demonstrates that an appropriate coating can enable separations which would not be feasible in an unmodified capillary under the same conditions. It is likely that such a switchable EOF can facilitate the separation of analytes of widely different mobilities using a single capillary. The use of nonaqueous systems clearly enhances the scope of inorganic oxide-coated capillary electrophoresis methods but there remains much to understand before the method can be used routinely.

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