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Short communication

Capillary electrophoretic separation enhanced by a macrocyclic dioxopolyamine additive

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Abstract

Macrocyclic polyamines, or in particular dioxopolyamines, are strong receptors for analytes such as metal ions, catechol and catecholamine derivatives. In this paper, based on their interaction with a dioxopolyamine compound: 1,4,7,10-tetraazacyclotridecane-11,13-dione (or dioxo[13]aneN$_4$), we report its use as an additive in capillary electrophoresis (CE) to improve the separation resolution and selectivity of various model analytes. Using an imidazole–acetic acid electrolyte with dioxo[13]aneN$_4$ as the only additive, alkali metal, alkaline earth metal and NH_4^+ ions can be effectively separated and detected by using CE with indirect UV detection, as opposed to the use of crown ether in which another additive, such as α-hydroxyisobutyric acid, is needed. The host–guest interaction between dioxo[13]aneN$_4$ and metal and NH_4^+ ions can modify their electrophoretic mobilities, and therefore can be used to differentiate the various cations, especially between K^+ and NH_4^+, and between Sr^{2+} and Ca^{2+}. We have also found that dioxo[13]aneN$_4$ is an effective additive in CE to resolve nitrophenols and, in particular, dihydroxybenzenes. Moreover, unlike previous reports, the separation of various biogenic monoamine neurotransmitters can be achieved at neutral or physiological pH. This work demonstrates that one of the macrocyclic dioxopolyamine derivatives: dioxo[13]aneN$_4$ is a promising additive in CE separations for any chemical species: cationic, anionic and neutral.

Keywords: Buffer composition; Inorganic cations; Tetraazacyclotridecanedione; Nitrophenols; Dihydroxy benzenes; Catecholamines

1. Introduction

In capillary electrophoresis (CE), capillary zone electrophoresis (CZE) is the most widely used operation mode due to its simplicity. The separation capillary is usually filled only with a simple electrolyte buffer (with no additives) that is used for separation of some inorganic ions or small organic molecules. However, since most electrolytes used in CZE do not have strong interaction with analytes, they cannot be used to effectively resolve the analytes that have similar electrophoretic mobilities. Hence, buffer additives are often employed to improve the separation resolution and selectivity. Surfactants are well-known additives in CE, and if the surfactant concentration is above the critical micelle concentration (CMC), micelles will be formed in the electrolyte, resulting in a new operation mode named micellar electrokinetic capillary chromatography

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(MECC) [1]. Other additives such as cyclodextrins, which do not form micellar structures, have also been utilized as the so-called pseudo-stationary phase in CE, resulting in the general electrokinetic capillary chromatography (ECC) operation mode [2]. Other ECC modes involve the use of synthetic macrocyclic compounds, such as crown ether [3], an CE additives which have aroused much interest recently. Their specific recognition to the analytes of interest is helpful to enhance separation resolution. Other macrocyclic compounds used as additives in CE include calixarenes [4] and macrocyclic antibiotics [5].

Macrocyclic polyamines, or in particular, dioxopolyamines, which have similar structures to crown ethers, are suitable models to perform studies of host–guest interaction. Dioxopolyamines, like crown ethers, are well-known host compounds for the recognition of metal ions [6]. CE with indirect UV detection has been commonly used for the separation of metal and ammonium ions. A highly UV-absorbing compound will be used as the background electrolyte and it is monitored at a wavelength of its absorbance maximum. When the zones of cations pass the detector region, they displace the positively charged UV absorbing species, which results in a decrease in absorbance. Many papers have reported CE with indirect UV detection of alkaline, alkaline earth, and NH_4^+ ions [7–15]. Imidazole is popularly used for CE with indirect UV detection of these inorganic cations because it has high UV absorbance and has an electrophoretic mobility close to that of the cations. In addition, organic acids, such as α-hydroxyisobutyric acid (HIBA), are often used as complexing agents to resolve alkaline and alkaline earth metal ions. However, these organic acids cannot effectively differentiate K^+ and NH_4^+ because these two ions still have very similar mobilities. Hence, another electrolyte additive such as crown ether is needed to improve the resolution of these two ions. In this work, it is shown that only the addition of dioxo[13]aneN_4 alone, without the need of organic acids, can provide the necessary resolution. As a result, the running buffer is simpler to prepare and use, which is essential for a constant and high background signal level employed in indirect UV detection.

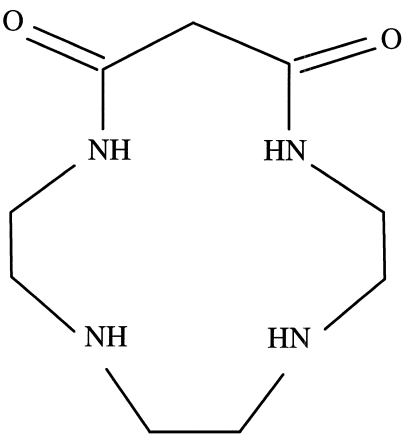

Fig. 1. Structure of 1,4,7,10-tetraazacyclotridecane-11,13-dione (or dioxo[13]aneN_4).

At neutral pH, macrocyclic polyamines have been demonstrated to be strong receptors with catechol or with the catecholamines, and they can form 1:1 complexes with these neurotransmitters [16]. In addition, these macrocyclic compounds have been reported to be selective receptors of biologically significant polyanions, and they probably associate with the amine and amide hydrogen atoms packed in the macrocyclic cavities via ionic hydrogen bonds, yielding highly stable and selective 1:1 anion complexes [17]. Based on their strong interaction with anions, polyamines have been utilized to covalently modify the fused-silica capillary for enhancement of the CZE separation of inorganic and organic anions [18]. Therefore, macrocyclic polyamines are versatile host molecules that can interact with positive, neutral and negative guest molecules

1,4,7,10-Tetraazacyclotridecane-11,13-dione (or dioxo[13]aneN_4) is a typical polyamine compound with good solubility in aqueous solutions. Its structure is shown in Fig. 1. It was anticipated that the two carbonyl functions in this dioxopolyamine compound might serve to increase selectivity toward cation separation [19]. This paper shows for the first time the use of a macrocyclic polyamine compound as a CE additive to improve separation resolution of metal and ammonium ions, catechol and related phenols, and biogenic monoamine neurotransmitters.

2. Experimental

2.1. Apparatus

All CE experiments were performed using the P/ACE 5000 system (Beckman, Fullerton, CA, USA) equipped with a UV absorbance detector. Separations were accomplished at room temperature under positive voltage. Uncoated fused-silica capillaries (Supelco, Bellefonte, CA, USA) with an inner diameter of 75 μm were used. The total capillary length was 47 cm, while the effective separation length was 40 cm. Indirect UV detection was carried out at 214 nm for cations, and direct UV detection was performed at 254 nm for catechol and related compounds and biogenic monoamine neurotransmitters. Sample introduction was accomplished by hydrodynamic injection at a pressure of 0.5 psi for 2 s. The data sampling rate was 5 Hz.

2.2. Reagents and procedures

Unless noted otherwise, all chemicals were of analytical grade. Imidazole and neurotransmitters: dopamine, epinephrine, norepinephrine, serotonin and 3-(3,4-dihydroxyphenyl)alanine (or DOPA) were purchased from Sigma (St. Louis, MO, USA). Isomeric (*o*-, *p*- and *m*-) dihydroxybenzenes and isomeric (*o*-, *p*- and *m*-) nitrophenols were obtained from Beijing Chemical Corp. (Beijing, China). Aqueous stock solutions of metal and ammonium ions were prepared using ultrapure (18 MΩ) water. Dihydroxybenzenes, nitrophenols and neurotransmitters standards were prepared in 0.1 *M* HCl solution. All analytes were diluted to the desired concentration using the electrophoretic running buffers before use. The buffer additive: 1,4,7,10-tetraazacyclotridecane-11,13-dione (or dioxo[13]aneN_4) was synthesized based on a standard procedure [20].

Before all CE experiments, the capillary was rinsed in turn with water, 0.5 *M* HCl, water and running buffer. The capillary was not washed using NaOH before CE separation of cations, otherwise it would result in systematic peaks and affect the detection of cations. Imidazole (5.0 m*M*) containing acetic acid (2.0 m*M*) at pH 6.0 was used as the background electrolyte for CE using indirect UV detection. Phosphate buffer (15 m*M* Na_2HPO_4–15 m*M* NaH_2PO_4 at pH 7.0) was used as the running buffer for CE with direct UV detection of dihydroxybenzenes, nitrophenols and neurotransmitters.

3. Results and discussion

3.1. CE separation of alkali metal, alkaline earth metal and ammonium ions

Since dioxo[13]aneN_4 can form stable complexes with inorganic cations, its addition in the background electrolyte modifies the electrophoretic behavior of these cations, thus enhancing their separation resolution. Fig. 2 (a) shows the electropherogram obtained for alkaline, alkaline earth and ammonium ions in imidazole–acetic acid electrolyte only. In this electrolyte, only a single electrophoretic peak was observed for K^+ and NH_4^+ ions. Moreover, the electrophoretic peaks of Ca^{2+} and Sr^{2+} overlap heavily due to their close electrophoretic mobilities. Fig. 2(b) shows the electropherogram obtained in imidazole–acetic acid electrolyte containing 2.5 m*M* dioxo[13]aneN_4 as an additive. Nine cations have been completely separated. Possible host–guest interaction between dioxo[13]aneN_4 and the cations decreases their electrophoretic mobilities to different extents, and thus enhancing their separation. Hence, the addition of dioxo[13]aneN_4 to the background electrolyte can effectively improve the separation resolution for these ions, especially between K^+ and NH_4^+ and between Ca^{2+} and Sr^{2+}. Note that K^+ migrates earlier than NH_4^+ when the dioxopolyamine was used as a buffer additive in this work; whereas the migration order was reversed when 18-crown-6-ether was used, as previously reported [7,13].

Table 1 gives the statistical data for the CE separation of nine cations obtained in two different buffer systems. In the case of imidazole–acetic acid buffer system in which there are no separations between K^+ and NH_4^+, and between Ca^{2+} and Sr^{2+}, the data of migration time and peak height were obtained via the injection of single cation standards. While improving separation, these data illustrate that the use of dioxo[13]aneN_4 did not cause any negative effect on the peak width as evident from the

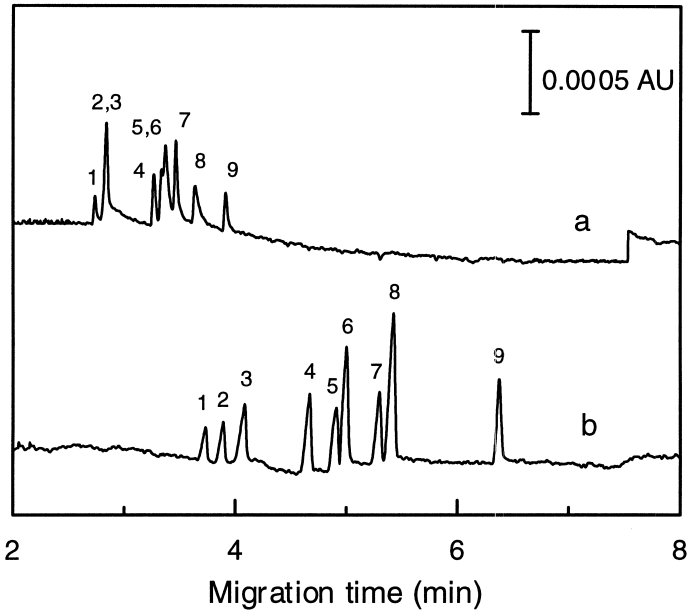

Fig. 2. Electropherograms obtained for the separation of alkali metal, alkaline earth metal and ammonium ions by CE with indirect UV detection using (a) 5.0 m*M* imidazole–2.0 m*M* acetic acid electrolyte and (b) 5.0 m*M* imidazole–2.0 m*M* acetic acid electrolyte containing 2.5 m*M* dioxo[13]aneN$_4$. Voltage: 10 kV; wavelength: 214 nm; analyte concentration: 50 μ*M*; buffer pH: 6.0. Peaks: 1=Cs^+, 2=K^+, 3=NH_4^+, 4=Ba^{2+}, 5=Sr^{2+}, 6=Ca^{2+}, 7=Na^+, 8=Mg^{2+}, 9=Li^+.

theoretical plate number which ranges from 43 900 to 140 500, as compared to the range of 43 500 to 104 000 in the case when no additive was used. Furthermore, the reproducibility of migration time in the case when dioxo[13]aneN$_4$ was added, which-when expressed in relative standard deviation (RSD), also known as coefficient of variation (C.V.)-ranges from 0.22 to 0.74%, is comparable to that in the case when no additive was used.

It was also observed that the use of dioxo[13]aneN$_4$ as an additive did not influence the detection sensitivity. In fact, the detection limits of all cations, except K^+, are improved. It is because the dioxopolyamine compound has a weak UV

Table 1
Migration time (*t*); theoretical plate number (*N*); limit of detection (LOD), based on *S*/*N*=3; RSD of migration time, (RSD$_t$), RSD of peak height (RSD$_H$), *n*=6; obtained for the CE separation of nine cations in two different electrolyte systems. (The left data at each column were obtained in imidazole–acetic acid electrolyte only, and the right data were obtained in imidazole–acetic acid electrolyte containing dioxo[13]aneN$_4$ as an additive. All other conditions are the same as in Fig. 2)

Cations	*t* (min)	*N*	LOD (μ*M*)	RSD$_t$ (%)	RSD$_H$ (%)
Cs^+	2.73, 3.71	62 600, 50 800	9.90, 8.49	0.56, 0.22	2.97, 1.90
K^+	2.84, 3.84	63 800, 54 500	7.09, 7.14	0.72, 0.54	2.38, 2.31
NH_4^+	2.84, 4.08	64 900, 44 300	6.55, 6.29	0.76, 0.74	2.44, 2.84
Ba^{2+}	3.26, 4.65	72 200, 72 900	6.41, 4.03	0.65, 0.40	2.38, 1.65
Sr^{2+}	3.33, 4.88	52 300, 43 900	7.02, 5.52	0.86, 0.30	1.96, 2.07
Ca^{2+}	3.37, 5.02	43 500, 59 600	4.68, 2.62	0.78, 0.58	2.49, 1.60
Na^+	3.46, 5.30	97 700, 89 800	4.15, 4.18	0.93, 0.67	2.90, 2.82
Mg^{2+}	3.64, 5.44	40 100, 61 100	8.44, 2.16	0.96, 0.43	2.93, 1.61
Li^+	3.91, 6.38	104 000, 140 500	8.37, 3.75	0.88, 0.58	3.33, 1.25

absorbance, as compared with other macrocyclic compounds. This is an important advantage of dioxo[13]aneN$_4$ comparing to another buffer additive: resorcarene, which is a highly UV-absorbing compound. When it is used as a buffer additive, it will cause a high background UV absorbance, resulting in a decrease of the detection sensitivity.

Fig. 3 illustrates the effect of different dioxo[13]aneN$_4$ concentrations on the migration time of the nine cations studied. It can be seen that their migration times increase or electrophoretic mobilities decrease as the dioxo[13]aneN$_4$ concentration increases, indicating that stronger host–guest interaction occurs at higher additive concentration. Apparently, this interaction reaches a maximum when dioxo[13]aneN$_4$ concentration is nearly 10 m*M*. Furthermore, separation between various cations is enhanced as the additive concentration increases. In order to illustrate the resolution achieved, only the electropherogram of the experiment performed at a dioxo[13]aneN$_4$ concentration of 2.5 m*M* was shown (see Fig. 2).

3.2. Separation of catechol and related compounds

Fig. 4 (a) shows the electropherogram obtained for the separation of dihydroxybenzenes and nitrophenols by CE with phosphate buffer only using direct UV detection. Since *o*-dihyroxybenzene (or catechol), *m*-dihyroxybenzene (or resorcinol) and *p*-dihyroxybenzene (or hydroquinone) have very similar mobilities, only a single electrophoretic peak was observed. The electropherogram shown in Fig. 4 (b) was obtained in phosphate buffer containing 0.25

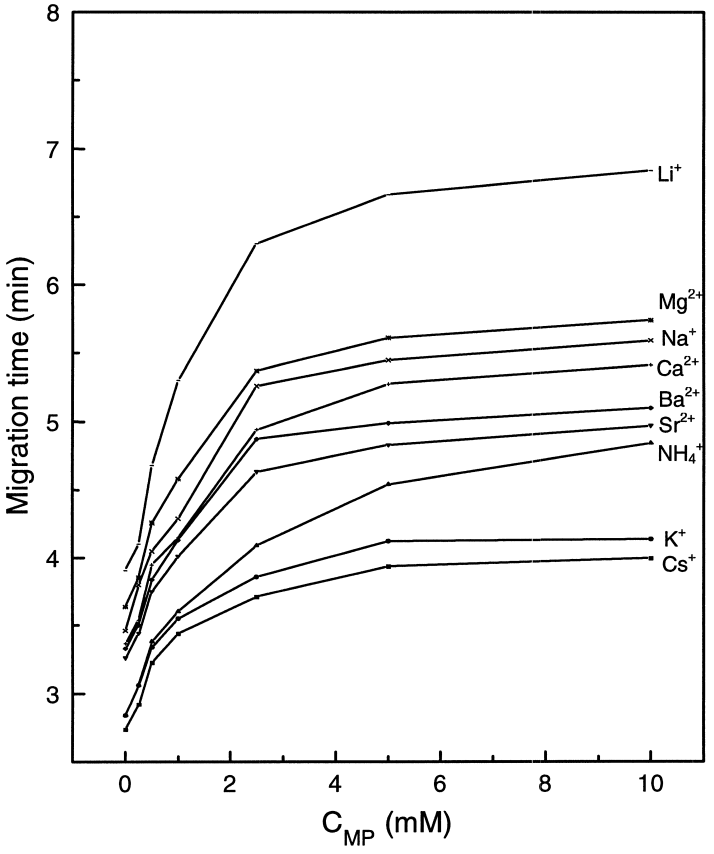

Fig. 3. Effect of different dioxo[13]aneN$_4$ concentrations (C$_{MP}$) on the migration times of nine cations. All conditions are the same as in Fig. 2.

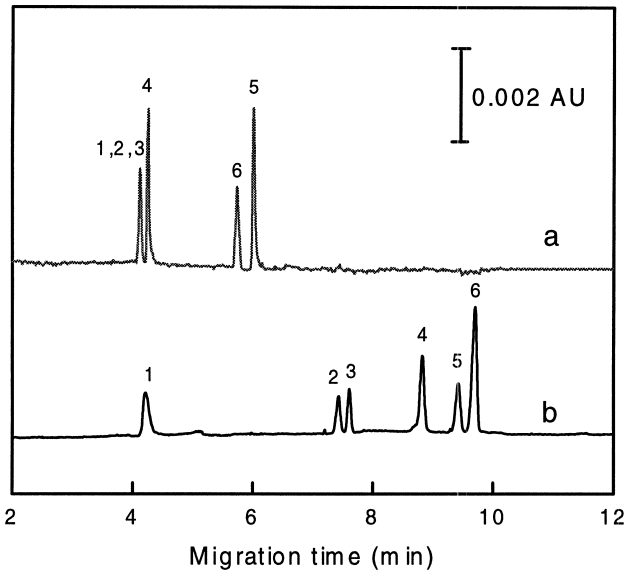

Fig. 4. Electropherograms obtained for the separation of *o*-, *m*-, *p*-dihydroxybenzenes and *o*-, *m*-, *p*-nitrophenol by CE with direct UV detection using (a) phosphate buffer and (b) phosphate buffer containing 0.25 m*M* dioxo[13]aneN$_4$. Voltage: 15 kV; wavelength: 254 nm; analyte concentration: 100 μM; buffer pH: 7.0. Peaks: 1=*p*-dihyroxybenzene (or hydroquinone), 2=*m*-dihyroxybenzene (or resorcinol), 3=*o*-dihyrdroxybenzene (or catechol), 4=*m*-nitrophenol, 5=*p*-nitrophenol, 6=*o*-nitrophenol.

m*M* dioxo[13]aneN$_4$. It shows that this additive can greatly improve the separation of three dihydroxybenzenes. They can be separated with baseline resolution, and their migration order is, hydroquinone, resorcinol and catechol. It is speculated that the two hydroxyl groups of hydroquinone are separated far from each other and they cannot complex effectively with dioxo[13]aneN$_4$. Accordingly, the compound has the weakest interaction with dioxo[13]aneN$_4$ and migrates first. While the two hydroxyl groups of catechol can form the strongest complexation with dioxo[13]aneN$_4$, possibly through ionic hydrogen bonds, so it migrates last. Although isomeric nitrophenols can already be resolved without the use of the additive, it modifies their electrophoretic mobilities because of the possible interaction between the polyoxo groups of the nitrophenols and the amine and amide hydrogen atoms of dioxo[13]aneN$_4$ at a neutral pH. As a result, in the buffer without additives, *o*-nitrophenol migrates faster than *p*-nitrophenol, while in the electrolyte with dioxo[13]aneN$_4$ added, *p*-nitrophenol migrates faster than *o*-nitrophenol.

3.3. Separation of neurotransmitters

Catecholamines (dopamine, norepinephrine and epinephrine), serotonin and DOPA have important physiological roles as neurotransmitters. Therefore, individual determination of these species is essential for monitoring their concentrations in biological fluids. In previous reports, borate has been used in the electrophoretic buffer to improve the resolution of catecholamines and serotonin, since the polyoxoanion can form complexes with these compounds [21]. Similarly, dioxo[13]aneN$_4$ can strongly complex with the catechol groups of catecholamines, as mentioned in the foregoing discussion. Accordingly, dioxo[13]aneN$_4$ was used as a buffer additive to improve the CE separation of catecholamines and related compounds. Fig. 5 (a) shows the electropherogram for the inadequate separation of dopamine (DA), serotonin or 5-hydroxytryptamine (5-HT), norepinephrine (NE), epinephrine (E) and DOPA obtained in a buffer containing phosphate only (pH 7.0). It is noted that the peaks of DA and 5-HT overlap significantly, so do those of NE and E.

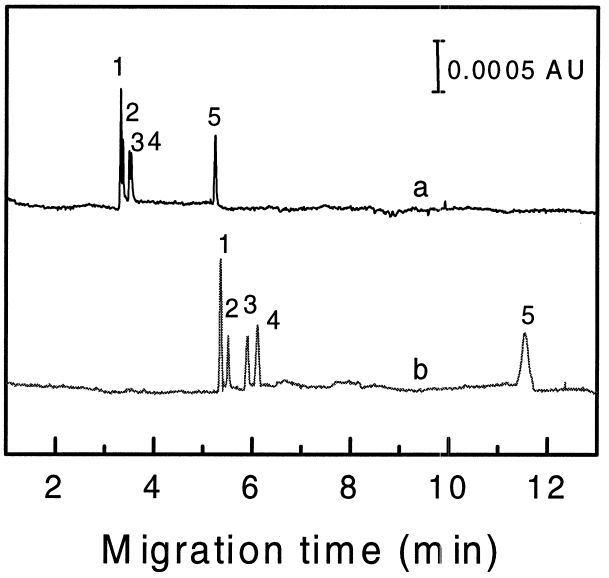

Fig. 5. Electropherograms obtained for the separation of catecholamines, 5-HT and DOPA by CE with direct UV detection using (a) phosphate buffer only and (b) phosphate buffer containing 0.25 m*M* dioxo[13]aneN$_4$. Voltage: 15 kV; wavelength: 254 nm; analyte concentration: 50 μ*M*; buffer pH: 7.0. Peaks: 1=serotonin or 5-hydroxytryptamine (5-HT), 2=dopamine (DA), 3=norepinephrine (NE), 4=epinephrine (E), 5=3-(3,4-dihydroxyphenyl)alanine (DOPA).

Fig. 5 (b) shows the electropherogram for the separation of three catecholamines, 5-HT and DOPA obtained in a phosphate buffer containing 0.25 m*M* dioxo[13]aneN$_4$ as an additive. It is speculated that the complete separation of these neurotransmitters is due to their different interaction with the additive. DA contains the catechol group and has strong complexation with dioxo[13]aneN$_4$, while 5-HT has no catechol group and its interaction is relatively weak. Consequently, DA migrated more slowly than 5-HT when dioxo[13]aneN$_4$ was used as a buffer additive. This method, which has been carried out at neutral pH, is possibly better than other reported methods which were conducted either at neutral pH requiring a surfactant additive to improve resolution [22], or at alkaline pH requiring other additives to prevent catecholamine oxidation [23]. This is important, especially in the case of direct sampling from test subjects, as this method allows the CE separation to be carried out directly at the physiological pH of biological fluids, without pH adjustment before analysis. Although UV detection is inadequate for the determination of trace amounts of these neurotransmitters present in physiological fluids, this work does illustrate the enhancement in capillary electrophoretic separation of these compounds by using a macrocyclic polyamine additive.

4. Conclusion

We have demonstrated for the first time that the addition of dioxo[13]aneN$_4$ to the electrophoretic electrolyte for the separation of inorganic cations, isomeric dihydroxybenzenes and nitrophenols, and biogenic monoamine neurotransmitters. The first advantage of dioxo[13]aneN$_4$ as a CE additive is that it can be applied not only to the separation of ionic species (cations and anions), but also to neutral compounds, indicating the macrocyclic dioxopolyamine compound is a versatile buffer additive. A possible reason is the availability of lone pairs of electrons on the nitrogen atoms for cationic interaction, and of acidic hydrogen on the nitrogen atoms for hydrogen bond interaction between dioxo[13]aneN$_4$ and anions or electron-pair rich

neutral molecules. Second, dioxo[13]aneN_4 has a weak UV absorbance and does not cause a negative effect on the UV detection limit. Accordingly, high UV detection sensitivity can be retained when the additive is used, as compared with other macrocyclic additives. Third, as a CE additive, dioxo[13]aneN_4 can provide good reproducibility in the separation of cations (RSD is less than 0.8%), possibly due to the use of a simple CE electrolyte system. Finally, only millimolar amount of the additive was used in the study, showing the low consumption of this additive. While detection sensitivity is not a main focus, this work demonstrates that one of the macrocyclic polyamine derivatives: dioxo[13]aneN_4 is a promising universal additive in CE separations for any chemical species: cationic, anionic and neutral.

Furthermore, in the separation of inorganic cations, only one macrocyclic additive is sufficient, as opposed to the use of crown ether in which another additive, such as HIBA, is needed. It was also found that dioxo[13]aneN_4 is an effective additive in CE to resolve dihydroxybenzenes. In the separation of neurotransmitters, the improvement lies not in enhancing resolution, but in allowing separation to be carried out at neutral or physiological pH without the need of surfactant additives.

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