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Use of dyes as indirect detection probes for the high-sensitivity determination of anions by capillary electrophoresis

Philip Doble, Miroslav Macka, Paul R. Haddad*

Department of Chemistry, University of Tasmania, GPO Box 252-75, Hobart, Tasmania 7001, Australia

Abstract

High sensitivity for indirect detection was achieved by utilising highly absorbing species as the displaced co-ion (or probe). Two highly absorbing dyes, bromocresol green and indigo-tetrasulfonate, were investigated as potential probes in the determination of small organic and inorganic anions. The concentration of these probes was kept as low as possible to ensure the signal-to-noise ratios were reasonable and the background absorbance was within the linear range of the detector. Four different protocols for buffering the electrolyte with such low probe concentrations were investigated. Buffering with agents that introduce co-anions [acetate or 2-(cyclohexylamino)ethanesulfonic acid (CHES) buffers] proved unsuitable as detection sensitivities were diminished due to competitive displacement by the analytes and system peaks were also induced. Buffering without introduction of co-anions was achieved using the buffering base, diethanolamine, or the use of ampholytes, lysine and glutamic acid. For separations performed with these two buffering approaches, migration time reproducibilities were less than 1% R.S.D. for most analytes. Minimal detectable amounts were in the low attomol region ($1 \cdot 10^{-18}$ mol), corresponding to sub-μM vacuum injected solution concentrations. These were an order of magnitude lower than the general detection limit reported for indirect photometric detection, and were comparable with detection limits achieved with indirect fluorescence detection. Finally, the detection limits were further improved by approximately three times for anions analysed with indigo-tetrasulfonate as the probe when a Z cell was employed as the detection cell.

Keywords: Buffer compositions; Dyes; Bromocresol green; Indigo-tetrasulfonate; Alkanesulfonic acids; Inorganic anions; Organic acids

1. Introduction

Analysis of low-molecular-mass and inorganic anions by capillary electrophoresis (CE) often utilises indirect photometric detection because these analytes usually exhibit poor chromophoric properties. In this form of detection, an absorbing ionic species (the probe ion) which has the same charge as the analyte of interest is added to the carrier electrolyte. The analyte displaces a proportion of the probe leading to a decrease in background absorbance and a measurable signal [1–4] The limit of detection (LOD) for a non-absorbing analyte is given by [5]:

$$C_{\mathrm{LOD}} = \frac{C_{\mathrm{p}}}{RD_{\mathrm{r}}} = \frac{N_{\mathrm{BL}}}{R\varepsilon l} \quad (1)$$

where C_{LOD} is the concentration limit of detection of the analyte, C_{p} is the concentration of the probe, R is the transfer ratio (the number of moles of the probe displaced by 1 mol of the analyte), and D_{r} is the dynamic reserve (i.e., the ratio of the background absorbance to the noise), N_{BL} is the baseline noise, ε is the molar absorptivity of the probe and l is the pathlength of the detection cell. Therefore, lowering C_{p} and increasing D_{r} will reduce the detection limit.

*Corresponding author.

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However, the D_r value also depends on C_p, so decreasing C_p will not reduce the detection limit because D_r is simultaneously reduced. The best approach to minimise detection limits is to increase D_r by increasing the molar absorbtivity (ε) of the probe [6]. Highly absorbing dyes therefore make ideal candidates as probes in order to maximise indirect detection sensitivity. When such probes are used C_p is also kept low by the necessity to maintain the background absorbance at such a level to keep the noise reasonable (increasing the background absorbance also increases the noise) and to operate within the linear range of the detector.

Several authors have explored various dyes as probes to minimise the detection limits for a variety of ions. Xue and Yeung [7] used bromocresol green for the analysis of the pyruvate anion, and malachite green for the detection of potassium, obtaining sub-femtomol LODs. However, both electrolytes were unbuffered. Mala et al. [8] also reported sub-femtomol LODs for cations analysed with the cationic dyes, Chlorophenol Red and Methyl Green. Tris(hydroxymethyl)aminoethane (Tris) was used as a buffering agent, but this species potentially acts as a competing co-cation and thereby decreases sensitivity. They also used Indigo Carmene as the anionic probe for the determination of some inorganic anions, obtaining LODs at the sub-picomol level. The disappointing LODs in this latter case were most probably due to the use of acetate as the buffering agent since this would act as a competing co-anion. More recently, Siren et al. [9] employed nitrosonaphthol dyes in unbuffered electrolytes for the determination of inorganic anions and organic acids, reaching LODs close to attomol levels. The authors report that attempts to buffer the electrolytes with phosphate at pH 8 were unsuccessful due to deleterious effects on sensitivity.

Thus, buffering of electrolytes for indirect detection provides something of a challenge. Recently, Doble and Haddad [10] showed that detection sensitivity could be severely compromised when co-anions are added to an electrolyte. This is due to competitive displacement between the probe and the added co-anions, resulting in a decrease of the transfer ratio. The extent to which the detection sensitivity is compromised was shown to be dependent upon the relative mobilities of the probe and the co-anion. It was concluded that it is possible to buffer an electrolyte that contained a fast moving probe and a slow moving buffering agent. A further problem with introduction of co-anions is inducement of system peaks, which can interfere with analytes of interest. Alternatively, electrolytes may be buffered for indirect detection of anions by titrating the acid form of a probe with a buffering base to a pH close to the pK_a of the base. Recently, a buffered chromate electrolyte was prepared using such an approach by titrating chromium trioxide with Tris and diethanolamine (DEA) [11,12]. Another possible alternative for buffering indirect detection electrolytes that has not been explored is the use of ampholytic buffers. When a free ampholyte is dissolved in pure water, the pH of the solution is close to the isoelectric point (pI) of the ampholyte. Under such conditions the ampholyte exists in a zwitterionic form having a net zero charge and therefore does not interfere with indirect detection. An ampholyte will buffer at its pI when the pK_a values of two buffering groups on either side of the pI are close together (within one pH unit). Further, the ampholyte at its pI does not contribute to the conductivity of the solution and may be added in sufficiently high quantities to provide good buffering capacity [13].

In this paper we present separations of C_2–C_8 alkanesulfonic acids obtained when bromocresol green is used as the probe. The four different buffering protocols mentioned above are investigated to demonstrate the most appropriate approach. These are buffering using a base as the counter-ion (DEA); buffering with an ampholyte (lysine); buffering with a low mobility co-anion [2-(cyclohexylamino)ethanesulfonic acid (CHES)], and buffering with a co-anion that has a mobility close to that of the probe (acetate). The relative merits and disadvantages of each are investigated and the analytical performance of the lysine- and DEA-buffered electrolytes are compared. Separations of inorganic anions and organic acids are also presented with indigo-tetrasulfonate as the probe. Two electrolytes were developed, the first buffered with the base 1,3 - bis[tris(hydroxymethyl)methylamino]propane (Bis-Tris), and the second with the ampholyte, glutamic acid. Analytical performance of each of the electrolytes is also presented. Finally, the further

improvement of detection limits is demonstrated when the effective pathlength of the detection cell is increased with a Z cell.

2. Experimental

2.1. Instrumentation

The capillary zone electrophoresis (CZE) instrument used was an Applied Biosystems Model 270A-HT (Perkin-Elmer, San Jose, CA, USA) interfaced to Turbochrome chromatography software (Perkin-Elmer). Separations were performed using two types of fused-silica capillaries: (1) 70 cm×75 μm I.D., optical pathlength 75 μm, effective length to detector of 50 cm (Polymicro Technologies, Phoenix, AZ, USA); and (2) a Z cell arrangement 70 cm×75 μm I.D., optical pathlength 3 mm, effective length to detector of 50 cm (Perkin-Elmer).

Injections were performed under vacuum at 5 in.Hg (1 in.Hg=3386.38 Pa). Injection times are given in each of the Figures.

2.2. Reagents and procedures

All carrier electrolytes and standards were prepared in water treated with a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus. Electrolytes were degassed using vacuum sonication and were filtered through a 0.45-μm syringe filter (Activon Thornleigh, Australia) prior to use.

The chemicals were of analytical reagent grade, unless stated otherwise. Heptanesulfonate, hexanesulfonate, pentanesulfonate, butanesulfonate, propanesulfonate, ethanesulfonate, perchloric acid, sodium thiocyanate, Bis-Tris, CHES, Carbowax 20M, potassium indigo-tetrasulfonate (ITS) – dye content 85%, bromocresol green (BCG) – dye content 95%, D-glutamic acid and lysine were obtained from Sigma–Aldrich (Milwaukee, WI, USA). Sodium salts of the desired anionic analytes were obtained from Ajax (NSW, Australia) or from BDH (Victoria, Australia). DEA and lysine were obtained from Fluka (Switzerland).

2.3. Preparation of lysine

The p*I* of lysine is 9.7, but dissolution of free lysine in water resulted in a solution having a pH of 10.2. This pH was too high for indirect detection methods since lysine would carry a net negative charge at this pH and therefore would act as a competing co-anion. Three different batches of lysine obtained from Fluka, Sigma and Aldrich showed similar behaviour and attempts at purifying lysine by recrystallisation procedures proved unsuccessful.

Cationic impurities were removed by utilising a dialysis apparatus which consisted of a 160 cm×0.6 mm I.D. DuPont (Wilmington, DE, USA) Nafion cation-exchange hollow fibre packed with polystyrene–divinylbenzene beads, coiled on a holder made of glass rods and placed in a housing tube made from clear acrylic material (20 cm×25 mM I.D.) [15]. The device contained a solution of 0.1 *M* camphorsulfonic acid solution and 25 g of Bio-Rad Dowex 50WX16 cation-exchange resin (H^+ form) which surrounded the hollow-fibre and acted as the hydrogen ion donating medium. When a 0.1 *M* solution of lysine was pumped through the fibre at 0.1 ml min^{-1}, the resulting effluent had a pH of 9.7.

2.4. Preparation of acid form of ITS

Five g of Bio-Rad Dowex 50WX16 cation-exchange resin was dry packed into a disposable 10-ml syringe. The packed syringe was washed with 200 ml of 0.1 *M* hydrochloric acid. A vacuum was applied to provide a flow-rate of approximately 30 ml min^{-1}. The syringe was then washed with 200 ml of Milli-Q water, or until the pH of the effluent was close to neutral. Fifty ml of a 10 m*M* solution of the potassium salt form of ITS was then passed through the packed syringe at a flow-rate of approximately 10 ml min^{-1}. The first 10 ml was discarded and the rest of the effluent collected. The collected fraction was used for subsequent preparation of electrolytes buffered with buffering bases.

2.5. Calculation of minimal detectable amounts (MDAs)

The MDAs were calculated at a signal-to-noise

Table 1
Selected characteristics of dyes used as probes

Dye	Experimental wavelength (nm)	Molar absorptivity[a] ($\cdot 10^3$ mol^{-1} l cm^{-1})	Mobility[b] (10^{-9} m^2 V^{-1} s^{-1})
BCG	618	45.0	−36.3
ITS	314	32.1	−70.7

[a] Calculated from spectra measured of 0.005 m*M* BCG buffered to pH 9.2 with DEA, and 0.05 m*M* of ITS buffered to pH 6.8 with Bis-Tris.
[b] Mobility of each dye was experimentally determined. Electrolyte: 1.0 m*M* phthalic acid buffered to pH 9.2 with DEA. Conditions: 30 kV, 618 nm, 0.6 s pressure injection at 5 in.Hg.

ratio of two. The noise was calculated as the root mean square of a 1 min segment of stable baseline using Turbochrome software. The amount of solute injected into the capillary was calculated from the Poiseuille equation [16]:

$$w = \frac{\Delta P \Pi r^4 C t_i}{8\eta L} \quad (2)$$

where w = amount of solute injected (mol), ΔP = pressure difference (Pa), r = radius of inner diameter of capillary (m), C = concentration of injected solute (mol l^{-1}), t_i = time of injection (s), η = viscosity (Pa s), L = total length of capillary (m).

If sample injection is accomplished by gravity the pressure difference (ΔP) is given by the hydrostatic pressure defined as:

$$\Delta P = \rho g \Delta h \quad (3)$$

where ρ = density of the electrolyte (kg m^{-3}), g = gravitational acceleration (9.8 N kg^{-1}), Δh = height difference between liquid levels of sample and buffer vials (m).

3. Results and discussion

3.1. Selection of dyes

For high sensitivity indirect detection, the UV absorbing component of the electrolyte (probe) must have a high molar absorptivity at the wavelength chosen and the molar concentration of the probe should be kept low. The charge of the probe must also be stable at the pH of the electrolyte. Two candidates were chosen to fulfil these requirements: bromocresol green (BCG) and ITS. Data relevant to these two dyes is given in Table 1, from which it can be seen that BCG has intermediate mobility and was therefore chosen for the determination of intermediate to low mobility anions. Further, BCG (pK_a = 4.9 [17]) has two negative charges and its molar absorbtivity is constant at pH 7 or higher, making it suitable for electrolytes buffered at pH > 7. ITS has a high to intermediate mobility and was therefore chosen as a candidate for the determination of anions of similar mobility. ITS has four negative charges and its molar absorptivity is constant over a very wide pH range, making it suitable for electrolytes buffered at most values of pH.

3.2. Buffering protocols

BCG was chosen as the probe for the investigation of the four buffering protocols detailed in the introduction. Table 2 presents the composition of each of the electrolytes. In all cases the probe concentration was kept at 0.5 m*M*. Electrolyte 1 consisted of the free acid of BCG titrated with DEA to the pK_a of DEA. This electrolyte provided a

Table 2
Electrolyte compositions for buffer investigation

	Probe	Buffer	pH
Electrolyte 1	0.5 m*M* BCG	2.0 m*M* DEA	9.20
Electrolyte 2	0.5 m*M* BCG	10 m*M* Lysine	9.70
Electrolyte 3	0.5 m*M* BCG	2.0 m*M* CHES	9.55
Electrolyte 4	0.5 m*M* BCG	2.0 m*M* Acetate, 4.0 m*M* DEA	9.20

moderately buffered two component electrolyte. Electrolyte 2 was buffered with the ampholyte, lysine. Electrolyte 3 was buffered with the low mobility buffer CHES. Electrolyte 4 was buffered with a DEA–acetate buffer.

A series of seven sulfonic acids (C_2–C_8) was used as analytes that encompassed the useful mobility range of BCG (10 to $40 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}). Fig. 1 shows the electropherograms obtained for the solutes with each of the above electrolytes. In all cases the analytes were migrating in a direction opposite to the flow of electroosmotic flow (EOF), but the EOF had sufficient electrophoretic velocity to sweep the analytes in the same direction as the EOF. Consequently the anion of lowest mobility (octanesulfonate) migrated from the capillary first, and the most mobile anion (ethanesulfonate) migrated last. Detection was performed at the cathodic end of the capillary. An EOF modifier such as tetradecyltrimethylammonium bromide (TTAB) could not be added to reverse the EOF because an insoluble precipitate was produced between the modifier and the dye.

The DEA buffered electrolyte provided baseline separation of all of the analytes, as well as very good sensitivity. The lysine buffered electrolyte also provided baseline resolution. For the CHES buffered electrolyte, peaks 1 and 2 were diminished in area, peak 3 completely disappeared, a system peak was induced that completely swamped peak 4, peaks 5 and 6 were relatively unaffected and peak 7 was halved in area. These effects were caused by competitive displacement of the buffer anion at the expense of the probe [10]. Consequently, CHES is unsuitable for buffering the moderately mobile BCG. The acetate–DEA buffer exhibited the same undesirable effects for similar reasons. This behaviour explains the observation [8] that relatively poor detection sensitivities for the detection of anions using the dye chlorophenol red as the probe in a buffer consisting of 1 m*M* Tris adjusted with acetic acid to pH 6.0–7.2. The concentration of acetate in this electrolyte was approximately 1 m*M*, which was two- to ten-times greater than the concentration of chlorophenol red (0.1–0.5 m*M*). The introduced co-anion acetate reduced the transfer ratio and therefore the detection sensitivity.

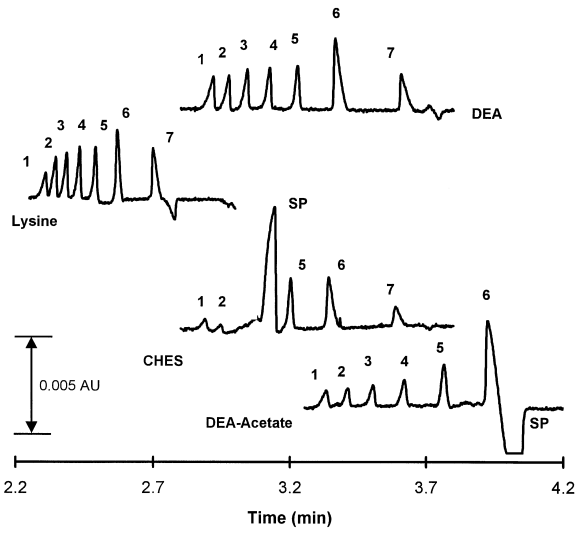

Fig. 1. Electropherograms obtained with different buffering protocols. Conditions: electrolyte given in Table 2, separation voltage 30 kV, 2 s pressure injection at 5 in.Hg, detection wavelength 618 nm, temperature 30°C. 10 μ*M* of each anion. Key: 1=octanesulfonate, 2=heptanesulfonate, 3=hexanesulfonate, 4=pentanesulfonate, 5=butanesulfonate, 6=propanesulfonate, 7=ethanesulfonate, SP=system peak.

3.3. Analytical performance parameters with BCG as probe

Maximum transfer ratios (and hence maximum theoretical sensitivity) as well as good reproducibility can be achieved when the acid form of the probe is titrated with a buffering base [11,18]. Accordingly, a comparison of sensitivity, calibration linearity and reproducibility obtained with BCG as the probe buffered either with DEA or lysine (electrolytes 1 and 2, Table 2) was performed. The results are given in Table 3.

Calibration curves of corrected area peak area versus molar concentration of the seven sulfonic acids used earlier were constructed from duplicate injections of 2–50 μ*M* of each sulfonic acid for both electrolytes, without replenishing the electrolyte reservoirs between injections. The correction made to peak area was to account for the different migration velocities of the sample bands and was achieved by dividing the peak area of each solute by its migration time. Comparisons of the resulting correlation coefficients for the calibration plots showed no significant difference between the two electrolytes,

Table 3
Analytical performance parameters of BCG electrolyte buffered with lysine or DEA

Solute (sulfonic acid)	Correlation coefficient (r^2)		Migration time precision (% R.S.D.)		Calibration slope ($\mu V\ s\ \mu M^{-1}$)		LOD			
							Lysine		DEA	
	Lysine	DEA	Lysine	DEA	Lysine	DEA	MDA (mol)	Conc. (μM)	MDA (mol)	Conc. (μM)
Octane	0.9977	0.9985	1.4	1.7	31	70	$3\cdot10^{-17}$	2	$2\cdot10^{-18}$	0.2
Heptane	0.9973	0.9992	1.5	1.6	45	64	$2\cdot10^{-17}$	1	$2\cdot10^{-18}$	0.2
Hexane	0.9974	0.9975	1.5	1.7	47	69	$2\cdot10^{-17}$	1	$2\cdot10^{-18}$	0.2
Pentane	0.9984	0.9991	1.5	1.8	49	68	$2\cdot10^{-17}$	1	$3\cdot10^{-18}$	0.3
Butane	0.9944	0.9986	1.5	1.8	50	65	$2\cdot10^{-17}$	2	$2\cdot10^{-18}$	0.2
Propane	0.9872	0.9967	1.2	2.0	51	83	$1\cdot10^{-17}$	1	$1\cdot10^{-18}$	0.1
Ethane	0.9965	0.9995	1.3	2.2	54	71	$1\cdot10^{-17}$	1	$2\cdot10^{-18}$	0.2

with linearity being maintained for each solute up to at least 50 μM. The migration time reproducibility was calculated from ten replicate injections for each analyte and in all cases the lysine buffered electrolyte gave superior migration time reproducibility compared to the DEA-buffered electrolyte. This was due to the higher buffering capacity of the lysine electrolyte. The DEA electrolyte was moderately buffered but its buffering capacity was limited to the concentration of the probe as a result of the titration method used to prepare the electrolyte. The probe concentration could not be further increased to increase the buffering capacity because the background absorbance became unacceptably high, increasing the baseline noise and moving the detector out of its linear response range.

The slopes of the calibration curves were quite different for the two electrolytes, with the lysine buffered electrolyte giving responses that were approximately 70% of those obtained with the DEA buffered electrolyte. The stock dialysed lysine solution was analysed using the buffered chromate electrolyte detailed in Ref. [11], and found to contain approximately 2 mM carbonate that presumably had been absorbed from the atmosphere during the dialytic clean-up step [19]. This resulted in an electrolyte containing 0.2 mM carbonate, which acted as a competing co-anion and thereby reduced the detection sensitivity [10]. Despite this drawback, the results showed that ampholytes could be used successfully to buffer electrolytes used for indirect detection.

The LODs for each of the sulfonic acids are reported in Table 3 as MDAs, and the corresponding solution concentrations in μM. Unlike LODs expressed as solution concentrations, MDAs are independent of the injection volume and provide a more reasonable comparison of sensitivities achieved between different studies. The MDAs were calculated using Eq. (1) and based on 0.6 s pressure injection at 5 in.Hg, and a 70 cm$\times$75 μm capillary. The MDAs for the sulfonic acids range from 1-3$\cdot 10^{-18}$ mol for the DEA buffered electrolyte, corresponding to solution concentrations of around 0.2 μM. Shamsi and Danielson [20] have reported detection limits for C_4–C_{14} alkanesulfonates of 0.5–2.0 mg l^{-1} using naphthalenemonosulfonic acid as the probe and detection at 214 nm. These convert to MDAs (calculation based upon 12 s pressure injection at 5 in.Hg, and 50 μM capillary) of approximately $1\cdot10^{-16}$ mol, or about 1.5-orders of magnitude higher than the MDAs obtained with BCG.

We believe the analysis of the sulfonic acids using BCG buffered with DEA to be the most sensitive yet reported for C_2–C_8 alkanesulfonic acids using CE and indirect photometric detection. Indeed, the LODs (10^{-6} M injected sample concentration) are at least an order of magnitude lower than the reported generalised detection limit for the technique of indirect detection (10^{-4} to 10^{-5} M) [14].

3.4. Use of ITS as the probe

The analysis of higher mobility solutes cannot be achieved in the same way as used for the low mobility analytes because the EOF does not have sufficient velocity to carry the high mobility analytes to the detector. Additionally, BCG does not have a

suitable mobility to provide adequate peak shapes for these anions [21]. Accordingly, the higher mobility probe ITS was investigated, with the separation polarity being reversed. In this case, suppression of the EOF was desirable but since this could not be achieved by the usual method of addition of a surfactant (due to precipitate formation), it was necessary to develop alternative electrolytes that suppressed the EOF. Two approaches were used to achieve this. The first was titrating the acid form of ITS with the buffering base, Bis-Tris which has been used earlier [18] in the determination of high mobility anions without the need for EOF flow reversal. The second approach for the analysis of fast anions was to use the low pH ampholytic buffer, glutamic acid.

The first approach is illustrated in Fig. 2a which shows an electropherogram of 14 anions obtained with ITS buffered with Bis-Tris, whilst Fig. 2b shows the same anions present at concentrations near their detection limit. Table 4 gives the analytical performance parameters obtained with ITS buffered with Bis-Tris. Calibration curves constructed for each of the analytes showed good linearity with the exception of citrate, which adsorbed onto the capillary wall and was not detected at concentrations less than 20 μ*M*. The linearity limits for bromide and chloride were approximately 100 μ*M*, and for the rest of the analytes were approximately 50 μ*M*. The migration time relative standard deviations (R.S.D.s) range from 0.1–1.4%, with the better R.S.D.s being associated with the most symmetrical peaks. The MDAs ranged from $1 \cdot 10^{-18}$ to $2 \cdot 10^{-17}$ mol, with the lowest values being obtained for the most symmetrical peaks and for analytes of higher charge. These values compared favourably with those obtained for the alkanesulfonic acids using BCG, and are again at least an order of magnitude lower than the general detection limit for indirect detection reported in Ref. [14].

Use of the second of the above mentioned approaches was straightforward since glutamic acid produced a solution of the expected pH (i.e., at the p*I* of 3.22). This allowed the indirect detection buffer to be prepared by dissolving the sodium salt of the probe in an appropriate concentration of the ampholyte, without the need to convert the probe into its acid form as was required when buffered with

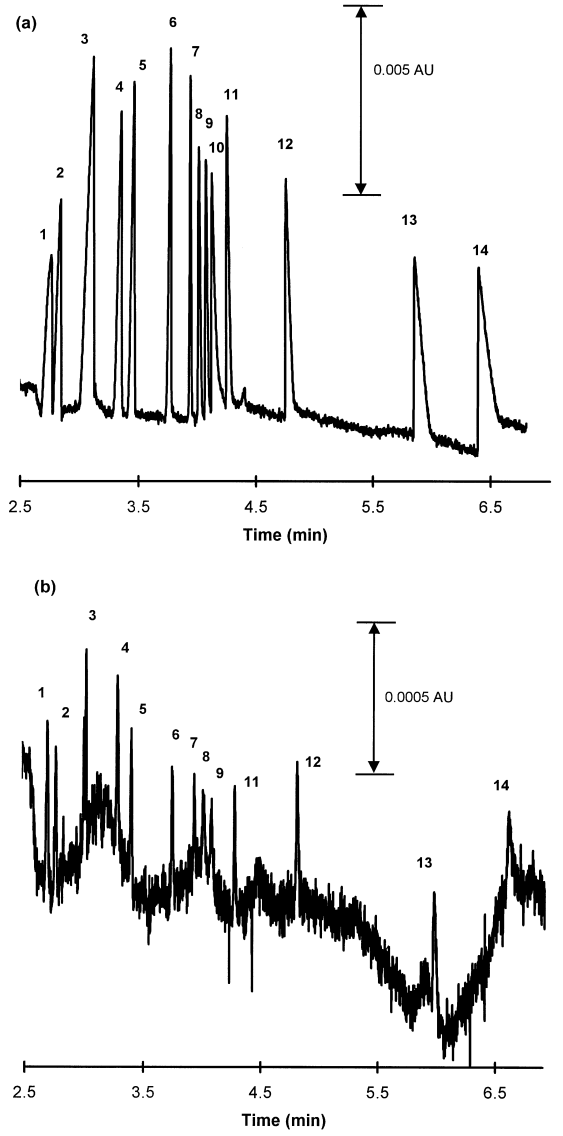

Fig. 2. Electropherogram of 14 anions obtained from the high concentration standard (a) and near their detection limits (b) with ITS as probe. Conditions: electrolyte 0.5 m*M* ITS, 2.67 m*M* Bis-Tris, pH = 6.8, separation voltage −30 kV, 0.6 s pressure injection at 5 in.Hg, detection wavelength 314 nm, temperature 30°C. Key: 1 = bromide (a) 200 μ*M*, (b) 10 μ*M*; 2 = chloride (a) 200 μ*M*, (b) 10 μ*M*; 3 = sulfate (a) 80 μ*M*, (b) 2 μ*M*; 4 = thiocyanate (a) 80 μ*M*, (b) 2 μ*M*; 5 = chlorate (a) 80 μ*M*, (b) 2 μ*M*; 6 = malonate (a) 40 μ*M*, (b) 1 μ*M*; 7 = tartrate (a) 40 μ*M*, (b) 0.5 μ*M*; 8 = bromate (a) 40 μ*M*, (b) 1 μ*M*; 9 = formate (a) 40 μ*M*, (b) 1 μ*M*; 10 = citrate (a) 30 μ*M*, (b) –; 11 = succinate (a) 30 μ*M*, (b) 0.8 μ*M*; 12 = phthalate (a) 30 μ*M*, (b) 0.8 μ*M*; 13 = iodate (a) 60 μ*M*, (b) 1.5 μ*M*; 14 = phosphate 60 μ*M*, (b) 1.5 μ*M*.

Table 4
Analytical performance parameters for ITS used as probe

Solute	Buffer	pH	Correlation coefficient (r^2)	Migration time precision (% R.S.D.)	LOD	
					MDA (mol)	Conc. (μM)
Bromide	Bis-Tris	6.80	0.9950	1.4	$2\cdot10^{-17}$	2
Chloride	Bis-Tris	6.80	0.9878	1.4	$2\cdot10^{-17}$	2
Sulfate	Bis-Tris	6.80	0.9989	1.7	$3\cdot10^{-18}$	0.3
Thiocyanate	Bis-Tris	6.80	0.9994	1.2	$5\cdot10^{-18}$	0.4
Chlorate	Bis-Tris	6.80	0.9996	1.1	$5\cdot10^{-18}$	0.4
Malonate	Bis-Tris	6.80	0.9998	0.7	$2\cdot10^{-18}$	0.2
Tartrate	Bis-Tris	6.80	0.9996	0.3	$1\cdot10^{-18}$	0.1
Bromate	Bis-Tris	6.80	0.9988	0.3	$3\cdot10^{-18}$	0.3
Formate	Bis-Tris	6.80	0.9792	0.2	$6\cdot10^{-18}$	0.5
Succinate	Bis-Tris	6.80	0.9999	0.1	$2\cdot10^{-18}$	0.2
Phthalate	Bis-Tris	6.80	0.9999	0.2	$2\cdot10^{-18}$	0.2
Iodate	Bis-Tris	6.80	0.9993	0.6	$5\cdot10^{-18}$	0.4
Phosphate	Bis-Tris	6.80	0.9976	0.7	$7\cdot10^{-18}$	0.6
Sulfate	Glutamic acid	3.22	0.9987	0.8	$1\cdot10^{-17}$	0.9
Nitrate	Glutamic acid	3.22	0.9994	0.4	$1\cdot10^{-17}$	1
Perchlorate	Glutamic acid	3.22	0.9989	0.2	$1\cdot10^{-17}$	1
Chlorate	Glutamic acid	3.22	0.9996	0.3	$1\cdot10^{-17}$	0.9
Bromate	Glutamic acid	3.22	0.9971	0.6	$8\cdot10^{-18}$	0.7

Bis-Tris. The EOF was suppressed sufficiently at pH 3.22 to allow migration of fast anions in a counter EOF mode with detection at the anode. However this method was limited to the determination of relatively strong acid anions due to the low pH of the electrolyte. Fig. 3a shows the separation of five inorganic anions obtained with 0.2 m*M* ITS buffered to pH 3.22 with 10 m*M* glutamic acid. Fig. 3b demonstrates the improvement in random baseline fluctuations (chemical noise) by addition of 0.1% Carbowax 20M to the electrolyte.

Analytical performance parameters were determined as before and are reported in Table 4. Calibration linearities were excellent for all solutes up to 100 μM, and the migration time reproducibilities are all less than 1%. Detection limits were at the $1\cdot10^{-17}$ mol level, which translates into solution concentrations around 1 μM. To illustrate the improvement in detection limits obtained using this approach, electropherograms of the same anions obtained with ITS and the commonly used chromate electrolyte are shown in Fig. 4. The same experimental conditions were used in each case with the exception of wavelength, which was set to the respective absorption maximum for each probe.

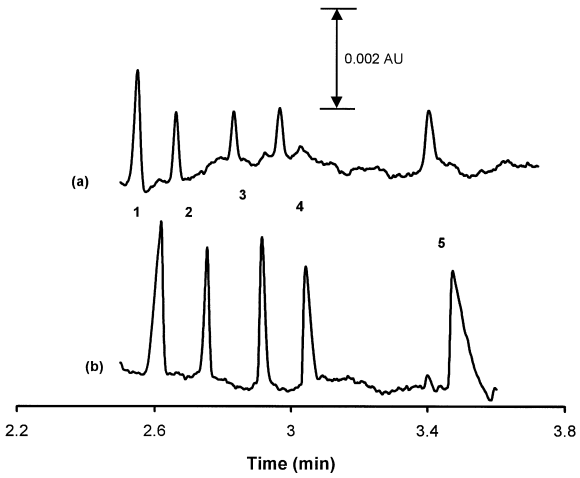

Fig. 3. Electropherogram of five anions obtained with ITS as probe (a), and improvement in baseline noise with Carbowax 20M added to the electrolyte (b). Conditions: electrolyte (a) 200 μM ITS, 10 m*M* glutamic acid, pH = 3.22, (b) 200 μM ITS, 10 m*M* glutamic acid, 0.1% carbowax, pH = 3.22, separation voltage −30 kV, 0.6 s pressure injection at 5 in.Hg, detection wavelength 314 nm, temperature 30°C, sample 20 μM of each anion. Key: 1 = sulfate, 2 = nitrate, 3 = perchlorate, 4 = chlorate, 5 = bromate.

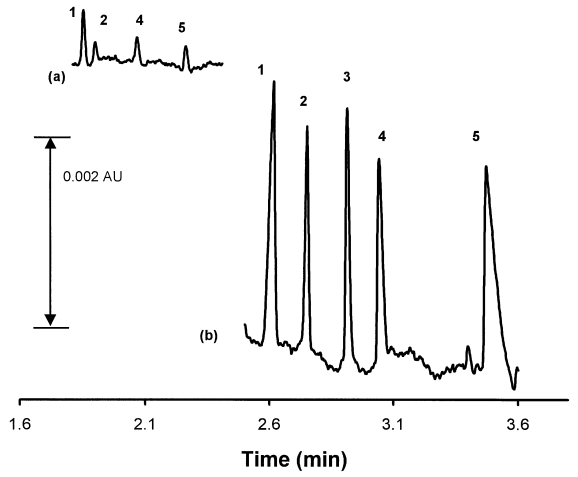

Fig. 4. Comparison of chromate (a) and ITS (b) as probes. Conditions: electrolyte (a) 5 m*M* chromic trioxide, 20 m*M* DEA, 0.5 m*M* TTAB, pH=9.1, (b) 200 μ*M* ITS, 10 m*M* glutamic acid, 0.1% Carbowax, pH=3.22, separation voltage −30 kV, 0.6 s pressure injection at 5 in.Hg, detection wavelength 314 nm, temperature 30°C, sample 20 μ*M* of each anion. Key: 1=sulfate, 2=nitrate, 3=perchlorate, 4=chlorate, 5=bromate.

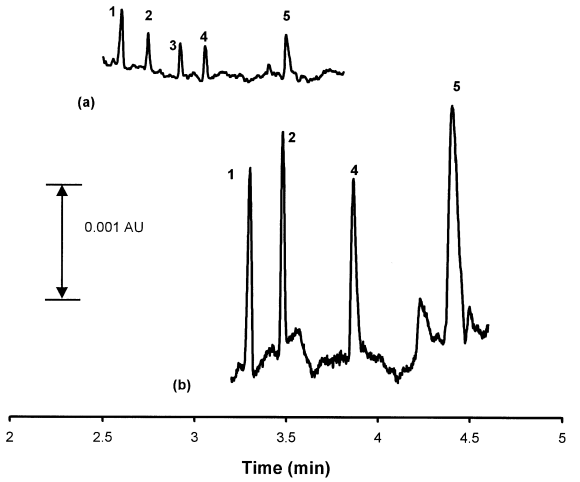

Fig. 5. Comparison of normal cell (a) and Z cell (b). Conditions: electrolyte (a) 200 μ*M* ITS, 10 m*M* ITS, 0.1% Carbowax, pH=3.22, (b) 50 μ*M* ITS 2.5 m*M* glutamic acid, 0.1% Carbowax, pH=3.22, separation voltage −30 kV, 0.6 s pressure injection at 5 in.Hg, detection wavelength 314 nm, temperature 30°C, sample 5 μ*M* of each anion. Key: 1=sulfate, 2=nitrate, 3=perchlorate, 4=chlorate, 5=bromate.

Perchlorate (peak 3) does not appear in the chromate trace because it co-migrates with chlorate. Noise levels in each electropherogram were approximately the same, but the peak heights were ten-times greater for ITS.

3.5. Z cell

The sensitivity of direct detection has been shown to improve by between 5- and twenty-times by bending a capillary and illuminating through the bend [14]. Such an arrangement is known as a Z cell. The increase in sensitivity is due to an increase in optical pathlength by about 40-fold. So far, Z cells have only been applied to direct detection [22,23].

Fig. 5 shows electropherograms for a selection of analytes of intermediate to high mobility obtained with ITS as the probe using either the normal cell arrangement (optical pathlength 75 μm) or the Z cell (optical pathlength 3 m*M*). The detection sensitivity was increased by about 3 times, giving MDAs of the order of $3 \cdot 10^{-18}$ mol for a singly charged solute. This is approximately in line with the detection limit achieved with indirect fluorescence detection [14].

Some practical problems must be resolved for the Z cell separation to work successfully. These mainly concern the increase in background absorbance (by a factor of 13) due to the increase in optical pathlength. This necessitated a reduction in the probe concentration to keep the detector noise to a reasonable level and to maintain a linear response. The concentration of ITS for this analysis was 50 μ*M*, and the corresponding background absorbance was 0.127. The only viable means to buffer such an electrolyte was to use an ampholyte. Due to the low probe concentration, titrating with a buffering base would result in a very weakly buffered electrolyte. Buffering with agents that introduce co-anions would not be suitable for reasons discussed above.

4. Conclusions

Indirect photometric detection sensitivity in CZE of low-molecular-mass anionic analytes was improved by at least an order of magnitude when highly absorbing dyes were used as probes. Buffering these types of electrolytes could be achieved with an ampholytic buffer or with a buffering base. Buffers that introduced co-anions were unsuitable

because the detection sensitivity was compromised and potentially interfering system peaks were induced.

Bromocresol green was found to be a suitable probe for the analysis of C_2–C_8 alkanesulfonic acids and when buffered with DEA gave detection limits at attomol levels. The detection limits were not as good when bromocresol green was buffered with the ampholyte lysine. This was caused by absorption of carbon dioxide from the air, resulting in an electrolyte that contained competing co-anions that reduced the transfer ratio. Nevertheless, the results have shown that ampholytic buffers were effective for buffering indirect detection electrolytes.

ITS proved to be a suitable probe for the analysis of small inorganic anions and organic acids when buffered with the base Bis-Tris. The detection limits achieved were again at attomol levels, with migration time reproducibilities generally less than 1% R.S.D. ITS was also a suitable probe for the analysis of strong acid inorganic anions when buffered at low pH with the ampholyte glutamic acid. This electrolyte gave ten-times higher sensitivities than the commonly used chromate electrolyte and migration time reproducibilities were also less than 1% R.S.D.

Finally the detection sensitivity could be further increased by three times when a Z cell was employed as the detection cell. However, when such a cell was used it was necessary to reduce the probe concentration to 50 μM. The only viable means to buffer this electrolyte was to use an ampholyte.

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