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Highly sensitive indirect photometric detection of cations by capillary electrophoresis with the cationic dye chrysoidine

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Abstract

The cationic dye, chrysoidine, has been used for the first time as a probe for the indirect photometric detection of cations. The dye has been used as a probe at concentrations of 5 m*M*, which is roughly an order of magnitude higher than for other cationic dyes used previously for the same purpose, in order to minimise electromigrational dispersion. Baseline instability was minimised by a combination of coating the capillary with poly(ethyleneimine), addition of a neutral polymer to the electrolyte, and the application of a small amount (20 mbar) of hydrodynamic pressure during the separation. Separation of a mixture containing alkali metals, alkaline earths, transition metals and lanthanides was achieved by the addition of 2-hydroxyisobutyric and lactic acid as complexing agents. Excellent peak shapes were observed over a wide range of analyte mobilities due to the moderate mobility of the probe. The high absorptivity (26 733 l mol^{-1} cm^{-1}) provided by chrysoidine in comparison with typically used, less absorbing probes, was reflected in limits of detection which were typically less than 0.5 μ*M*. These are amongst the lowest reported using hydrodynamic injection without the use of large volume stacking methods. The use of 2-hydroxyisobutyric and lactic acids as complexing agents at pH values close to their pK_a values provided suitable buffering which was highlighted by very good reproducibility of migration time, corrected peak area and peak height.

Keywords: Indirect detection; Detection, electrophoresis; Dyes, Chrysoidine; Inorganic cations; Organic acids

1. Introduction

Indirect photometric detection is a well-established detection mode for capillary electrophoresis [1–4]. It operates by the displacement of an absorbing ion (often referred to as the probe ion) in the background electrolyte by an analyte ion of the same charge sign as the probe. The resultant decrease in background absorbance is monitored and quantified. A non-absorbing analyte will have a limit of detection given by [5]:

$$C_{LOD} = \frac{C_p}{RD_r} = \frac{N_{BL}}{R\varepsilon l} \quad (1)$$

where C_{LOD} is the concentration limit of detection, C_p is the probe concentration, R is the transfer ratio (the number of moles of the probe displaced by 1 mol of analyte), D_r is the dynamic reserve (the ratio of background absorbance to noise), N_{BL} is the baseline noise, ε is the molar absorptivity of the probe, and l is the detection cell path length. It has been clearly shown [6,7] that the most effective

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means of decreasing limits of detection and thereby improving sensitivity is to increase the absorptivity of the probe. This approach has not yet been fully applied to the detection of cations.

Typically used cationic probes, such as imidazole, benzylamine, 4-methylbenzylamine (marketed as UV Cat 1 by Waters) and creatinine, have absorptivities of 5000–10 000 l mol^{-1} cm^{-1}. The use of cationic dyes with greater absorptivities would lead to increased peak heights when compared to traditional probes. Xue et al. [8] detected potassium using malachite green as a probe, but without buffering the electrolyte. Zhang et al. detected potassium, sodium and three polyamines using quinine sulfate as a probe [9]. Mala et al. [10] used methyl green for the detection of caesium, potassium, calcium, magnesium, sodium and lithium, but, tris(hydroxylaminomethane) (Tris) and acetic acid were used to provide buffering at pH 6.5, which meant that Tris would act as a competing co-ion. This method was adapted by Butler et al. [11] who substituted pyronine G in place of methyl green in order to better match the emission wavelength of a light emitting diode (LED) light source. Potassium, calcium, sodium and lithium were separated in a background electrolyte (BGE) comprising 0.15 m*M* pyronine G and 1 m*M* Tris at pH 4.0. In the rare occurrences of the use of cationic probes, they have been used at concentrations of less than 1 m*M* in order to remain within the linear range of the absorbance detector. The adsorption problems that can be encountered during the use of cationic dyes were illustrated by the work of Higashijima et al. [12] who used Methylene Blue as an indirect fluorescence probe for amino acids. An alkylsilane derivatised capillary was used to reduce adsorption of the dye.

Recent work [13] has shown that most modern CE instruments are capable of maintaining a linear response at absorbance values which are far in excess of those used commonly. The use of highly absorbing anionic dyes for detection of anions at increased probe concentrations has shown that significant improvements in peak shape, separation efficiency, resolution and increased detection sensitivity can be achieved [14,15]. Associated problems such as baseline instability can be minimised by careful manipulation of the BGE composition, together with the capillary and operating conditions. In the present work, a highly absorbing dye, chrysoidine, has been used to provide very sensitive indirect photometric detection of cations. Separation of a wide range of metals was made possible by the addition of hydroxycarboxylic acids which acted as a complexing agent. Comparisons of this system with a method based on a traditional probe, imidazole, showed that chrysoidine provided more sensitive detection.

2. Experimental

2.1. Instrumentation

The capillary electrophoresis instrument used during this work was an Agilent Technologies ^{3D}CE (Waldbron, Germany). This instrument was equipped with a deuterium lamp with a photodiode array detector. A voltage of +30 kV was applied during all separations, with temperature maintained at 25 °C. Injections were carried out hydrodynamically with a pressure of 50 mbar used with various injection times. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 75 μm I.D., 375 μm O.D., and 50 μm I.D., 375 μm O.D. of varying lengths were used in conjunction with the appropriate capillary alignment interfaces. Capillaries of length 48.5 cm (40.0 cm to detector) were used.

Spectrophotometric measurements were conducted using a Cary UV–Vis–near IR (NIR) spectrophotometer (Varian Australia) with 1-cm pathlength quartz cells.

2.2. Reagents

Chrysoidine (4-phenylazo-*m*-phenylenediamine hydrochloride) was obtained from Fluka, Switzerland. Hydroxypropylmethylcellulose (HPMC), 2-hydroxyisobutyric acid (HIBA) (98%), imidazole (99%) and lactic acid (85+%) were obtained from Aldrich (Milwaukee, WI, USA). A 50% (w/w) solution of poly(ethyleneimine) of average molecular mass 50 000–60 000 was obtained from Acros Organics (Geel, Belgium). Solutions of potassium, barium, strontium, calcium, sodium, magnesium,

manganese, chromium, iron, cobalt and lithium were prepared from nitrate and chloride salts obtained from APS (NSW, Australia), Sigma–Aldrich (Milwaukee, WI, USA) and BDH (Victoria, Australia). Solutions of lanthanum, cerium, praseodymium, neodymium, samarium, europium and gadolinium were prepared from nitrate salts obtained from Koch-Light Labs. (Colnbrook, UK). Water treated with a Millipore (Bedford, MA, USA) Milli-Q system was used to prepare standard solutions and electrolytes.

2.3. Procedures

Fused-silica capillaries were coated with poly-(ethyleneimine) (PEI) by flushing with 1 *M* sodium hydroxide for 30 min, water for 30 min followed by a 4% poly(ethyleneimine) solution for 1 h which was then left to stand in the capillary for 30 min. The capillary was finally flushed with water for 30 min before use.

Chrysoidine was purified using the following approach. A solution of 3 g of chrysoidine dissolved in 150 ml of Milli-Q water was placed in a separating funnel. Sodium hydroxide (1 *M* solution) was added dropwise until a colour change from red to yellow occurred. The deprotonated dye was then extracted with three successive 50-ml portions of chloroform. The organic extracts were collected and were washed with four 100-ml portions of Milli-Q water in order to back-extract inorganic salts and impurities. The organic layer was then dried over anhydrous sodium sulfate and was gravity filtered through a fluted filter paper. The solution was evaporated to dryness using rotary evaporation. The solid product was then dissolved in 100 ml of ethanol and was acidified with hydrochloric acid to regenerate the dye as the hydrochloride salt. The solution was rotary evaporated to dryness with successive 100-ml portions of ethanol to remove excess hydrochloric acid. The final product was dried at 100 °C for 2 h. A final yield of 1.8 g was obtained and the product was stored under nitrogen.

Detection limits were calculated at a signal-to-noise ratio of two.

Separation efficiencies were calculated based on the peak width at half-height. Plate numbers for a 48.5-cm capillary length are given.

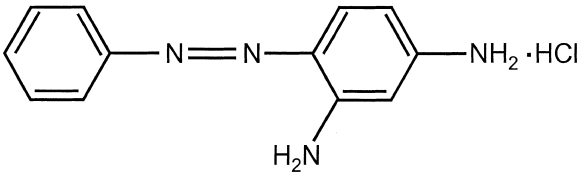

Fig. 1. Structure of chrysoidine.

3. Results and discussion

3.1. Selection of the dye to be used as a probe

Chrysoidine (Fig. 1) was chosen for investigation as it is highly absorbing, monovalent and has a moderate mobility. All three factors were expected to result in improved sensitivity when compared with other probes used for cationic detection. Chrysoidine has an absorption maximum at 453 nm and an absorptivity at this wavelength of 23 427 l mol^{-1} cm^{-1}, which is approximately five to 10 times greater than the absorptivities of commonly used probes. Increased detection sensitivity and peak heights should follow (Eq. (1)). In order to obtain well-shaped and symmetrical peaks, the mobility of the probe should match the mobility of the analytes as closely as possible. Cationic dyes typically have mobilities significantly less than the electrophoretic mobilities of most metal ions. Accordingly, it would be beneficial to use a cationic dye with a relatively high mobility. As the vast majority of commercially available dyes are monovalent, those with lower molecular masses should offer the highest possible mobilities. The mobility of chrysoidine measured at pH 4 with an imidazole BGE was found to be -32×10^{-9} m^2 V^{-1} s^{-1}, which offers an acceptable mobility match for most metal ions. The use of a monovalent probe will also provide the highest possible transfer ratios, which should maximise detection sensitivity.

Analysis of chrysoidine (as supplied) using an imidazole–HIBA electrolyte showed that it contained a significant amount of sodium. It has been clearly demonstrated that introduction of co-ions must be avoided in indirect detection systems [16]. The dye was therefore purified using the procedure outlined in the Experimental section. Analysis of the purified

dye by capillary electrophoresis with an imidazole–HIBA electrolyte showed that the purity was increased from 91 to 99.3% using this method, resulting in a suitable level of purity for use as an indirect detection probe for cations. The absorptivity of the purified dye was determined to be 26 733 l mol^{-1} cm^{-1}.

Initial investigations were made using BGEs containing only chrysoidine without the addition of a complexing agent in order to determine suitable operating conditions. It was to be not viable to perform detection at the absorption maximum of chrysoidine (453 nm) as baseline noise was far too high due to the low light intensity provided by the deuterium lamp in the visible region. A compromise wavelength of 230 nm was therefore chosen to provide the best combination of low baseline noise and chrysoidine absorptivity. The absorptivity of chrysoidine at this wavelength was only about 40% of its maximum absorptivity. An arbitrary probe concentration of 5 m*M* was used for initial studies and the background absorbance of BGEs containing this chrysoidine concentration was well inside the upper detector linearity limit of the particular CE instrument used (780 mAU). This moderately high dye concentration was chosen in order to decrease electromigration dispersion, improve stacking effects and potentially to increase detection sensitivity. It has also been shown previously that dyes can be used at this concentration only if adsorption of the dye to capillary walls can be prevented or at least reduced to an acceptable level [14,15]. When a 5 m*M* chrysoidine BGE without any additives was used on an uncoated capillary, the resultant baseline was unstable and irreproducible. Baseline stability was improved markedly by coating the capillary with PEI [15]. Addition of HPMC to the BGE also helped to further stabilise the baseline and to suppress the electroosmotic flow (EOF). In a similar fashion to the use of Orange G at a high BGE concentration [15], the application of a small amount of inlet pressure assisted the attainment of a stable baseline. The use of a capillary of reduced inner diameter helped to maintain separation efficiency. Hence, all further work was carried out on a 50-μm PEI-coated capillary with the addition of 0.05% HPMC to the BGE and the application of a small amount of pressure to the inlet side of the capillary.

3.2. Separation with addition of complexing agents to the BGE

The conditions described above were found to be suitable for the separation of a small number of metal ions. However, separation of mixtures of several metal ions was not possible without addition of a complexing agent. The addition of HIBA to a 5 m*M* chrysoidine solution caused the pH to decrease from 4.1 to 3. At this lowered pH, the concentration of H^+ present causes competitive displacement with the probe, leading to a loss of sensitivity, especially for the high-mobility cations. Adjustment of the pH of the BGE by addition of a buffer was not possible without introduction of unwanted co-ions. Titration with anion-exchange resin in the hydroxide form was investigated as a method of avoiding the addition of co-ions but the dye was found to undergo strong and irreversible adsorption onto the resin. Similarly, replacement of the dye counter-anion (chloride) for hydroxide or a weak acid complexing agent using anion-exchange was unsuccessful.

This problem was solved by modifying the dye purification process. The neutral, free-base form of the dye was isolated by evaporating the chloroform-containing dye solution (see Experimental) to dryness prior to the reformation of the dye hydrochloride. The solid product was collected, oven dried and stored under nitrogen. Analysis of the purified dye showed the absence of chloride or any other anions, suggesting that the dye was present as a free base. In this form, the dye could be titrated with a solution of a weak acid complexing agent without the pH falling below 4. The dye was therefore used with the desired complexing agent present as the dye counter-anion.

The two complexing agents chosen for use in conjunction with chrysoidine were HIBA and lactic acid. Both have been used widely in the separation of a range of metal ions [17–20]. Since the emphasis of the present study was on detection sensitivity rather than on separation selectivity, the concentrations of HIBA and lactic acid were not optimised and arbitrary concentrations at 5 and 10 m*M*, respectively, were used. These values were chosen on the basis of previous successful applications of the two complexing agents. The necessity for BGEs for indirect detection to be properly buffered without the

introduction of co-ions has been well documented. In order to provide sufficient buffering, the pH of the electrolyte was adjusted to the pK_a of the complexing agent used. Hence the complexing agent provided both complexation effects to enhance separation selectivity and also the required buffering capacity.

3.3. Performance of the BGE

A mixture of alkaline earth, transition metals and lanthanides was chosen to investigate the performance of the BGE described above. The separation with HIBA is given in Fig. 2 and shows that good peak shapes were obtained for analytes having a very wide mobility range, indicating that the mobilities of the complexed metal ions matched the mobility of chrysoidine. A separation of 18 cations was achieved in 4.5 min. Good peak shapes were also observed in the separation of 16 cations in less than 3.5 min using lactic acid as the complexing agent (Fig. 3). Complexation with lactic acid did not reduce the mobilities of the analytes as markedly as did HIBA. It should be pointed out that neither the lactate nor HIBA systems were optimised with regard to the concentration of the probe and the complexing agent and it would be expected that such optimisation

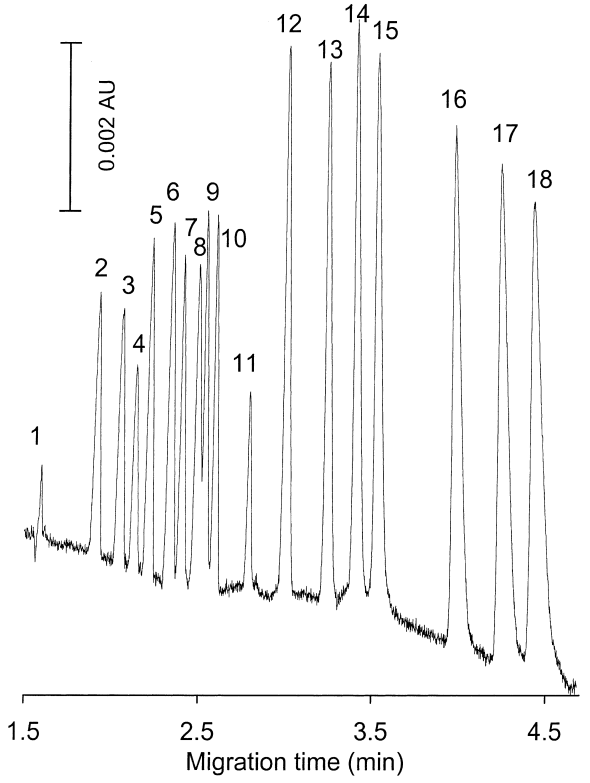

Fig. 2. Electropherogram of 50 μ*M* cation mixture with HIBA as complexing agent. Peak identification: 1 = potassium, 2 = barium, 3 = strontium, 4 = calcium, 5 = sodium, 6 = magnesium, 7 = manganese, 8 = chromium, 9 = iron, 10 = cobalt, 11 = lithium, 12 = lanthanum, 13 = cerium, 14 = preaseodymium, 15 = neodymium, 16 = samarium, 17 = europium, 18 = gadolinium. Conditions: capillary: PEI coated fused-silica, 0.485 m (0.40 m to detector) × 50 μm I.D.; electrolyte: 5 m*M* chrysoidine, 5 m*M* HIBA, 0.05% HPMC, pH 4.0; separation voltage: +30 kV (current 3 μA at 25 °C); separation pressure: 20 mbar, detection: indirect photometric at 230 nm; pressure injection: 50 mbar for 3 s.

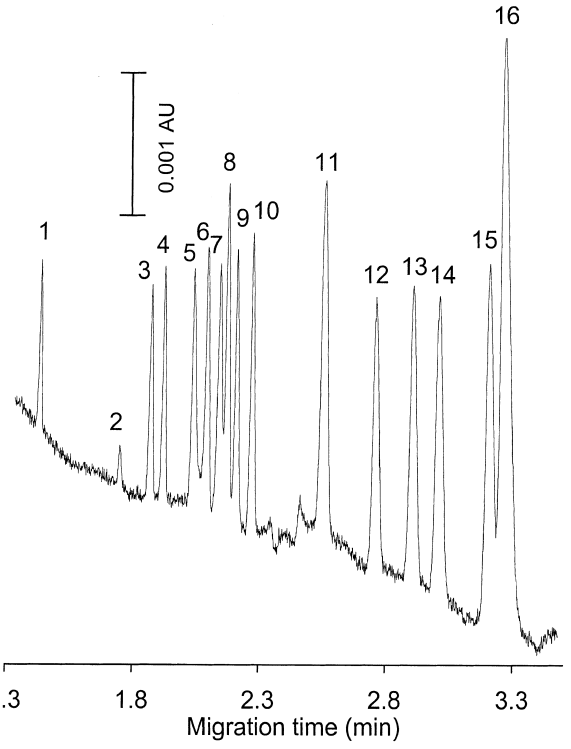

Fig. 3. Electropherogram of 10 μ*M* cation mixture with lactic acid as complexing agent. Peak identification: 1 = potassium, 2 = barium, 3 = strontium, 4 = calcium, 5 = sodium, 6 = magnesium, 7 = manganese, 8 = chromium, 9 = iron, 10 = cobalt, 11 = lanthanum, 12 = cerium, 13 = praseodymium, 14 = neodymium, 15 = samarium, 16 = europium. Conditions: electrolyte: 5.0 m*M* chrysoidine, 10.0 m*M* lactic acid, 0.05% HPMC, pH 3.8, voltage: +30 kV (current 6 mA at 25 °C), separation pressure: 20 mbar, injection: 50 mbar for 8 s. All other conditions as in Fig. 2.

would lead to improved separations and better sensitivity. Detection limits and separation efficiencies were calculated from an injection of a 2 μ*M* mixture (Figs. 4 and 5) and are recorded in Table 1. For comparison purposes, the same 2 μ*M* mixture was analysed using a previously reported optimised 5.0 m*M* imidazole, 6.75 m*M* HIBA electrolyte and is included for comparison purposes in Figs. 4 and 5. Peak heights with chrysoidine are larger than those obtained with imidazole, especially considering the fact that a 75-μm I.D. capillary was used with imidazole compared to a 50-μm capillary with chrysoidine. Injection volumes in all three cases were the same. Significantly, peak heights and shapes for low mobility analytes were very poor when the relatively high mobility imidazole probe was used. On the other hand, peak shapes were consistently good for all analytes when chrysoidine

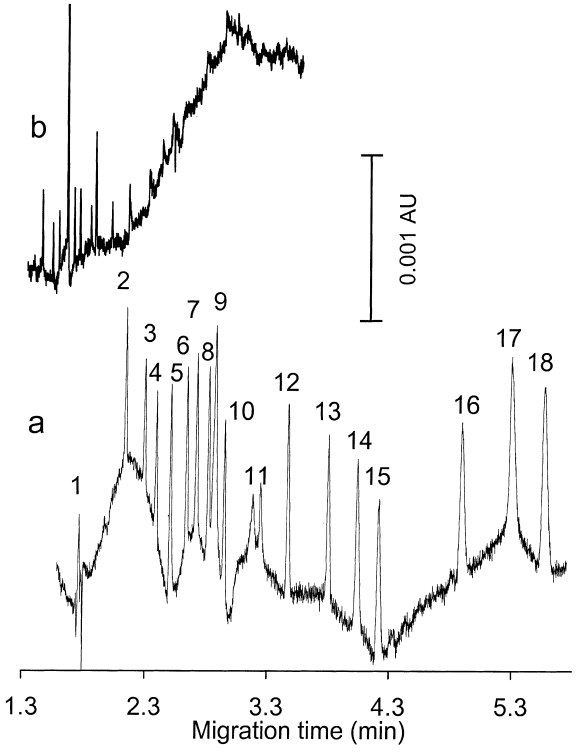

Fig. 4. Electropherogram of 2 μ*M* cation mixture with (a) 5.0 m*M* chrysoidine, 5.0 m*M* HIBA BGE and (b) 5.0 m*M* imidazole, 6.75 m*M* HIBA BGE. Conditions: (b) 5.0 m*M* imidazole, 6.75 m*M* HIBA, 75 μm bare fused-silica capillary; injection: (a) 50 mbar for 15 s, (b) 50 mbar for 3 s. All other conditions as in Fig. 2.

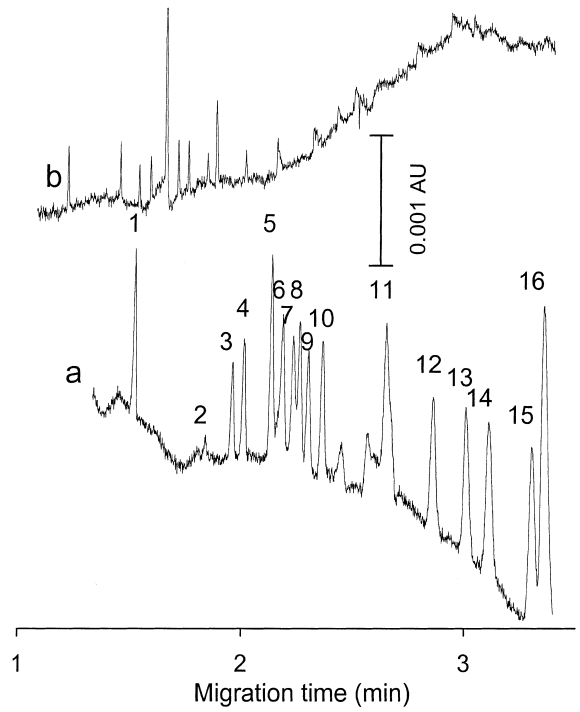

Fig. 5. Electropherogram of 2 μ*M* cation mixture with (a) 5.0 m*M* chrysoidine, 10 m*M* lactic acid BGE and (b) 5.0 m*M* imidazole, 6.75 m*M* HIBA BGE. Conditions: (b) 5.0 m*M* imidazole, 6.75 m*M* HIBA, pH 4.0, 75 μm bare fused-silica capillary; injection: (a) 50 mbar for 15 s, (b) 50 mbar for 3 s. All other conditions as in Fig. 3.

was used as a probe. Detection limits when HIBA or lactate was used were in the range 0.22–0.61 and 0.12–1.43 μ*M*, respectively. This sensitivity is remarkable considering that chrysoidine does not have an absorption maximum compatible with the use of a deuterium lamp light source. The use of a more suitable light source, such as an appropriate LED, could be expected to lead to even lower detection limits. Better signal-to-noise ratios may also result from careful optimisation of the probe concentration. Regardless of these restrictions, the detection limits achieved here are superior to most other previously reported values obtained using hydrodynamic injection without any sample preconcentration.

Separation efficiencies were acceptable, with values ranging from 72 170 to 164 210 for HIBA and 40 300–100 530 for lactic acid. Increased efficiencies were observed for electrolytes containing HIBA because of a better correlation between the mobilities

Table 1
Detection limits (LODs, μ*M*) and efficiencies (plate numbers, *N*) obtained with 5.0 m*M* chrysoidine electrolytes with (a) HIBA and (b) lactic acid as complexing agents; reproducibility results obtained with chrysoidine–HIBA BGE

Analyte	HIBA		Lactate		Reproducibility (%RSD)		
	LOD (μ*M*)	Efficiency (*N*)	LOD (μ*M*)	Efficiency (*N*)	Time	Corrected peak area	Peak height
Potassium	0.52	96 180	0.21	83 640	0.23	2.67	3.05
Barium	0.30	132 040	1.43	45 500	0.27	2.30	2.92
Strontium	0.32	137 620	0.36	85 580	0.22	2.79	3.48
Calcium	0.29	135 990	0.29	79 520	0.22	2.56	3.02
Sodium	0.22	99 990	0.19	97 780	0.24	2.21	3.57
Magnesium	0.25	153 350	0.29	78 340	0.24	2.38	3.82
Manganese	0.25	139 000	0.31	61 570	0.23	2.08	2.90
Chromium	0.26	164 210	0.26	100 530	0.23	2.50	2.89
Iron	0.22	59 400	0.31	92 740	0.24	2.76	3.23
Cobalt	0.26	142 660	0.26	87 050	0.23	2.42	2.37
Lithium	0.61	89 930			0.27	3.49	2.95
Lanthanum	0.23	126 620	0.24	40 300	0.26	2.92	2.80
Cerium	0.27	138 270	0.27	64 160	0.26	2.09	2.53
Praseodymium	0.27	88 000	0.24	65 880	0.29	2.12	2.38
Neodymium	0.29	95 870	0.23	55 390	0.29	2.60	2.46
Samarium	0.30	81 580	0.23	82 300	0.30	2.67	2.98
Europium	0.28	75 470	0.12	56 990	0.34	2.56	2.83
Gadolinium	0.25	72 170			0.35	2.42	2.13

Conditions: sample: 2 μ*M* of each cation, electrolyte: (a) 5.0 m*M* chrysoidine, 5.0 m*M* HIBA, 0.05% HPMC, pH 4.0 or (b) 5.0 m*M* chrysoidine, 10.0 m*M* lactic acid, 0.05% HPMC, pH 3.8, injection 15 s at 50 mbar. All other conditions as in Fig. 2.

of the metal–ligand complexes and that of chrysoidine. Increasing the chrysoidine concentration in order to improve stacking effects could further increase the separation efficiencies of both systems.

In order to demonstrate that the use of complexing agents operated at a pH value close to their pK_a provided proper buffering, a 50 μ*M* mixture was analysed for 20 consecutive runs without electrolyte replacement or replenishment. Very little change in migration times, peak areas and peak heights occurred between the first and the 20th runs. Relative standard deviations (RSDs) for migration time, corrected peak area and peak height are recorded in Table 1. Migration time reproducibility ranged from 0.22 to 0.35%, which was less than the values typically reported for CE, particularly considering the large number of consecutive runs used to generate the reproducibility data. Similarly, reproducibility for corrected peak areas (2.08–3.49%) and peak heights (2.13–3.82%) was very satisfactory. The use of a coated capillary and a weak acid complexing agent operated at its pK_a has therefore provided a stable, reproducible, and well buffered system.

4. Conclusions

This work has shown that significant improvements in indirect detection sensitivity for cations can be attained using a highly absorbing dye as a probe. Associated problems such as baseline stability, lack of probe purity and pH effects can be overcome. Isolation of the cationic dye as a free base was found to be necessary to allow the addition of a weak acid to act as a complexing agent without decreasing pH to a region where competitive displacement with H^+ would become significant. The dye could then be titrated with the weak acid and adjusted to a desired pH. This allowed excellent separations of a metal mixture and highly sensitive detection to be carried out. The detection limits achieved were amongst the best reported for indirect detection of cations. Reproducibility and buffering was provided using complexing agents at pH values close to their pK_a values.

It could be expected that further increases in sensitivity could be gained by optimisation of the concentrations of the probe and complexing agent. A significant decrease in detection limits would also

occur if the dye could be used at a wavelength at which it absorbs more strongly. This was not possible for chrysoidine with the particular instrument used in this study. Peak heights at the absorption maximum of 453 nm were observed to be roughly twice those at 230 nm, but baseline noise was about four times greater due to the lower output of the deuterium lamp at 453 nm. The use of an LED light source operating in the visible region of the spectrum could solve this problem.

In summary, the use of the highly absorbing dye chrysoidine as a probe has led to sensitive detection of a wide range of metal cations. Sensitivity was enhanced compared with less absorbing, commonly used probes for the indirect detection of cations. This study has laid the foundation for further use of dyes for the indirect detection of cations in capillary electrophoresis.

Acknowledgements

The website for the Australian Centre for Research on Separation Science is http://www.acros-s.utas.edu.au/

References

[1] M.T. Ackermans, F.M. Everaerts, J.L. Beckers, J. Chromatogr. 549 (1991) 345.
[2] M.W. Nielen, J. Chromatogr. 588 (1991) 321.
[3] G.J.M. Bruin, A.C. Van Asten, X. Xu, H. Poppe, J. Chromatogr. 608 (1992) 97.
[4] J.L. Beckers, J. Chromatogr. A 679 (1992) 153.
[5] M. Macka, P.R. Haddad, Electrophoresis 18 (1997) 2482.
[6] F. Foret, S. Fanali, L. Ossicini, P. Bocek, J. Chromatogr. 470 (1989) 299.
[7] P. Doble, M. Macka, P.R. Haddad, J. Chromatogr. A 804 (1998) 327.
[8] Y.J. Xue, E.S. Yeung, Anal. Chem. 65 (1993) 2923.
[9] R. Zhang, C.L. Cooper, Y. Ma, Anal. Chem. 65 (1993) 704.
[10] Z. Mala, R. Vespalec, P. Bocek, Electrophoresis 15 (1994) 1526.
[11] P.A.G. Butler, B. Mills, P.C. Hauser, Analyst 122 (1997) 949.
[12] T. Higashijima, T. Fuchigami, T. Imasaka, N. Ishibashi, Anal. Chem. 64 (1992) 711.
[13] C. Johns, M. Macka, P.R. Haddad, M. King, B. Paull, J. Chromatogr. A 927 (2001) 237.
[14] C. Johns, M. Macka, P.R. Haddad, Electrophoresis 23 (2002) 43.
[15] C. Johns, M.J. Shaw, M. Macka, P.R. Haddad, Electrophoresis, in press.
[16] C. Johns, M. Macka, P.R. Haddad, Electrophoresis 21 (2000) 1312.
[17] Y. Shi, J.S. Fritz, J. Chromatogr 640 (1993) 473.
[18] T.-I. Lin, T.-H. Lee, Y.-C. Chen, J. Chromatogr. A 654 (1993) 167.
[19] M. Chen, R.M. Cassidy, J. Chromatogr. 640 (1993) 425.
[20] N. Shakulashvili, T. Faller, H. Engelhardt, J. Chromatogr. A 895 (2000) 205.