ELSEVIER

Journal of Chromatography A, 671 (1994) 397–402

JOURNAL OF
CHROMATOGRAPHY A

Evaluation of carrier electrolytes for capillary zone electrophoresis of low-molecular-mass anions with indirect UV detection

Sarah M. Cousins, Paul R. Haddad*, Wolfgang Buchberger

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

---

Abstract

Indirect UV detection in capillary zone electrophoresis has been investigated with respect to transfer ratios for low-molecular-mass analyte anions and chromate or various aromatic carboxylic acids acting both as carrier electrolytes and detection probes. The transfer ratio can be regarded as a measure of the effectiveness by which analyte ions displace the UV-absorbing probe ions and has been found to depend on the relative mobilities of the analyte and the carrier. Highest values of transfer ratios have occurred when the carrier ion had a higher mobility than the analyte ion. This trend follows the Kohlrausch theory, but still considerable deviations between calculated and experimental data have been encountered. Consideration of the transfer ratio, the molar absorptivity of the probe and the degree of separation achieved, suggests that chromate is the best electrolyte for high-mobility inorganic anions. The results obtained enable predictions of the most suitable carrier electrolyte for various applications with indirect UV detection.

---

1. Introduction

In recent years, capillary zone electrophoresis (CZE) has become a highly efficient separation technique for inorganic and low-molecular-mass organic anions [1–3]. Many of these species have no or only negligible UV absorbance, so that indirect UV detection is currently the preferred detection mode. In this form of detection, a UV-absorbing species (or "probe") having the same charge as the analyte is used as electrolyte. Displacement of the probe by the migrating analyte creates a region where the concentration of the probe is less than that in the bulk electrolyte, and this forms the basis of indirect detection [4]. Optimal sensitivity can be achieved if several conditions are fulfilled. First, the detection wavelength should be such that the carrier electrolyte ion generates a UV-absorption background and the sample ion absorption is minimal. Second, the molar absorptivity of the carrier electrolyte ion should be high in order to ensure high sensitivity; the concentration of the carrier electrolyte must be high enough to ensure a high dynamic range but should be such that the background absorbance of the electrolyte falls within the linear range of the detector and is at a level where baseline noise remains low. Third, the mobility of carrier electrolyte ions should be similar to that of analyte ions in order to maintain the shape of the migrating zone as an approximately Gaussian distribution and so avoid a deterioration in resolution.

Sensitivity in indirect UV detection is gov-

---

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1223-M

erned by the molar absorptivity of the carrier electrolyte and its charge. One might expect that the displacement of the probe ion by the migrating analyte would occur on an equivalent-per-equivalent basis. Nevertheless, investigations into quantitative aspects of indirect UV detection published by Nielen [5] show that, from the Kohlrausch theory, the change concentration ($\Delta[B]$) of a probe ion (B) caused by an analyte concentration [A] is given by:

$$\Delta[B] = -\frac{z_A}{z_B} \cdot \frac{\mu_B[\mu_A + \mu_C]}{\mu_A[\mu_B + \mu_C]} \cdot [A] \qquad (1)$$

where z is the charge of the analyte (A) and the probe (B) and μ the effective electrophoretic mobility of the analyte (A), the probe (B) and the counter-ion (C). Eq. 1 indicates that equivalent-per-equivalent displacement can be expected only if the analyte has the same mobility as the background ions. When this is not the case, sensitivity of detection will be affected to some extent.

In this paper we report the determination of transfer ratios (TR) for several carrier electrolytes commonly used for anion separation by CZE, including chromate, benzoate, phthalate, trimellitate and pyromellitate. The term "transfer ratio" as used throughout this paper is defined as the number of moles of probe ions displaced by one mole of analyte ions. The data obtained have been used to investigate the degree to which transfer ratios calculated from the Kohlrausch theory can be verified. The general pattern arising from these data should be useful for predictions of optimal carrier electrolyte compositions for various applications.

2. Experimental

2.1. Instrumentation

The CZE instrument employed was a Quanta 4000 (Waters, Milford, MA, USA) interfaced to a Maxima 820 data station (Waters). Separations were carried out using an AccuSep (Waters) fused-silica capillary (60 cm × 75 μm I.D., effective length 52 cm). Injection was performed hydrostatically by elevating the sample at 10 cm for 30 s at the cathodic side of the capillary. The run voltage was −20 kV. Indirect UV detection at 254 nm was used.

2.2. Reagents and procedures

Carrier electrolytes (5 m*M*) were prepared in water treated with a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus using sodium chromate, benzoic acid, phthalic acid, trimellitic acid (1,2,4-benzenetricarboxylic acid) or pyromellitic acid (1,2,4,5-benzenetetracarboxylic acid). The pH was adjusted to 8.0 using sodium hydroxide or hydrochloric acid. All chemicals used were of analytical-reagent grade. OFM Anion BT (Waters) was used as an electroosmotic flow modifier in all carrier electrolytes at a concentration of 0.5 m*M*, except in the case of the pyromellitate electrolyte which was used in combination with 0.5 m*M* hexamethonium bromide (Sigma, St. Louis, MO, USA).

Relative migration times of probe anions were obtained by injecting these anions into a 5 m*M* phosphate buffer pH 8 and measuring migration times relative to bromide by direct UV detection at 214 nm. Molar absorptivities of the probes were determined using a Varian 5E UV–Vis–near-IR spectrophotometer with 1 cm path length quartz cells.

3. Results and discussion

3.1. Characteristics of the probe ions

At the electrolyte pH used (8.0) in this work, all of the probes are fully ionized, so that the charge varies between −1 (for benzoate) and −4 (for pyromellitate). The molar absorptivities and relative migration times (with respect to bromide) of the probe ions are listed in Table 1, which shows that chromate has the highest mobility, followed by the series of aromatic carboxylic acids arranged in order of decreasing charge. A schematic representation of the relative migration times of the probes and those of

Table 1
Experimentally determined molar absorptivities and relative migration times

Probe	Molar absorptivity ($l\,mol^{-1}\,cm^{-1}$)	Relative migration time[a]
Chromate	3180	1.000
Pyromellitate	7062	1.125
Trimellitate	7147	1.232
Phthalate	1357	1.365
Benzoate	809	1.804

[a] Measured with respect to bromide.

the analyte anions used in this study is given in Fig. 1. It can be seen that only three of the probes (chromate, pyromellitate and trimellitate) have relative migration times (and hence mobilities) which fall within the approximate range covered by the analyte anions, with the remaining two probes having significantly longer relative migration times (*i.e.* lower mobilities).

3.2. Determination of transfer ratios

The determination of transfer ratios was based on the following sequence of steps.

The first step involved the injection of the probe ions (*i.e.* chromate, benzoate, phthalate, trimellitate and pyromellitate) into a UV-transparent electrolyte, which was a phosphate buffer pH 8, using direct UV detection at 254 nm. Calibration plots (peak area *versus* molar concentration) were established for each probe and in order to account for the influence of different migration velocities on the peak area monitored by the detector, all peak areas were multiplied by the apparent velocities of the ions. These velocities were calculated from the migration times and the length of the capillary. The slopes of the calibration plots were calculated from these data and these slopes provided quantitative values for the area counts per mole of each probe.

The next step involved the injection of analyte ions (chloride, sulphate, citrate, fluoride and phosphate) using one of the probes as carrier electrolyte and indirect UV detection at 254 nm. A calibration plot of peak area of analyte ion *versus* molar concentration were prepared for each analyte ion and the slope calculated. As can be seen from Fig. 2A, the slopes for analyte ions of the same charge (which would be expected to be the same if equivalent-per-equivalent displacement of the probe occurred) differed considerably unless the areas were corrected for the varying migration velocities of the sample band. However, even after multiplication of peak areas by migration velocities some differences in slopes still remain, such as for sulphate and phosphate (see Fig. 2B). These variations must be attributed to differences in the transfer ratios of the analyte anions.

In the final step, transfer ratios were calcu-

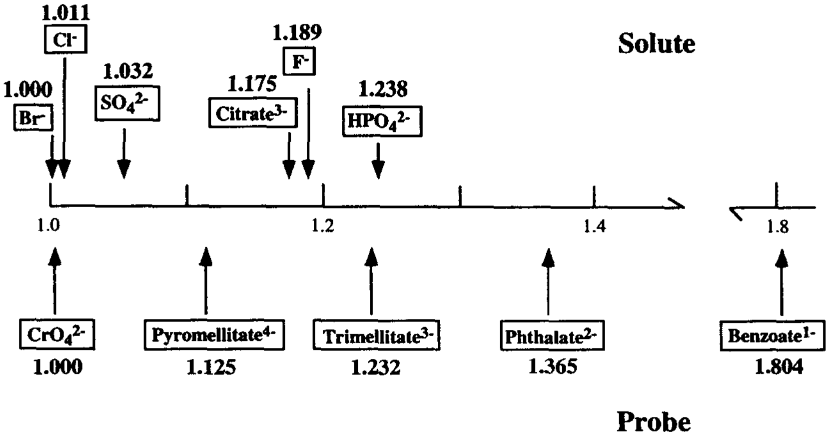

Fig. 1. Relative migration times of the probes and analyte anions used in this study.

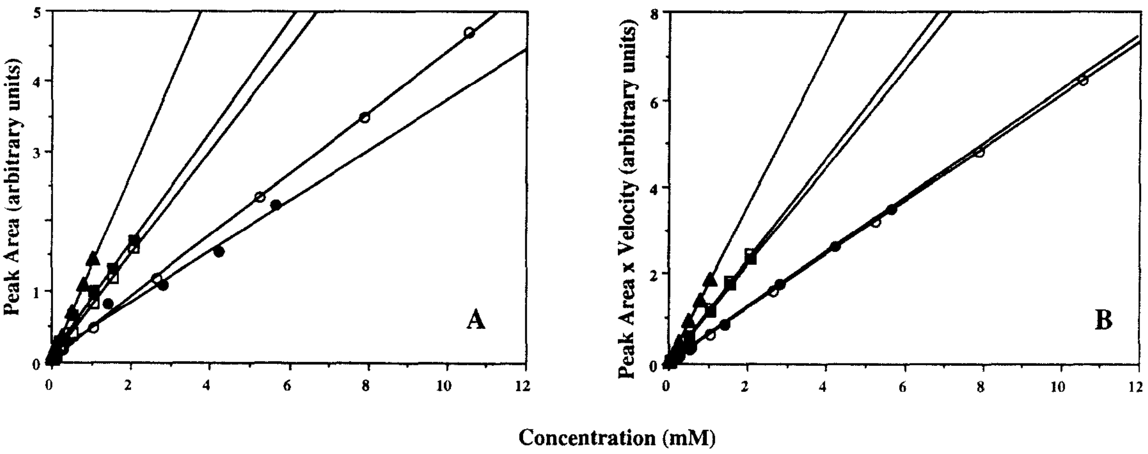

Fig. 2. Calibration plots using trimellitate as probe. (A) Without and (B) with correction for differing migration velocities of the analyte anions. ○ = Fluoride; ● = chloride; □ = sulphate; ■ = phosphate; ▲ = citrate.

lated for each analyte ion. The transfer ratio is the quotient of the slope of an analyte calibration plot and the slope of the probe calibration plot. The entire process was repeated for each probe.

Table 2 summarises the results for transfer ratios of the analyte ions using different probes. The experimentally determined transfer ratios are compared with the ratios expected on the basis of an equivalent-to-equivalent exchange

Table 2
Experimentally determined transfer ratios and comparison with calculated data

		Probe				
		Benzoate	Phthalate	Trimellitate	Pyromellitate	Chromate
Chloride	a	0.184	0.205	0.325	0.366	0.608
	b	1.000	0.500	0.333	0.250	0.500
	c	0.651	0.423			0.521
Fluoride	a	0.421	0.233	0.319	0.356	0.718
	b	1.000	0.500	0.333	0.250	0.500
	c	0.748	0.486			0.599
Sulphate	a	0.897	0.575	0.612	0.625	1.124
	b	2.000	1.000	0.667	0.500	1.000
	c	1.277	0.831			1.023
Phosphate	a	1.065	0.463	0.585	0.584	1.218
	b	2.000	1.000	0.667	0.500	1.000
	c	1.476	0.960			1.182
Citrate	a	1.629	0.614	0.931	0.593	1.756
	b	3.000	1.500	1.000	0.750	1.500
	c	2.019	1.313			1.617

a = Transfer ratios from experiment; b = transfer ratio calculated on the basis of an equivalent-to-equivalent exchange; c = transfer ratio calculated according to ref. 5.

between probe and analyte. Table 2 also includes the transfer ratios calculated from Eq. 1 [5], in which the calculations have been based on mobility data from the literature [6,7]. The experimental data follow the general trend seen in the calculated data, but the fit of the experimental data to the model is poor.

An overview of the transfer ratios and their dependencies on different probes can be achieved if the transfer ratios of the analytes are multiplied by the charge of the probes (in order to normalise variation in transfer ratios arising from the different charges of the probes) and plotted against the relative mobilities of the probes (expressed as the inverse of the relative migration times shown in Fig. 1). This plot is given in Fig. 3. Considering the two univalent analytes (chloride and fluoride), it can be seen that the transfer ratio, corrected for the charge on the probe, should ideally be unity (that is, the lowermost broken line in Fig. 3). The observed values are less than unity for benzoate and phthalate (identified as probes 1 and 2, respectively, in Fig. 3), close to unity for trimellitate (probe 3) and greater than unity for pyromellitate and chromate (probes 4 and 5, respectively). A similar pattern is evident for the two divalent analytes, sulphate and phosphate, with the transfer ratio values being less than the expected value (namely 2) for the probes of low mobility and greater than this value for the probes of higher mobility. The same can be said for the behaviour of the trivalent analyte, citrate. Whilst this trend is in general accordance with Eq. 1, the data show some anomalies. For example, the transfer ratios for chloride and fluoride are almost unity for the trimellitate probe, however its mobility is close only to that of fluoride. Other examples of such anomalies can be seen. Nevertheless, Fig. 3 demonstrates clearly that the transfer ratio of an ion generally increases as the mobility of the probes increases (provided that the charge of the probes remains the same).

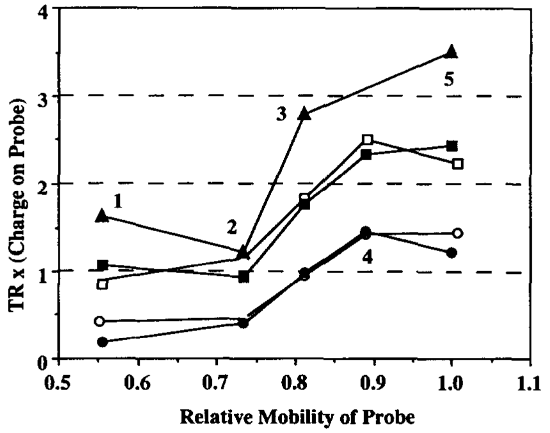

Fig. 3. Dependence of transfer ratios (multiplied by the charge on the probe) upon the relative mobility of the probe. Probes: 1 = benzoate; 2 = phthalate; 3 = trimellitate; 4 = pyromellitate; 5 = chromate. The broken lines show the theoretical values for solutes having a single, double and triple charge. Symbols as in Fig. 2.

3.3. Maximising detection sensitivity

In addition to the transfer ratio, the molar absorptivity of the probe must be taken into account if the sensitivity of indirect UV detection with different probes is to be evaluated. In Fig. 4, the observed transfer ratios multiplied by the absorptivity of the probe are plotted against the relative mobilities of the probes. The higher the value of this product, the higher the sensitivity of indirect UV detection. The fact that the transfer ratios of trimellitate, pyromellitate and chromate

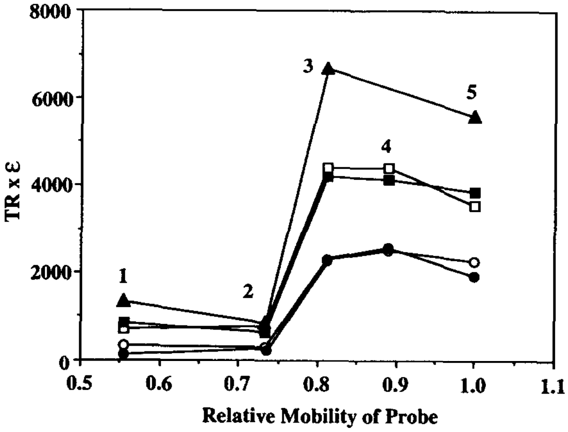

Fig. 4. Dependence of transfer ratios multiplied by the absorptivity of the probe upon the mobility of the probe. Probes: 1 = benzoate; 2 = phthalate; 3 = trimellitate; 4 = pyromellitate; 5 = chromate. Symbols as in Fig. 2.

exceed the equivalent-per-equivalent values, combined with their high molar absorptivities, suggest that these probes should be preferred to benzoate and phthalate. Fig. 2 suggests that optimal sensitivity for most analyte anions will result with trimellitate as probe.

Since the probe also acts as a background electrolyte, selection of a suitable probe must include not only the consideration of high sensitivity but also of separation efficiency. It might become necessary to compromise on sensitivity in order to obtain a desired degree of resolution between species to be separated. A comparison of the separations achieved with chromate, trimellitate and pyromellitate showed that chromate provided the best overall performance for the separation of the analyte anions used in this paper and is therefore the preferred probe, despite a small loss in sensitivity resulting from the fact that its molar absorptivity is less than that for trimellitate.

The results obtained in this paper can be used to predict suitable probes for the indirect detection of a variety of anions. For example, alkanesulphonic acids can be separated and detected using probes such as *p*-phenolsulphonate, *p*-toluenesulphonate or *p*-hydroxybenzoate. The relative migration times (with respect to bromide) of these probes in a phosphate buffer are 1.85, 2.01 and 2.06, respectively. The alkane sulphonates from ethanesulphonate to pentanesulphonate have relative migration times between 1.67 and 2.06. The use of *p*-phenolsulphonate as probe should therefore give the highest transfer ratios. However, *p*-hydroxybenzoate is likely to be the best probe on the basis that its molar absorptivity at 254 nm is much greater than that of the other two probes (ϵ of *p*-hydroxybenzoate: 10299 $\mathrm{l\ mol^{-1}\ cm^{-1}}$; ϵ of *p*-phenolsulphonate: 783 $\mathrm{l\ mol^{-1}\ cm^{-1}}$; ϵ of *p*-toluenesulphonate: 344 $\mathrm{l\ mol^{-1}\ cm^{-1}}$).

4. References

[1] W.R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445.
[2] W.R. Jones, *J. Chromatogr.*, 640 (1993) 387.
[3] M.P. Harrold, M.J. Wojtusik, J. Riviello and P. Henson, *J. Chromatogr.*, 640 (1993) 463.
[4] F. Foret, S. Fanali, L. Ossicini and P. Boček, *J. Chromatogr.*, 470 (1989) 299.
[5] M.W.F. Nielen, *J. Chromatogr.*, 588 (1991) 321.
[6] J.A. Dean (Editor), *Lange's Handbook of Chemistry*, McGraw-Hill, New York, 14th ed., 1992, p. 8.158.
[7] D.R. Lide (Editor), *Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 72nd ed., 1991, p. 5-96.