Journal of Chromatography, 588 (1991) 321–326
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 625

Quantitative aspects of indirect UV detection in capillary zone electrophoresis

M. W. F. Nielen

AKZO Research Laboratories Arnhem, Corporate Research, Analytical Chemistry Department, P.O. Box 9300, 6800 SB Arnhem (Netherlands)

(First received April 29th, 1991; revised manuscript received July 5th, 1991)

ABSTRACT

Quantitative aspects of indirect UV absorbance detection in capillary zone electrophoresis were studied using the separation of sodium alkylsulphate surfactants as a model system. Nearly uniform response factors, excellent reproducibility of electrophoretic mobilities and linearity of the detection signal can be obtained when veronal buffer is used as the UV-absorbing background electrolyte. The feasibility of the system for the analysis of commercial mixtures of primary sodium alkyl sulphates is demonstrated.

INTRODUCTION

Capillary zone electrophoresis (CZE) offers rapid and efficient separations of ionic and ionizable compounds [1]. One of the major weak points in CZE is the lack of a sensitive and universal detection system. Indirect detection modes [2] might be a relatively simple solution to the detection of both organic and inorganic compounds without a suitable chromophore.

Several indirect detection approaches have been described, including indirect UV absorbance [3–5], indirect fluorescence [6–8] and indirect amperometric [9] detection.

Despite the impressive detection limits obtained and the interesting applications shown, the applicability of indirect detection in CZE in quantitative analysis might be questioned. Foret *et al.* [4] discussed the selection of a suitable UV-absorbing background electrolyte and the relationship with electromigration dispersion. They concluded that the highest sensitivity is obtained for those analytes having an effective electrophoretic mobility close to the mobility of the absorbing background ion. However, for a given background ion, the useful dynamic range of this detection mode was reduced to approximately one order of magnitude for analytes with mobilities deviating from the mobility of the background ion.

A second drawback for quantitative analysis using the (universal) indirect detection mode follows from the Kohlrausch theory [10]. The change in concentration of the absorbing background ion, d[B], caused by an analyte concentration [A] can be calculated using the following equation:

$$d[B] = -\frac{\mu(B)\{\mu(A) + \mu(C)\}}{\mu(A)\{\mu(B) + \mu(C)\}} \cdot [A]$$

where the μ terms are the effective electrophoretic mobility of the analyte (A), the UV-absorbing background ion (B) and the counter ion (C). Consequently, one-to-one displacement will only occur for analytes having the same mobility as the background ion. Other analytes will have different response factors, which have to be calculated from accurately determined mobility data.

An additional disadvantage of indirect detection systems is their poor stability. Owing to the low concentrations of the background electrolyte usually applied, electroosmosis is very sensitive towards

0021-9673/91/$03.50

impurities, pH shifts, etc. Baseline problems and irreproducible mobilities might be observed [5].

In this work, we used indirect UV absorbance detection with veronal buffer as a background electrolyte. The CZE separation of aliphatic anionic surfactants was used as a model system, in order to study quantitative aspects such as the dependence of the response factor on the mobilities of the analytes, the reproducibility of the electrophoretic mobilities and the coefficient of electroosmotic flow, and the linear dynamic range. The feasibility of the analysis of commercial mixtures of primary sodium alkyl sulphates is demonstrated.

EXPERIMENTAL

Apparatus

An Applied Biosystems (San Jose, CA, USA) Model 270A capillary electrophoresis system [11] was used, equipped with a variable-wavelength UV absorbance detector, operated at 240 nm and a 0.2 s rise time. CZE was performed in a 70 cm × 50 μm I.D. fused-silica capillary (Applied Biosystems) at +25 kV (constant-voltage mode), thus yielding a current of 2–6 μA, depending on the buffer concentration used. The capillary was thermostated at 30.0°C. Samples were introduced into the capillary via a controlled vacuum system; the injection time was 0.5 s, which corresponds to a volume of *ca.* 1.5 nl.

The coefficient of electroosmotic flow was calculated using the migration time of the system peak. The effective electrophoretic mobilities and the plate numbers were calculated using the equations in ref. 12. Data were recorded using a Nelson Analytical Model 4400 integration system.

Chemicals

Ultrex-grade water was obtained from J. T. Baker (Deventer, Netherlands). Veronal (5,5-diethylbarbituric acid) buffer, Teepol HB7 and sodium octanesulphonate (further referred to as C_8) were obtained from Sigma (St. Louis, MO, USA). Sodium *n*-dodecyl sulphate (C_{12}), sodium decyl hydrogensulphate (C_{10}) and sodium nonyl hydrogensulphate (C_9) were obtained from Merck (Darmstadt, Germany). Technical-grade sodium chloroethylsulphonate (C_2) was obtained from laboratory stock.

Methods

The UV-absorbing veronal buffers were prepared in water, yielding either 6 or 12 m*M* solutions having a pH value of 8.6. Buffers were filtered through 0.45-μm Spartan 30/B membrane filters (Schleicher & Schüll, Dassel, Germany) prior to use. A stock solution of the C_2–C_{12} analytes, $2 \cdot 10^{-3}$ *M* each (except C_2, *ca.* $4 \cdot 10^{-3}$ *M*) was prepared in water. Sample solutions were prepared by dilution of the stock solution with the veronal buffer.

RESULTS AND DISCUSSION

Performance of the separation and detection limit

The performance of the CZE system using indirect UV absorbance detection is clearly shown in Fig. 1. All components of the test mixture (10^{-4} *M* of each C_8–C_{12} surfactant and *ca.* $2 \cdot 10^{-4}$ *M* of C_2) were baseline resolved, even those differing by only one CH_2 group. The efficiencies of the C_8–C_{12} peaks were 360 000–440 000 theoretical plates in the 12 m*M* and 350 000–390 000 in the 6 m*M* veronal system. The lower plate numbers in the latter situation and also the lower plate number for C_2 (*cf.*, Tables I and II) have to be attributed to electromigration dispersion caused by the less favourable analyte-to-buffer concentration ratio (60 *versus* 120).

The dynamic reserve, as determined for the 6 m*M* veronal system, was calculated to be 1100, yielding a theoretical detection limit of *ca.* $1 \cdot 10^{-5}$ *M* or

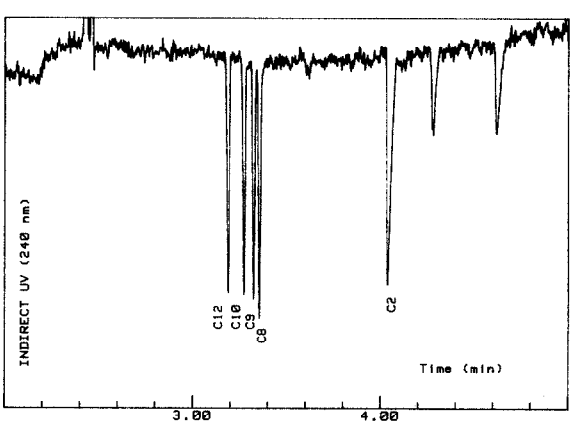

Fig. 1. CZE separation of a mixture of C_2–C_{12} alkyl sulphates using 12 m*M* veronal buffer at pH 8.6. For other conditions, see Experimental.

TABLE I

REPEATABILITY OF MOBILITIES AND DETECTION SIGNAL

Mean values of six analyses ± relative standard deviation (R.S.D.). μ(eo) = 0.849 · 10^{-3} cm^2 V^{-1} s^{-1} ± 0.14% R.S.D. Conditions: 12 m*M* veronal buffer (pH 8.6); other conditions as under Experimental.

Component	μ(ep) (10^{-3} cm^2 V^{-1} s^{-1})	Peak height (μV)	Area/time (μV)	Plate number
C_{12}	−0.2172 ± 0.15%	117 ± 2.2%	4.59 ± 3.0%	360 000
C_{10}	−0.2352 ± 0.15%	112 ± 2.9%	4.46 ± 3.1%	380 000
C_9	−0.2458 ± 0.15%	108 ± 4.8%	4.24 ± 4.4%	395 000
C_8	−0.2520 ± 0.15%	114 ± 4.4%	4.55 ± 3.9%	440 000
C_2	−0.3569 ± 0.14%	128 ± 2.6%	6.85 ± 4.3%	230 000

15 fmol (signal-to-noise ratio 2). The detection limits obtained experimentally [(1–2) · 10^{-5} *M*] were in good agreement with this value.

Repeatability of the mobilities and the detection

The repeatability of the system was evaluated for both 12 and 6 m*M* veronal buffers. The test mixture (10^{-4} *M* each, except C_2, *ca.* 2 · 10^{-4} *M*) was analysed six times and the coefficient of electroosmotic flow, μ(eo), the electrophoretic mobilities, μ(ep), the peak heights and the peak areas were determined.

Contrary to ref. 8, the peak areas were corrected for the different velocities of the zones in the detection window by dividing their values by the corresponding migration times [3]. The calculated mean values and standard deviations are presented in Table I (12 m*M* buffer) and Table II (6 m*M* buffer).

It can be concluded from Tables I and II that the mobilities are very constant. Obviously, a buffering UV-absorbing background ion is very beneficial, even at concentrations as low as 6 m*M*. Both peak heights and corrected peak areas can be used for precise quantification. The detector signal decreased by a factor of *ca.* 1.5 (instead of 2) on changing from 6 to 12 m*M* buffer. This discrepancy can be explained when the corresponding UV background levels are taken into account; 0.11 absorbance for 6 m*M* and 0.20 absorbance for 12 m*M* veronal at 240 nm. According to Foret *et al.* [4], it can be calculated that the linearity of Beer's law is limited in CZE to *ca.* 0.1 absorbance. From this point of view, a background electrolyte of 6 m*M* veronal will be the practical upper limit at 240 nm.

An impression of the reproducibility was obtained by the analysis of a freshly prepared C_9–C_{12} mixture using a 12 m*M* veronal CZE system, re-assembled 1 year later. The coefficient of electroosmotic flow, μ(eo), was 0.810 · 10^{-3} cm^2 V^{-1} s^{-1}, only 5% less than the value given in Table I. The electrophoretic mobilities of C_{12}, C_{10} and C_9 were calculated to be $-0.221 \cdot 10^{-3}$, $-0.239 \cdot 10^{-3}$ and $-0.250 \cdot 10^{-3}$ cm^2 V^{-1} s^{-1}, respectively; only 2% less than

TABLE II

REPEATABILITY OF MOBILITIES AND DETECTION SIGNAL

Conditions: 6 m*M* veronal buffer (pH 8.6); other conditions and explanation as in Table I.

Component	μ(ep) (10^{-3} cm^2 V^{-1} s^{-1})	Peak height (μV)	Area/time (μV)	Plate number
C_{12}	−0.2203 ± 0.17%	150 ± 3.3%	6.20 ± 3.4%	365 000
C_{10}	−0.2382 ± 0.14%	151 ± 3.0%	6.01 ± 3.9%	350 000
C_9	−0.2488 ± 0.16%	145 ± 2.7%	5.73 ± 3.3%	395 000
C_8	−0.2550 ± 0.13%	159 ± 1.8%	6.07 ± 3.0%	370 000
C_2	−0.3678 ± 0.08%	151 ± 1.7%	9.10 ± 2.2%	175 000

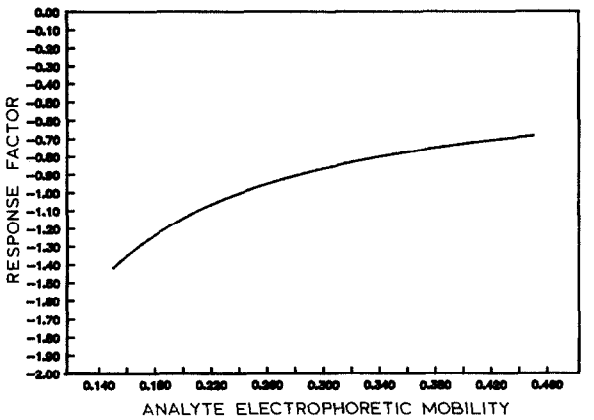

Fig. 2. Theoretical dependence of the response factor of an individual analyte on its electrophoretic mobility. The mobilities of the background ion and the counter ion are assumed to be $-0.241 \cdot 10^{-3}$ and $0.528 \cdot 10^{-3}$ cm^2 V^{-1} s^{-1}, respectively.

the values in Table I. The corresponding area/time data were found to be 5–10% less than the data presented in Table I.

Response factors

The effective electrophoretic mobility of veronal was determined using 12 m*M* borate buffer and found to be $-0.241 \cdot 10^{-3}$ cm^2 V^{-1} s^{-1}. The electrophoretic mobility of the counter ion, sodium, was taken from the literature ($0.528 \cdot 10^{-3}$). Assuming that these mobility data are applicable to the CZE veronal system, and using the equation presented in the Introduction, a theoretical curve showing the dependence of the individual response factor of an analyte on its corresponding electrophoretic mobility can be derived (Fig. 2).

From Fig. 2, it can be seen that this dependence is very moderate for analytes differing only slightly in mobility. The C_8–C_{12} surfactants would show a difference in response factor of less than 10%. Consequently, one can conclude that indirect UV detection of homologues, such as alkyl sulphates, might yield nearly uniform response factors, provided that the effective electrophoretic mobility of the veronal ion is carefully adjusted using the pH value.

The experimental data presented in Table I show a significantly higher peak height and area/time for C_{12} than for C_9 and C_2 (after correction for the two-fold higher concentration of C_2) and are in good agreement with the theoretical differences in the response factors, as shown by Fig. 2. The other data (except for C_8, which showed too high response factors throughout this study, possibly owing to a concentration error) show the same theoretical trend but more data will be required to support the significance of these differences statistically. The peak-height data for C_9, C_{10}, C_{12} and C_2 in Table II show significantly lower response factors than C_8. This discrepancy must be attributed to electromigration dispersion of the former components (the ratio of the concentrations of the background ion and the analytes is only 60 in Table II). Note that this discrepancy also implies that the electrophoretic mobility of C_8 must be very close to the effective electrophoretic mobility of veronal, *i.e.*, the mobility of veronal in the present CZE system will be *ca.* $-0.255 \cdot 10^{-3}$ instead of $-0.241 \cdot 10^{-3}$ cm^2 V^{-1} s^{-1} (which value had been determined in a borate CZE system; see above).

The area/time data in Table II show again for C_{12} a significantly higher response than for C_9 and C_2: C_{12} shows an 8% higher area/time than C_9 (8–9% predicted by Fig. 2); the area/time of C_2 is 79% relative to C_9 (80–81% is predicted by Fig. 2). As in Table I, the other data (except for C_8) showed the same theoretical trend, but the differences were statistically too small to be significant.

Linearity of the detector response

The linearity of the detection of the C_8–C_{12} surfactants was studied using a 6 m*M* veronal buffer and sample concentrations of 0, $2 \cdot 10^{-5}$, $6 \cdot 10^{-5}$, $2 \cdot 10^{-4}$, $6 \cdot 10^{-4}$ and $2 \cdot 10^{-3}$ *M*. Each analysis was carried out in duplicate.

The data for C_8, C_9 and C_{10} at $2 \cdot 10^{-3}$ *M* and for C_8 and C_9 at $2 \cdot 10^{-4}$ *M*, representing partially overlapping asymmetric peaks, could not be integrated reliably with our integration software and the corresponding area/time data were therefore rejected from the data sets.

Curve fitting of the mean peak heights yielded a second-order equation owing to eletromigration dispersion (overloading) at higher concentrations. Interestingly, curve fitting of the mean area/time data yielded straight lines, regression data for which are shown in Table III. The slopes for C_{12} and C_{10} are higher, which supports the theory about the response factors. However, correlations of the slopes

TABLE III

CURVE FITTING OF THE MEAN AREA/TIME DATA

Concentrations ranging from 0 up to $2 \cdot 10^{-3}$ *M*. For other conditions, see text.

Component	Slope	Intercept	Regression (r^2)
C_{12}	0.63	+0.53	1.000
C_{10}	0.64	−0.01	0.999
C_9	0.54	+0.15	1.000
C_8	0.57	+0.12	1.000

with the theoretical response factors are risky because of the curve fitting data, which are based on duplicate measurements only (contrary to the repeatability data above and the associated discussion about response factors), and the impact of the lower accuracy of the data points at concentrations near the detection limit on the regression analysis.

Application

As an application, we analysed a commercial sample of C_9–C_{13} primary sodium alkyl sulphates. The Teepol HB7 sample was diluted 5000-fold with veronal buffer and analysed using the 6 m*M* veronal system. The electropherogram thus obtained is shown in Fig. 3. The relative content of each surfactant in the sample was calculated using both peak-height and area/time data, and using both assumed uniform response factors and the real

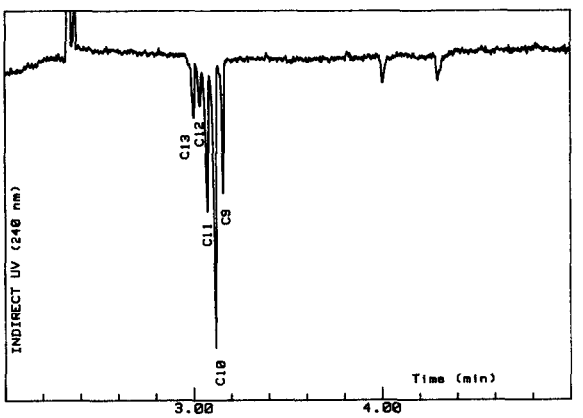

Fig. 3. CZE separation of primary sodium alkyl sulphates in Teepol using 6 m*M* veronal buffer at pH 8.6. For other conditions, see Experimental.

TABLE IV

RELATIVE COMPOSITION OF A COMMERCIAL SAMPLE

Conditions: 6 m*M* veronal CZE system; sample, Teepol HB7; calculation with either assumed uniform response factors or after correction for the theoretical factors.

Component	Relative content (%) based on			
	Peak height		Area/time	
	Uncorrected	Corrected	Uncorrected	Corrected
C_{13}	9.1	8.4	8.6	8.0
C_{12}	7.2	6.9	6.1	5.8
C_{11}	22.2	21.8	23.7	23.3
C_{10}	42.1	42.7	44.2	44.9
C_9	19.4	20.1	17.2	17.9

response factors, as calculated with the theoretical equation. The results are presented in Table IV. It can be concluded that the use of uniform response factors yields only small errors (less than 1% absolute). The area/time data will be less influenced by electromigration dispersion and are to be preferred.

CONCLUSIONS

CZE with indirect UV absorbance detection can provide constant mobility data provided that a UV-absorbing ion is selected with buffering capability under the pH conditions used. Veronal buffer was found to fulfil this requirement. Quantitative analysis with nearly uniform response factors can be achieved for a series of homologues, *e.g.*, sodium alkyl sulphates, provided that the effective electrophoretic mobility of veronal is carefully adjusted. The linear dynamic range of indirect UV detection in CZE covers more than two orders of magnitude, as long as area/time data are considered. The veronal system can be successfully applied to the determination of the relative composition of technical mixtures of aliphatic anionic surfactants.

REFERENCES

1 J. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
2 E. S. Yeung and W. G. Kuhr, *Anal. Chem.*, 63 (1991) 275A.

3 S. Hjerten, K. Elenbring, F. Kilar, J.-L. Liao, A. J. C. Chen, C. J. Siebert and M. D. Zhu, *J. Chromatogr.*, 403 (1987) 47.
4 F. Foret, S. Fanali, L. Ossicini and P. Bocek, *J. Chromatogr.*, 470 (1989) 299.
5 G. J. Bruin, A. C. van Asten, J. C. Kraak and H. Poppe, presented at the *3rd International Symposium on High-Performance Capillary Electrophoresis, San Diego, CA, February 3–6, 1991*, poster PM57.
6 W. G. Kuhr and E. S. Yeung, *Anal. Chem.*, 60 (1988) 1832.
7 W. G. Kuhr and E. S. Yeung, *Anal. Chem.*, 60 (1988) 2642.
8 L. Gross and E. S. Yeung, *J. Chromatogr.*, 480 (1989) 169.
9 T. M. Olefirowicz and A. G. Ewing, *J. Chromatogr.*, 499 (1990) 713.
10 F. Kohlrausch, *Ann. Phys. (Leipzig)*, 62 (1987) 208.
11 S. E. Moring, J. C. Colburn, P. D. Grossman and H. H. Lauer, *LC · GC Int.*, 3 (1990) 46.
12 M. W. F. Nielen, *J. Chromatogr.*, 542 (1991) 173.