Isotachophoretic desorption of anionic compounds from solid-phase adsorbents

P.J.M. Hendriks*, H.A. Claessens and J.T.H. Verhoeven

Department of Chemical Engineering, Laboratory of Instrumental Analysis, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven (Netherlands)

T.H.M. Noij

KIWA NV Research and Consultancy, P.O. Box 1072, 3430 BB Nieuwegein (Netherlands)

C.A. Cramers

Department of Chemical Engineering, Laboratory of Instrumental Analysis, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven (Netherlands)

ABSTRACT

The isotachophoretic (ITP) desorption of anionic compounds adsorbed on solid-phase extraction (SPE) columns is described. Octadecyl (C_{18})-modified silica and styrene–divinylbenzene (ST–DVB) packings were used as the SPE adsorbents. The influence of methanol on the ITP desorption of the test compounds was also examined. The ITP desorption of amaranth red as the test compound from both the ODS and ST–DVB adsorbents showed good results. In contrast, the ITP desorption of bromophenol blue resulted in a discontinuous desorption and no sharp zones were obtained. However, the addition of methanol to both the leading and terminating electrolytes during the ITP desorption in combination with a counter flow improved the desorption of bromophenol blue significantly. The ITP desorption of benzoic acid from the ST–DVB phase showed no reproducible results, whereas its desorption yields from the ODS phase was significantly better.

INTRODUCTION

The application of high-performance liquid chromatography (HPLC) or capillary zone electrophoresis to the determination of compounds in complex matrices or at low concentration levels often necessitates a sample pretreatment step. As demonstrated previously, both zone electrophoresis and isotachophoresis (ITP) can be used as on-line sample pretreatment steps for ionic compounds prior to HPLC analysis [1–3].

Another interesting application of electrophoresis is solid-phase extraction (SPE), followed by the desorption of the compounds by ITP. This technique, which can be termed isotachophoretic desorption, was first described by Kašička and Prusík [4–7]. They used ITP to desorb some proteins from an affinity adsorbent. Other groups have reported the use of zone electrophoresis as the desorption step after SPE (*e.g.*, [8–18]). In principle, the electrophoretic desorption of solutes adsorbed on SPE materials may contribute to a more selective desorption of the target compounds in smaller sample volumes.

In this paper, the preliminary results of the

---

* Corresponding author.

ITP desorption of some anionic compounds from C_{18}-modified silica and styrene–divinylbenzene as SPE adsorbents are presented. To correlate the ITP desorption data with the adsorption capacities of the test compounds on both SPE adsorbents, breakthrough curves were also determined by HPLC experiments. Bromophenol blue, benzoic acid and amaranth red were used as test compounds. Because of the limited detection sensitivity of ITP equipment, the experiments were performed in the 10^{-5} mol/l range. The results of the breakthrough and desorption experiments are compared and discussed.

EXPERIMENTAL

Breakthrough volumes

The breakthrough volumes of the test solutes on the investigated SPE phases were measured by frontal analysis. The retention volume of a specific test solute minus 2.33 times the standard deviation was taken as the breakthrough volume [19]. A schematic diagram of the LC equipment for the determination of breakthrough volumes is presented in Fig. 1. This equipment consisted of a Model 100A pump (Beckman Instruments, Palo Alto, CA, USA), a Model PU4225 UV detector (Unicam, Cambridge, UK) and a Valco N-60 HPLC injection valve (VICI-AG Valco Europe, Schenkon, Switzerland). The 10×2 mm I.D. dry-packed Kel-F column was positioned in a C270 stainless-steel high pressure column cartridge holder (Upchurch Scientific, Oak Harbor, WA, USA). UV detection was performed at 225 nm. The detector output was recorded with a

BD41 potentiometric recorder (Kipp & Zonen, Delft, Netherlands) and a DC268 computer (Tulip, 's-Hertogenbosch, Netherlands) equipped with a laboratory-made Multilab-TS interface. Data handling was performed with Caesar software (B-Wise Software, Geleen, Netherlands). To measure the breakthrough volumes the column was conditioned with methanol. After washing the column with 0.25 ml of acidified demineralized water (pH 3.0), the equipment was flushed with the sample solution. By switching the six-way valve, adsorption of a compound could be measured. The flow-rate through the column was 0.5 ml/min. The samples and the demineralized water for the washing steps were acidified to pH 3.0 by adding 1 M hydrochloric acid.

Isotachophoresis

The ITP equipment for the desorption experiments consisted of a laboratory-constructed ITP apparatus [20] equipped with an in-line laboratory-made SPE column, two three-way Hamilton (Bonaduz, Switzerland) valves and a laboratory-made a.c. conductivity detector. Fig. 2 shows schematic diagrams of the ITP desorption equipment with and without the membrane pump. The I.D. of the 13-cm long PTFE capillary was 1.0 mm (Alltech, Laarne, Belgium) and the applied electric current during the ITP desorption was 100 μA. Kel-F columns of 7×2 mm
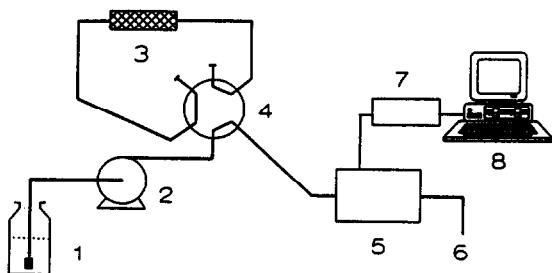

Fig. 1. Schematic diagram of the equipment for the determination of breakthrough volumes. 1 = Eluent; 2 = HPLC pump; 3 = SPE column; 4 = six-way valve; 5 = UV detector; 6 = waste; 7 = interface; 8 = computer.
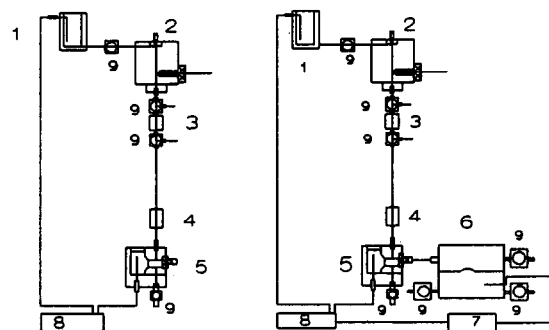

Fig. 2. Schematic diagrams of the ITP desorption equipment, (right) with and (left) without the membrane pump. 1 = Terminating electrolyte compartment; 2 = injection block; 3 = SPE column; 4 = conductivity detector; 5 = leading electrode compartment; 6 = membrane pump; 7 = interface; 8 = high-voltage power supply; 9 = Hamilton valve.

I.D. and 7 × 1 mm I.D. were used for the ITP desorption experiments. The 2-μm A113 polyethylene frits and the Tefzel C283 low-pressure column cartridge holder were from Upchurch Scientific. A number of ITP desorption experiments were also performed by applying a counter-flow membrane pump to increase the ITP desorption time. This pump was controlled by a Brandenburg (Thornton Heath, UK) 807R power supply, which also delivered the constant driving electric current for the ITP desorption experiment. The output of the conductivity detector to monitor the ITP desorption process was recorded and processed with the same data-handling equipment as described under *Breakthrough volumes*.

The leading electrolyte was 0.01 M HCl (pH 6.0). The counter ion was histidine and either 0.2% (w/w) hydroxyethylcellulose or 0.05% (w/w) poly(vinyl alcohol) was added to suppress the electroosmotic flow. The terminating electrolyte was a 0.005 M morpholinoethanesulphonic acid (MES) solution (pH 6.4) with histidine as the counter ion. If a counter-flow was applied, the terminating electrolyte was 0.00645 M MES solution (pH 6.43) with histidine as the counter ion. Conditioning and washing of the column were performed as described under the section *Breakthrough volumes*. After the column had been loaded with the sample, the column was washed with 0.25 ml of water (pH 3.0). Next, the ITP capillaries were filled with the leading and the terminating electrolytes and the desorp-

tion was started by applying the high-voltage power supply across the ITP equipment.

Chemicals

Benzoic acid (pK_a = 4.2), bromophenol blue (pK_a = 4.0), amaranth red, L-histidine and hydrochloric acid were from Merck (Darmstadt, Germany), HPLC-grade methanol from FSA Laboratory Supplies (Loughborough, UK), poly(vinyl alcohol) (Mowiol, M_r = 44 000) from Hoechst (Frankfurt, Germany), morpholinoethanesulphonic acid from Sigma (St. Louis, MO, USA) and hydroxyethylcellulose from Polysciences (Warrington, UK). Water was demineralized with a Milli-Q water purification system (Waters–Millipore, Milford, MA, USA). The 8-μm styrene–divinylbenzene (ST–DVB) copolymer was obtained from Polymer Labs. (Zeist, Netherlands) and the 10-μm C$_{18}$-modified silica (Zorbax ODS) from Rockland Technologies (New Port, DE, USA).

RESULTS AND DISCUSSION

Breakthrough volume experiments

The capacities of the stationary phases for the test solutes were determined under defined conditions in order to establish what concentration could be loaded on to the SPE columns to perform the subsequent ITP desorption experiments. The results of the measurements of the breakthrough volumes of the test solutes are summarized in Table I. Large differences in the

TABLE I

BREAKTHROUGH VOLUMES OF THE TEST COMPOUNDS ON THE TWO ADSORBENTS

Kel-F column (10 × 2 mm I.D.). All samples were acidified to pH 3.0 by the addition of hydrochloric acid. Flow-rate, 0.5 ml/min.

Experiment	Compound	Concentration (M)	Breakthrough volume (ml)	
			ODS	ST–DVB
1	Benzoic acid	$1 \cdot 10^{-6}$	0.8	10.4
2	Benzoic acid	$1 \cdot 10^{-5}$	0.7	7.2
3	Benzoic acid	$1 \cdot 10^{-4}$	0.7	5.7
4	Bromophenol blue	$1 \cdot 10^{-5}$	114	34
5	Amaranth red	$1 \cdot 10^{-5}$	24	9

capacities of the two SPE packing materials for bromophenol blue were obtained. These and similar differences for the other two compounds can be understood from the large differences in the interaction forces between these solutes and the SPE adsorbents, resulting in significant differences in their chromatographic behaviour.

Isotachophoretic desorption experiments

To study the efficiency of the ITP desorption of amaranth red and bromophenol blue from the SPE adsorbents the effluents, from these adsorbents were monitored visually. First 1 ml of a solution containing $1 \cdot 10^{-5}$ M of either amaranth red or bromophenol blue was adsorbed on the C_{18}-modified silica or the ST–DVB adsorbent using the 7×2 mm I.D. column as the SPE cartridge. Amaranth red was desorbed by ITP from both the SPE adsorbents as a narrow red zone showing sharp zone boundaries. In addition, after each experiment the remaining SPE packing was extracted with methanol in order to determine whether the ITP desorption process was completed. In the extracts no amaranth red could be observed visually, indicating that the desorption of this compound had been performed efficiently. In contrast, the desorption of bromophenol blue resulted in a non-continuous desorption process with diffuse zones. To investigate whether a local change in the electric field, by the connection of the applied 2 mm I.D. column to a 1 mm I.D. ITP capillary, might disturb the electrodesorption process, the experiments were repeated using the 7×1 mm I.D. column. Also in these experiments the ITP desorption of bromophenol blue resulted in broad diffuse zones.

To establish whether the electroosmotic flow (EOF) could be responsible for the observed irregular desorption patterns of bromophenol blue, 0.05% (w/w) of Mowiol was added to the washing water to suppress the EOF. Also in these experiments no improvement of the desorption of bromophenol blue was obtained.

Generally, ITP processes are considered to be completed when the situation indicated as the steady state has been achieved. To obtain this steady state in relatively short ITP capillaries, a counter flow, opposite to the migration direction

of the target compounds, can be applied. ITP desorption experiments of 1 ml of $1 \cdot 10^{-5}$ M bromophenol blue solutions applying a counter-flow were performed on both SPE packings in the 7×1 mm I.D. column. In these experiments a significant improvement of the desorption of bromophenol blue from both packings was obtained. These observations indicate that the ITP desorption of this compound can be improved by using longer ITP capillaries.

Next, the effects of the addition of 10% (v/v) of methanol to both the leading and terminating electrolytes on the desorption efficiency of bromophenol blue were investigated. In these experiments also a counter flow was applied during a short period of time. Again a significant improvement of the desorption of bromophenol blue was obtained. We assume that this might be explained by the decreased capacity of the stationary phase for this compound.

It is concluded that the ITP desorption of amaranth red from both SPE adsorbents could easily and completely be performed and resulted in sharp narrow zones. In contrast, the ITP desorption of bromophenol blue proved to be much more complicated and did not result in complete desorption from the SPE adsorbents. Reversed-phase liquid chromatographic stationary phases, which were used as the SPE adsorbents here, may show strongly different behaviour towards these compounds, especially under the aqueous conditions applied here. Therefore, we assume that the large differences in the ITP desorption of amaranth red and bromophenol blue may be explained by their different interaction forces towards the adsorbents.

In the above studies, the isotachophoretic desorption was monitored visually. Applying a conductivity detector in ITP analysis, electropherograms are obtained in which the conductivity signal is plotted on the ordinate as a function of time. In ITP the relative step height of the detector signal provides qualitative information about a solute while the length of a zone provides quantitative information.

To investigate the ITP desorption process further, experiments with benzoic acid as the test compound and applying a conductivity detector
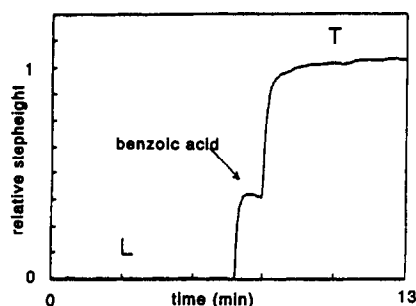
Fig. 3. Isotachopherogram of the electrodesorption of benzoic acid ($4 \cdot 10^{-8}$ mol) from the ST–DVB stationary phase (experiment 4, Table II); L = leading electrolyte, T = terminating electrolyte.

were carried out. The step height of benzoic acid in the applied operational system was 0.43. As an example, Fig. 3 shows an isotachopherogram of benzoic acid. Because the 10×2 mm I.D. column was too large to be fit into the cartridge holder and no evidence was found that a reduced I.D. (using the 7×1 mm I.D. column) improved the ITP desorption process, these electrodesorption experiments were carried out in 7×2 mm I.D. column, packed with either the C_{18}-modified silica or the ST–DVB adsorbent. The calibration graph of the amount of benzoic acid (A, in moles) versus the resulting ITP zone length (l, in seconds), determined by repeated direct injections of benzoic acid solutions in the injection compartment of the ITP equipment, was $l = 2.06 \cdot 10^9 A + 9.2$, with a regression coefficient $r = 0.994$.

The SPE columns were loaded with aqueous solutions of benzoic acid of concentration $2 \cdot 10^{-5}$ or $1 \cdot 10^{-4}$ M. The loading volumes ranged from 0.25 to 2.0 ml of standard solution. As can be seen in Table I, in most instances the amount of benzoic acid loaded on the SPE columns did not exceed the maximum capacity of these columns. The results of the ITP desorption of benzoic acid from both SPE adsorbents are summarized in Table II. From these results, it can be concluded that the recoveries and the repeatabilities of the desorption of benzoic acid from the adsorbents are limited in most instances. The reasons for these observations are not yet clear. Further studies will be focused on the elucidation of the ITP desorption process and especially the role of the nature of the SPE adsorbent.

CONCLUSIONS

Preliminary results on the desorption of amaranth red, bromophenol blue and benzoic acid by ITP from two different SPE adsorbents have been presented. From the data, it can be concluded that for amaranth red and benzoic acid the ITP desorption from both adsorbents resulted in narrow zones with sharp boundaries. However, the recoveries and the repeatability of the ITP desorption process with benzoic acid were poor. The ITP desorption of bromophenol blue showed poor results on both SPE adsorbents. However, a significant improvement of the desorption efficiency could be obtained by the application of a counter flow and the addition of 10% (v/v) of methanol to both ITP

TABLE II

RECOVERY EXPERIMENTS OF BENZOIC ACID BY ITP DESORPTION FROM THE ODS AND THE ST–DVB PHASES AS SPE ADSORBENTS IN THE 7×2 mm I.D. COLUMN

Experiment	Concentration (M)	Adsorbed volume (ml)	Adsorbed amount (mol)	Recovery (%)[a]	
				ODS	ST–DVB
1	$2 \cdot 10^{-5}$	0.25	$5 \cdot 10^{-9}$	90, 110	
2	$2 \cdot 10^{-5}$	0.5	$1 \cdot 10^{-8}$	103, 93	78
3	$2 \cdot 10^{-5}$	1.0	$2 \cdot 10^{-8}$	47, 21	62
4	$2 \cdot 10^{-5}$	2.0	$4 \cdot 10^{-8}$		56
5	$1 \cdot 10^{-4}$	0.5	$5 \cdot 10^{-8}$		84, 34, 66
6	$1 \cdot 10^{-4}$	1.0	$1 \cdot 10^{-7}$		62, 37, 39

[a] ODS-phase: experiments 1–3 are duplicate measurements; ST-DVB-phase: experiments 5 and 6 are triplicate measurements.

330

P.J.M. Hendriks et al. / J. Chromatogr. A 655 (1993) 325–330

electrolytes. Finally, it can be concluded that the ITP desorption of ionic substances from SPE adsorbents may have prospects as a sample treatment technique, although a more extensive study of the background of this technique is necessary.

ACKNOWLEDGEMENT

REFERENCES

1 P.J.M. Hendriks, H.A. Claessens, T.H.M. Noij, F.M. Everaerts and C.A. Cramers, Chromatographia, 11/12 (1992) 539.
2 A.J.J. Debets, R.W. Frei, K.P. Hupe and W.Th. Kok, J. Chromatogr., 465 (1989) 315.
3 A.J.J. Debets, H. El Arabi, K.-P. Hope, U.A.Th. Brinkman, P.J.M. Hendriks, H.A. Claessens, F.M. Everaerts and Th.M. Noij, Analyst, 117 (1992) 1355.
4 V. Kašička and Z. Prusík, J. Chromatogr., 273 (1983) 117.
5 V. Kašička and Z. Prusík, J. Chromatogr., 320 (1985) 75.
6 Z. Prusík and V. Kašička, J. Chromatogr., 320 (1985) 81.
7 Z. Prusík and V. Kašička, J. Chromatogr., 390 (1987) 39.
8 P. Atrat, V. Deppmeyer and C. Horhold, J. Chromatogr., 189 (1980) 279.
9 L.A. Haff, Electrophoresis, 2 (1981) 287.
10 M.R.A. Morgan, N.A. Slater and P.D.G. Dean, Anal. Biochem., 92 (1979) 144.
11 M.R.A. Morgan, N.A. Slater and P.D.G. Dean, Anal. Biochem., 105 (1980) 1.
12 R. Palluk, R. Muller and M. Kempfle, Fresenius' J. Anal. Chem., 311 (1982) 475.
13 J. Cai and Z. El Rassi, J. Liq. Chromatogr., 15-6/7 (1992) 1179.
14 J. Cai and Z. El Rassi, J. Liq. Chromatogr., 15-6/7 (1992) 1193.
15 H. Sioni, T. Tsuda and M.N. Novotny, J. Chromatogr., 559 (1991) 547.
16 N.A. Guzman, M.A. Trebilcock and J.P. Advis, J. Liq. Chromatogr., 14 (1991) 997.
17 A.J.J. Debets, M. Mazereeuw, W.H. Voogt, D.J. van Ieperen, H. Lingeman, K.P. Hupe and U.A.Th. Brinkman, J. Chromatogr., 608 (1992) 151.
18 M.E. Swartz and M. Merion, J. Chromatogr., 632 (1993) 209.
19 P. Subra, M.C. Hennion and R. Rosset, J. Chromatogr., 456 (1988) 121.
20 F.M. Everaerts, J.L. Beckers and Th.P.E.M. Verheggen, Isotachophoresis —Theory, Instrumentation and Applications (Journal of Chromatography Library, Vol. 6), Elsevier, Amsterdam, 1976.