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Anomalous behaviour of sodium in isotachophoresis

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ABSTRACT

Using a cationic operational system in capillary isotachophoresis, millimolar concentrations of sodium were determined in the absence and presence of serum proteins. With several internal standards, the reproducibility of the sodium determination was significantly poorer in the presence of proteins, even after partial thermal degradation. The results are explained by protein–sodium interaction in the injection port, where the electric field strength was sixteen times lower than in the separation capillary.

INTRODUCTION

The latest instrumental developments in capillary zone electrophoresis (CZE) prompted us to undertake a reassessment of the potential use of capillary isotachophoresis (ITP). In our view, future uses of ITP will mainly be focused on the following applications: (a) as a preparative, concentrating separation technique; (b) for the determination of non-UV-absorbing components at lower millimolar concentration levels as no sensitive universal detector is available for use in CZE; and (c) applications requiring high accuracy and precision, *e.g.*, as a reference method; this is not possible with any diluting separation technique, such as chromatography.

Our recent work has been focused on the third aspect. An example is the use of ITP as a reference method for the determination of barium and strontium in glass standards. In addition, the possibility of using ITP as a reference method for sodium in serum was the subject of an earlier publication [1]. Aqueous samples were analysed using different ITP equipment and a routine determination based on flame atomic emission spectrometry (FAES). The results were fairly promising. Determination in serum samples, however, showed a slight but significant intercept in favour of FAES [2]. Nevertheless, the calibration lines were still excellent with ITP.

It is well known that binding of many species to serum proteins does occur. Up to now, we have assumed that the electric field strength in the separation compartment was sufficiently high to free the bound components. In our example, irreversible binding of a tiny fraction of the sodium to serum proteins would explain the discrepancies found in the experiments mentioned. In this present work, experiments were designed to confirm this theory.

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EXPERIMENTAL

ITP equipment and system

Experiments were performed using laboratory-made ITP equipment described previously [3]. The separation compartment was a PTFE capillary with a length of 200 mm and 0.2 mm I.D. The detector was an a.c. conductivity detector. Chemicals used were all of analytical-reagent grade and were obtained from Merck (Darmstadt, Germany) unless stated otherwise. The operational system consisted of a solution of 0.01 mol/l $KHCO_3$ adjusted to pH 5.0 with citric acid as a leading electrolyte. The reason for not using KOH (Titrisol quality) was that the latter was difficult to keep sodium-free in a stock solution, essential in this experiment. In this system, calcium did not interfere in the separation, owing to complexation with the citric acid. The terminator was a solution of 0.005 mol/l creatinine, adjusted to pH 5 with citric acid.

The stabilized driving current of 30 μA was delivered by a modified Brandenburg (Thornton Health, U.K.) power supply. The conductivity detector signal and its differential were recorded with a Kipp (Delft, The Netherlands) Type BD41 flat-bed potentiometric recorder, with a paper speed of 5 mm/s.

The samples were injected with a 10-μl syringe (Hamilton, Bonaduz, Switzerland), equiped with a device to inject reproducibly at exactly the same location at the leading/terminator interface. The injection volume was 1 μl, which was adjusted and read under a microscope.

Samples and internal standards

Samples consisted of a healthy pooled serum and a solution of 40 g/l human serum albumin fraction V, HSA (Sigma, St. Louis, MO, U.S.A.). The effect of sodium binding to proteins was likely to be detected preferably at a low sodium content. Therefore, 1 ml of each of the samples was dialysed against 2 l of deionized water for 24 h to decrease the sodium content to *ca.* 1 mmol/l.

In addition, it was considered that if binding of sodium to serum proteins does occur, the effect will at least alter on degradation of the proteins. Therefore, half of each of the dialysed samples (after addition of the internal standard) was subjected to heat treatment for 30 min on a water-bath of 90°C. The protein-containing solutions turned opaque, indicating at least partial thermal degradation.

A blank sample, with respect to serum proteins, was prepared using a 2.50 mmol/l solution of sodium chloride. There were several reasons for using a mixture of three internal standards (I.S.). Solvent evaporation during thermal treatment can then be corrected for, using Na/I.S. zone-length ratios. Possible interactions between one of the internal standards and any of the other sample constituents can be verified by measuring the I.S./I.S. zone-length ratios. A solution of *ca.* 1 mmol/l of each of the following components was used as an internal standard: tetramethylammonium iodate (TMA), tetraethylammonium iodate (TEA) and histidine (HIS). Sample and internal standards were mixed in equal amounts of 1000 μl using a fixed-volume pipette with a disposable tip.

RESULTS AND DISCUSSION

A blank run indicated that the operational system was essentially sodium-free:

no additional zone transitions could be observed in the differential signal. In a typical isotachopherogram, only zones of sodium, TMA, TEA and HIS are visible (Fig. 1). Zone lengths were measured from the differential using a ruler. Zone-length ratios were calculated and are given in Table I.

The relative zone-length reproducibility of the internal standards was satisfactory, even for histidine, the mobility of which was close to that of the terminator. The I.S. reproducibility in the HSA solution was slightly lower less. It was concluded that all three internal standards performed satisfactorily.

Solvent evaporation during thermal treatment was *ca.* 3%, a seen from the average increase in absolute I.S. zone lengths. The determination of 2.50 mmol/l of sodium in the blank was found to be highly reproducible, with a 1% relative standard deviation. A small amount of sodium was found in both pooled serum and albumin solutions. The reproducibility of sodium determination in HSA-containing solutions was considered to be very poor.

Contrary to expectations the standard deviations for sodium determination in albumin-containing solutions did not allow conclusions regarding the effect of heat treatment. The interaction apparently is not manifested as an irreversible binding of a constant amount of sodium. In that event a significant increase on heat treatment would be expected.

The better reproducibility in the pooled serum than in the HSA solutions may be misinterpreted. It should be noted that with the pooled serum, a considerable dilution of the original protein content was the result of osmosis during dialysis, because of the high initial sodium content. It remains to be seen if further dilution of the sample, prior to injection, would decrease the effect to acceptable proportions.

In our view, the results indicate an irreproducible interaction between sodium

TABLE I

RELATIVE ZONE LENGTHS AND CORRESPONDING RELATIVE STANDARD DEVIATIONS (R.S.D.) OF SODIUM AND THE INTERNAL STANDARDS

Sample	Na/TMA	R.S.D. (%)	TMA/TEA	R.S.D. (%)	TEA/HIS	R.S.D. (%)
Pooled	0.81		0.84		0.81	
	0.76	9	0.85	1	0.80	1
	0.68		0.85		0.83	
Pooled, treated	0.67		0.85		0.81	
	0.67	5	0.84	1	0.84	1
	0.73		0.85		0.81	
HSA	0.73		0.85		0.83	
	0.67	11	0.85	1	0.83	1
	0.58		0.82		0.83	
HSA, treated	0.56		0.82		0.85	
	0.59	83	0.85	2	0.93	5
	1.47		0.83		0.81	
Blank	1.21		0.85		0.99	
	1.20	1	0.84	1	0.99	1
	1.21		0.84		0.97	
Blank, treated	1.23		0.86		0.98	

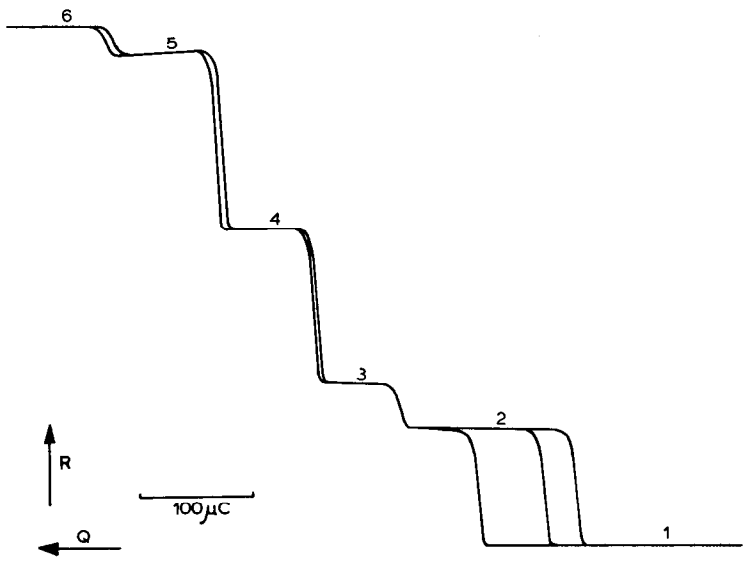

Fig. 1. Irreproducible results of the determination of millimolar concentrations of sodium in the presence of partially degradated pooled serum proteins. 1 = Potassium; 2 = sodium; 3 = trimethylammonium; 4 = triethylammonium; 5 = histidine; 6 = creatinine.

and albumin during ITP separation, inside the injection compartment. Here, the electric field strength is sixteen times lower than in the separation capillary. This sample matrix effect must be taken into account in the trace analysis of biological samples.

REFERENCES

1 A. A. G. Lemmens, J. C. Reijenga, F. M. Everaerts, R. T. P. Janssen, J. A. R. J. Hulsman and C. A. M. Meijer, *J. Chromatogr.*, 320 (1985) 193.
2 A. A. G. Lemmens, unpublished results.
3 F. M. Everaerts, Th. P. E. M. Verheggen and J. L. Beckers, *Isotachophoresis, Theory, Instrumentation and Applications*, (*Journal of Chromatography Libarary*, Vol. 5), Elsevier, Amsterdam, 1978.