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ISOTACHOPHORESIS AS A CANDIDATE REFERENCE METHOD IN ANALYTICAL CHEMISTRY

DETERMINATION OF SODIUM IN SERUM

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SUMMARY

Isotachophoresis seems a likely candidate for a reference method, as it is more accurate and precise than common routine methods. The response is highly linear and depends on a well defined transport number of the leading ion, stability of the driving current, mobility of the separand, which is well controlled by the leading electrolyte and the use of a high-resolution detector. The suitability of isotachophoresis as a reference method was investigated for the determination of sodium in human serum. The operational conditions were 0.01 *M* K^+/citrate (leading electrolyte) at pH 5.5 and 0.01 *M* creatinine · HCl (terminating electrolyte). Both *n*-butylamine and ammediol could be used as internal standards. The calibration graph constructed from standard solutions, diluted by weight with the internal standard, yielded a correlation coefficient of 0.99994 ($n = 50$) in the working range. The method seems especially useful for the determination of any ionic solute in, *e.g.*, clinical samples (lithium, calcium, creatinine or drugs).

INTRODUCTION

In general, analytical methods can be classified as routine, reference or definitive methods[1]. A definitive method is one that gives the best approximation of the so-called "true value". The definitive method for sodium, for example, consists of an ion-exchange pre-separation, followed by a gravimetric determination[2]. Such a method can only be carried out in well equipped laboratories and, because it needs laborious work and requires specialists, it is not attractive for routine determinations. For a good correlation of routine methods and definitive methods, reference methods can be used.

To be a candidate reference method, it is important that the method has a well defined theoretical basis and has high accuracy and precision. In this respect, iso-

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tachophoresis seems a likely candidate because of its high linearity, which depends on a well defined transport number of the leading ion, stability of the driving current, mobility of the separand, which is well controlled by the leading electrolyte, and the use of a high-resolution detector.

The suitability of isotachophoresis as a reference method was investigated for the determination of sodium in human serum[3]. Isotachophoresis may be an attractive alternative for another candidate reference method, *viz.*, flame atomic-emission spectrometry (FAES)[4].

EXPERIMENTAL

For the determination of sodium by isotachophoresis the choice of the operational system is important. Small amounts of sodium in the electrolytes make the method less accurate. In conventional electrolyte systems calcium would migrate near sodium and will often disturb the sodium zone. For this reason, citrate was used to lower the effective mobility of calcium. Potassium hydrogen carbonate and citric acid used for the leading electrolyte were obtained from Merck (Darmstadt, F.R.G.). Creatinine · HCl from Sigma (St. Louis, MO, U.S.A.) was used as the terminating electrolyte. Highly purified sodium chloride from Aldrich (Milwaukee, WI, U.S.A.) was used for standard sodium solutions. Ammediol and *n*-butylamine from Fluka (Buchs, Switzerland), used as internal standards, were sodium free. All chemicals were of analytical-reagent grade. Using the operational system summarized in Table I, there were no interferences from other ions in serum (see Fig. 1).

TABLE I

OPERATIONAL SYSTEM FOR THE DETERMINATION OF SODIUM IN SERUM

No further additives needed.

Parameter	*Leading electrolyte*	*Terminating electrolyte*
Cation	Potassium	Creatinine
Concentration	0.01 *M*	0.01 *M*
Counter ion	Citrate	Chloride
pH	5.50	7.0

The analyses were performed using the isotachophoresis equipment developed and built by Everaerts *et al.*[5]. The separation compartment consisted of a PTFE tube (*ca.* 250 × 0.2 mm I.D.). The direct constant electrical driving current was taken from a Brandenburg (Thornton Heath, U.K.) high-voltage power supply. The separated zones were detected by measuring the a.c. conductivity. The zone lengths were measured on recorder paper (peak-to-peak measurement) using the differential signal of the conductivity trace, with an accuracy of 0.1 sec.

RESULTS AND DISCUSSION

The use of isotachophoresis as a reference method for sodium determinations

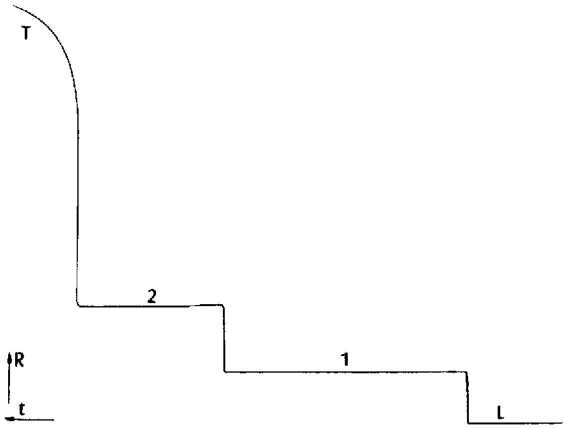

Fig. 1. Isotachopherogram of serum and internal standard (*n*-butylamine). 1 = Sodium; 2 = *n*-butylamine.

was investigated with standard sodium chloride solutions, by determination of the linearity and transferability.

A reference method should be free from interferences from other sample constituents. In this instance a distinction can be made between either drugs and metabolites or other metals. For the former category, each serum sample should be analysed without an internal standard to verify interference from the internal standard. Owing to the high mobility of sodium, interferences from drugs are extremely unlikely. Potassium and ammonium do not interfere because they migrate in the leading zone. Of the other metals, Li(I), Al(III), Ba(II), Cd(II), Co(II), Fe(II), Fe(III), Mg(II), Ni(II), Pb(II), Sr(II) and Zn(II) were investigated. Most bi- and trivalent metals show a marked decrease in effective mobility owing to complexation with the counter ion (citrate), and consequently migrate in the terminator. Of these, only Ba, Sr and Li migrate in the operational system used. Ba and Sr did not interfere with either sodium or the internal standard. The internal standard was chosen such that it did not interfere with either of these ions. The linearity of the method was determined by diluting three sodium concentrations, 129.67, 141.68 and 149.3 m*M* with 2000 mg of 5 m*M* *n*-butylamine solution. Each standard solution was diluted six times and each dilution was injected three times.

In Fig. 2, ratio of the zone lengths of sodium and *n*-butylamine is plotted against the concentration ratio. The correlation coefficient is 0.99994. For a 95% confidence interval[6] the slope is 1.119 (±0.003).

The transferability of the method was checked by evaluation with three different isotachophoresis equipments (Table II), two of which were developed by Everaerts *et al.*, with inner diameters of the separation compartments of 0.2 and 0.45 mm. The third instrument was the commercially available Tachophor 2127 (LKB, Bromma, Sweden) with a separation compartment of I.D. 0.5 mm. The instruments were operated by three different persons, all of whom prepared their own electrolytes.

For these measurements ammediol was used as an internal standard instead of *n*-butylamine, which forms a mixed zone with lithium. A total of six standard solutions of sodium chloride (119.75, 135.52, 140.45, 146.52, 150.25 and 158.24 m*M*)

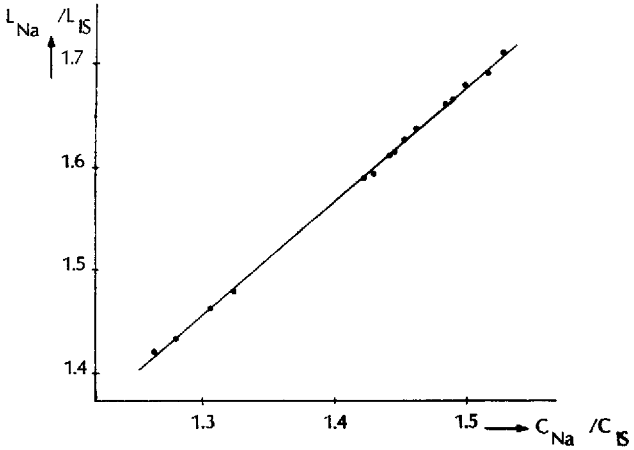

Fig. 2. Ratio of zone-lengths of sodium and internal standard (*n*-butylamine) *versus* their concentration ratio.

were diluted by volume with a Micro-Lab 1000 (Hamilton, Bonaduz, Switzerland). From each standard solution 80 μl were diluted three times with 2240 μl of 5 m*M* ammediol solution. Each dilution was injected once.

In Fig. 3 the ratios of the zone lengths are plotted against the concentration of the standard solution. The slope of the line obtained with the Tachophor differs significantly from those obtained with the other equipments. A statistical test revealed no significant differences between the slopes of the two other equipments. Consequently, the difference in inner diameter of the separation compartment has no influence.

In these experiments, the results were not corrected for instabilities of the driving current. Short-term instability of commercial isotachophoresis power supplies can amount to 0.1%. The long-term instability depends on the voltage supply, the voltage and the current; these instabilities can amount to 1–2%. In most instances, the driving current will decrease during the analysis. These values refer to the total

TABLE II

RESULTS OF THE TRANSFERABILITY INVESTIGATION

Equipment: A, I.D. = 0.2 mm (Eindhoven University of Technology); B, I.D. = 0.45 mm (Eindhoven University of Technology); C, I.D. = 0.5 mm (Tachophor 2127, LKB).

Parameter	*Equipment*		
	A	*B*	*C*
n	18	18	14
Slope (l/mol)	4.995	4.791	5.313
Intercept	0.039	0.025	−0.024
Correlation coefficient	0.996	0.996	0.997
Current (μA)	25	80	100

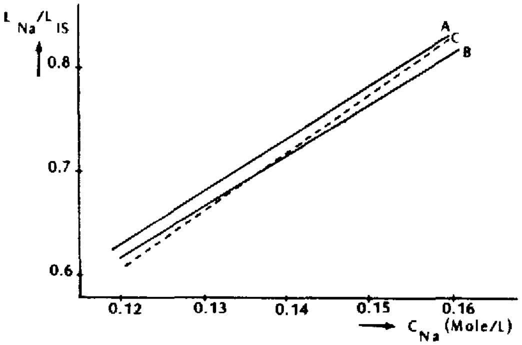

Fig. 3. Ratio of zone lengths of sodium and internal standard (ammediol) *versus* sodium standard solution concentration. A, B, C refer to three different isotachophoresis equipments (see Table II).

analysis time. Only the instability during detection is important, being only a fraction of the total value. The rate of decrease of the current is highest during detection.

This problem can be solved using a coulometric device for recording the signals[7,8], making the speed of the recording paper directly proportional to the driving current. Short- and long-term current instabilities are consequently corrected for. A coulometer was constructed[8] with a resolution of 1 μC. Together with a potentiometric recorder with a stepping motor, Type BD41 (Kipp, Delft, The Netherlands), the current instability was reduced to 0.004% within 15 min at 25 μA[9].

CONCLUSION

Sodium could be determined specifically and with great accuracy and precision in the operational system given. Although there is a significant difference in the slopes between the Tachophor and the two other equipments, the method has proved to be transferable. The present study justifies an inter-laboratory investigation for the candidateship of isotachophoresis as a reference method. Moveover, isotachophoresis seems very useful as a reference method for other ions such as calcium, potassium, lithium and creatinine. It is even possible to determine some of them simultaneously. The use of a coulometric device improves the analytical results.

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