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Influence of Protein on the Determination of Sodium, Potassium and Chloride in Serum

by Ektachem DT 60 with the DTE Module; Evaluation with Special Attention to a Possible Protein Error by Flame Atomic Emission Spectrometry and Ion-Selective Electrodes; Proposals to Their Calibration

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Summary: The reliability of the Ektachem DT 60 with the DTE module was evaluated. The precision of the determination of sodium, potassium and chloride in serum was adequate. The relative standard deviation for precision between days was Na^+ 0.7%, K^+ 1.5% and Cl^- 1.0%. The means of the Ektachem results for 7 control sera differed from those of the reference method values by 0.9% (Na^+), −0.9% (K^+) and +4.4% (Cl^-). Similar results were obtained for the analysis of patient sera.

The influence of protein was investigated, using sera of increasing protein concentration prepared by ultracentrifugation. The results from the Ektachem corresponded to the values obtained by flame atomic emission spectrometry, even at high protein concentrations, although Ektachem measurements are performed by ion-selective electrodes without predilution. In paraproteinaemia, the Ektachem and flame atomic emission spectrometry results disagreed. Chloride determinations by Ektachem distinctly differed from measurements of the chloride concentration in total serum.

It is proposed that ion-selective electrodes should be calibrated and linearized with respect to sodium chloride, in order to obtain an accurate value for the concentration of electrolyte in serum water. Concentrations are easier to interpret than "activities" for therapeutical purposes, and they can be used to define protein- and lipid-independent reference intervals for these electrolytes. With this calibration procedure, the results from ion-selective electrodes are never lower than values obtained by flame atomic emission spectrometry. The accuracy of ion-selective electrode measurements should be evaluated by applying reference methods for sodium, potassium and chloride to the ultracentrifugation supernatant of the corresponding serum. This approach can help to settle the dispute concerning the influence of protein on the residual liquid junction potential.

Introduction

Fifteen years ago determinations of sodium and potassium were almost exclusively performed by flame atomic emission spectrometry. Since then measurements by ion-selective electrodes have become more and more popular. More recently, it has become possible to determine electrolytes with ion-selective electrodes in combination with carrier-bound reagents, using the KODAK Ektachem system. In this study the reliability of the sodium, potassium and chloride determinations by the KODAK Ektachem system was evaluated, by using the reference methods of the Na-

tional Institute of Standards and Technology (NIST) (1–3), and by comparing the Ektachem results with those from traditional ion-selective electrodes and those from flame atomic emission spectrometry. The specificity of the ion-selective electrodes was checked by determination of the same samples adjusted to different pH. The influence of the protein concentration on the electrolyte measurements by flame atomic emission spectrometry, ion-selective electrodes and coulometry was investigated, and the values obtained by the different methods were clearly biased in proportion to the amount of protein.

Materials and Methods

The sodium, potassium and chloride concentrations of the following control sera were determined.

Standard Reference Material 909 (SRM 909) (National Institute of Standards and Technology, Washington, D. C.); Kontrollogen L, lot no. 623126 (Behring, Frankfurt); Kontrollogen LP, lot no. 623213 (Behring, Frankfurt); Moni-trol I, lot no. 208 (AHS/Deutschland, München); Moni-trol II, lot no. 108 (AHS/Deutschland, München); Pathonorm H, lot no. 21 (Merck, Darmstadt); Pathonorm L, lot no. 20 (Merck, Darmstadt); QCS Abnormal Control Serum Assayed lot no. 025505 E (Ciba Corning, Fernwald); Seronorm lot no. 166 (Merck, Darmstadt); Validate-N, lot no. 4 × 023 (Gödecke, Freiburg).

Comparison of methods

The sera used originated from healthy employees during the annual medical check.

Flame atomic emission spectrometry

For flame atomic emission spectrometry an AFM 5052 was used (Eppendorf, Hamburg) calibrated according to the recommendations of the manufacturer.

Ion-selective electrode

Samples were measured, without predilution, with the Nova I (Nova Biomedical, Darmstadt), calibrated as recommended by the manufacturer.

Coulometry

Chloride determinations by coulometry were performed with the Chloride Analyser 925 (Ciba Corning, Fernwald), calibrated according to the instructions of the manufacturer.

KODAK Ektachem DT 60 Analyser with DTE module (Kodak, Stuttgart)

The electrodes in the slides are dry, multilayered, analytical elements coated on a polyester support. Two identical electrodes form a complete concentration cell on a single slide which is intended to be used for one test and then discarded. A reference fluid with a fixed ionic concentration is required for measurement. Determinations are performed by depositing 10 µl each of a reference fluid and a serum on separate halves of the slide. A polymer-coated paper bridge connects the reference electrode and the indicator electrode which receives the serum. A small volume of water from the sample fluid penetrates to the reference layer. The electrical potential is poised at the reference layer/silver chloride layer while the potential across the membrane is determined by the ratio of the ion activity in the deposited solution to that in the reference layer. The electrometer measures the potential difference between the reference and indicator electrodes. The analyser's microcomputer uses this measurement and the stored calibration parameters to determine the concentration value of the ion in the sample fluid.

Sodium

The ion-selective membrane contains a sodium ionophore, methyl monensin, dissolved in a carrier solvent and a polymeric binder.

Potassium

The ion-selective membrane is composed of valinomycin dissolved in a polymer mixture.

Chloride

The chloride ions in the reference and sample fluid migrate to the silver/silver chloride layers and affect the equilibrium between free chloride and chloride bound to silver ions. A protective layer inhibits the effect of interfering substances (e. g. bromide, uric acid) on the electrode.

Measurements were performed at room temperature. The analyser was calibrated with standard specimens: KODAK Ektachem DT Calibrator 1, lot no. 0283501 and Calibrator 2, lot no. 0285502. The KODAK Ektachem DT Electrolyte Reference Fluid Gen 01, lot no. 0333400 was used. GEN-51 00830 slides were used for sodium determinations; GEN-51 00671 for potassium determinations; and GEN-51 01673 for chloride determinations. The reliability of the analyser was checked by KODAK Control liquid lot no. 0401400.

Sample preparation

Samples containing different amounts of protein were prepared as follows.

A homogeneous pool serum (100 ml) was adjusted to the appropriate pH by addition of glacial acetic acid p. a. (Merck, Darmstadt). Pool serum (8 ml) was pipetted into each of the 10 polycarbonate ultracentrifuge tubes (Beckman, München). The samples were centrifuged in a Beckman ultracentrifuge L8-80M (Beckman, München) at 106000 *g* during 24 h at 24 °C.

The sample with the highest protein concentration was obtained by removing the protein-free supernatant completely; samples with lower protein content were obtained by only partly discarding the supernatant; a low protein content was obtained by addition of supernatant to the original pool serum. All prepared samples were thoroughly homogenized.

Results

1. Sodium

1.1 *Precision*

A control material was analysed on 21 consecutive days. The relative standard deviation was 0.7% (tab. 1).

Tab. 1. Precision between days[1]) of Ektachem DT 60.

Analyte	Number of determinations	Mean value $\bar{x}$ mmol/l	Relative standard deviation CV %	Allowable rel. standard deviation[2]) %
Sodium	21	150.4	0.7	≤2.0
Potassium	21	6.04	1.5	≤2.7
Chloride	21	113.2	1.0	≤2.0

[1]) as determined by use of KODAK Control liquid lot 040 1400
[2]) according to l. c. (4)

1.2 *Accuracy*

The Ektachem values were compared with the *definitive-*, the *reference method- and method-dependent assigned values* (tab. 2). Satisfactory agreement was observed, especially with the reference method values, and the requirements of the new guidelines – maximal bias ± 6% from the target values (4) – were satisfied with the exception of SRM 909.

Tab. 2. Accuracy of Ektachem: Comparison with target values (definitive values, reference method values, method-dependent assigned values).

Control serum	Sodium			Potassium			Chloride		
	Target value mmol/l	d%[6])	D%[7])	Target value mmol/l	d%[6])	D%[7])	Target value mmol/l	d%[6])	D%[7])
SRM 909	134.1[1])	+ 5.9		3.52[1])	+2.3		108.4[1])	+0.6	
Seronorm	138.9[1])	+ 2.2		4.54[1])	+3.5		104.9[1])	+3.9	
			+4.1			+2.9			+2.3
SRM 909	133.7[2])	+ 6.2		3.53[2])	+2.0		108.5[2])	+0.5	
E[8])	139.8[2])	− 0.6		4.94[2])	+1.2		99.2[2])	+4.8	
Seronorm	140.5[2])	+ 1.1		4.59[2])	+2.4		104.3[2])	+4.5	
A[8])	141.3[2])	+ 0.5		4.93[2])	−0.6		97.6[2])	+5.5	
D[8])	144.1[2])	− 2.2		4.90[2])	−4.0		86.1[2])	+3.4	
C[8])	162.9[2])	+ 0.1		6.00[2])	−6.7		103.2[2])	+5.6	
B[8])	163.6[2])	+ 0.9		6.82[2])	−0.3		114.3[2])	+6.7	
			+0.9 (0.0)[9])			−0.9			+4.4
Pathonorm L	115[3])	± 0.0		2.55[3])	−6.0		79.9[5])	+1.4	
Moni-trol II	119[3])	+ 0.8		6.70[3])	−3.0		90.0[5])	+2.2	
Kontrollogen L	139[3])	+ 0.7		4.97[3])	−1.4		98.5[5])	+3.6	
Moni-trol I	139[3])	+ 2.9		4.31[3])	−0.2		107.0[5])	+5.6	
Seronorm	141[3])	+ 0.7		4.60[3])	+2.2		104.0[5])	+4.8	
Validate N	145[3])	+ 5.5		5.00[3])	−4.0		107.0[5])	+5.6	
QCS Abnormal	149[3])	+11.4		7.05[3])	+0.7		112.5[5])	+7.6	
Pathonorm H	156.8[3])	+ 4.6		6.62[3])	−0.3		116.2[5])	+6.7	
Kontrollogen LP	163[3])	+ 4.3		6.85[3])	−5.1		115.0[5])	+5.2	
			+3.4 (2.4)[10])			−1.9			+4.7
Pathonorm L	115.1[4])	− 0.1		2.52[4])	−4.8				
Moni-trol II	117[4])	+ 2.6		6.73[4])	−3.4				
Moni-trol I	141[4])	+ 1.4		4.47[4])	−3.8				
Validate N	150[4])	+ 2.0		5.10[4])	−5.9				
Pathonorm H	159.3[4])	+ 3.0		6.66[4])	−0.9				
QCS Abnormal	161[4])	+ 3.1		7.20[4])	−1.4				
			+2.0			−3.4			

[1]) Definitive value
[2]) Reference method value
[3]) Method-dependent assigned value: flame atomic emission spectrometry (Dt. Gesellschaft f. Klin. Chemie)
[4]) Method-dependent assigned value: ion-selective electrode without predilution of the sample
[5]) Method-dependent assigned value: Coulometry
[6]) Deviation of the value obtained by KODAK Ektachem from the target value in %
[7]) Mean deviation of the values obtained by KODAK Ektachem from the target values in %
[8]) Control sera, commercially not available
[9]) in ( ): D% without SRM 909
[10]) in ( ): D% without QCS Abnormal

Tab. 3. Comparison of results obtained by flame atomic emission spectrometry and ion-selective electrodes with Ektachem DT 60.

Methods (y) vs. (x)		Number of determinations	Standardized principal component analysis		$s_{y\cdot x}$[1]) mmol/l	$\bar{y}$[2]) mmol/l	$\bar{x}$[3]) mmol/l	t[4])	Correlation coefficient r
			Slope	Intercept mmol/l					
a) Sodium									
ISE-E[5])	FAES[6])	61	0.936	10.6	2.01	142.8	141.3	3.99	0.496
ISE-E[5])	ISE[7])	61	1.102	−15.1	1.99	142.8	143.3	1.43	0.505
b) Potassium									
ISE-E[5])	FAES[6])	61	1.048	−0.11	0.090	4.40	4.31	5.99	0.973
ISE-E[5])	ISE[7])	61	0.985	0.01	0.091	4.40	4.46	3.31	0.972
c) Chloride									
ISE-E[5])	Coulometry	61	1.124	− 6.7	2.484	109.7	103.5	14.46	0.543

[1]) standard error of residuals
[2]) arithmetic mean of y-values
[3]) arithmetic mean of x-values
[4]) t-value (paired t-test)
[5]) Ektachem DT 60
[6]) Flame atomic emission spectrometry
[7]) Ion-selective electrode measurement without predilution of the sample

Patient sera ($\bar{n}$ = 61) were analysed by ion-selective electrode and flame atomic emission spectrometry. The values obtained by the different methods were comparable (tab. 3). The mean values of results from the Ektachem were higher than those from flame atomic emission spectrometry (+1.06%) and lower than those from the ion-selective electrode (−0.35%). Standardized principal component analysis and correlation coefficient could not be interpreted conclusively, because these data are based on a very narrow range of results.

Tab. 4. Determination of sodium, potassium and chloride at different protein concentrations obtained by ultracentrifugation.

Sodium

Protein g/l	FAES[1]) mmol/l	ISE[2]) mmol/l	ISE-E[3]) mmol/l
33	145.5	143.3	146.3
65	146.9	148.1	145.7
80	147.6	150.4	145.5
120	149.3	156.4	144.8

Potassium

Protein g/l	FAES[1]) mmol/l	ISE[2]) mmol/l	ISE-E[3]) mmol/l
33	4.13	4.10	4.12
65	4.17	4.24	4.17
80	4.20	4.31	4.20
120	4.24	4.48	4.27

Chloride

Protein g/l	Coulometry mmol/l	ISE-E[3]) mmol/l
33	114.0	110.3
65	109.6	111.3
80	107.5	111.7
120	101.9	113.0

[1]) Flame atomic emission spectrometry
[2]) Ion-selective electrode without predilution of the sample
[3]) Ion-selective electrode KODAK DT 60

Sera containing different *protein concentrations* were obtained by ultracentrifugation (see methods). At 120 g/l protein — a concentration, which is observed in every thousandth patient according to our files — the Ektachem results were 3.0% lower than those from flame atomic emission spectrometry and even 7.4% lower than those from the ion-selective electrode (tab. 4) (fig. 1). Samples of different protein concentration,

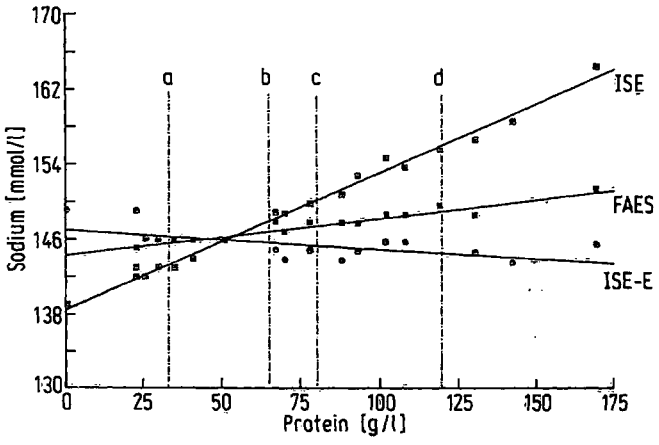

Fig. 1. Determination of sodium in serum preparations (pH 7.6) of different protein concentrations (prepared by ultracentrifugation, see "Methods") by ion-selective electrode (ISE ⊞), flame atomic emission spectrometry (FAES ■), and Ektachem DT 60 (ISE-E ⊕).
a) 33 g/l protein; b) 65 g/l protein; c) 80 g/l protein; d) 120 g/l protein.

prepared from paraproteinaemic sera by ultracentrifugation, were analysed for sodium with the ion-selective electrode and the Ektachem. The two methods then showed better agreement, at high protein concentrations (fig. 2).

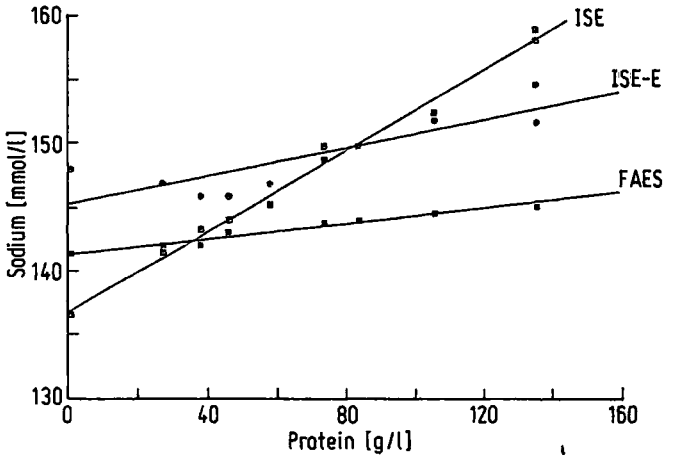

Fig. 2. Determination of sodium in preparations of paraproteinaemic sera (pH 7.4) of different protein concentrations (prepared by ultracentrifugation, see "Methods") by ion-selective electrode (ISE ⊞), flame atomic emission spectrometry (FAES ■), and Ektachem DT 60 (ISE-E ⊕).

The *influence of pH* on Ektachem measurements was studied by addition of acetic acid to outdated sera. When the pH of fresh serum (pH 7.5) rose to pH 8.5 on standing, the sodium value showed a corresponding decrease of 2.7%. For the same pH change, the ion-selective electrode (Nova) sodium values dropped by 1.2% (tab. 5).

Tab. 5. pH-Dependency.

	n[6])	FAES[1]) d%[4])	ISE[2]) d%[4])	ISE-E[3]) d%[4])	Coulometry d%[4])
Sodium	20	+0.1	−2.5	−5.5	n.d.[5])
Potassium	20	−0.2	−1.0	−3.5	n.d.[5])
Chloride	20	n.d.[5])	n.d.[5])	+0.7	+0.3

[1]) Flame atomic emission spectrometry
[2]) Ion-selective electrode without predilution of the sample
[3]) Ektachem DT 60
[4]) Change of values in percent if pH changes from 6.5 to 8.5
[5]) not determined
[6]) number of determinations

2. Potassium

2.1 *Precision*

The precision between days was determined by measurement of Ektachem Control liquid on 21 consecutive days. The relative standard deviation was 1.5% (tab. 1).

2.2 *Accuracy*

Various *control sera* were analysed by the Ektachem DT 60/DTE and the results were compared with the definitive values, the reference method values and the method-dependent assigned values. All measurements were within the allowable limits of ± 8% deviation (4) (tab. 2).

In the analysis of *patient sera*, the values from the Ektachem were significantly higher than those from flame atomic emission spectrometry. Results from "direct potentiometry" were on average 0.06 mmol/l higher than those from measurement with the slides (tab. 3).

At the high *protein concentrations (120 g/l)* obtained by ultracentrifugation, the potassium results from the Ektachem were 0.7% higher than those from flame atomic emission spectrometry and 4.7% lower than those from the ion-selective electrode. At a protein concentration of 33 g/l — a concentration, which is observed in every thousandth patient according to our files — the ion-selective electrode values were the lowest (tab. 4, fig. 3). The results of potassium measurements in paraproteinaemic samples prepared by ultracentrifugation (fig. 4) were analogous to those for the determination of sodium (see fig. 2).

The effect of pH on potassium measurements was not clinically relevant (tab. 5).

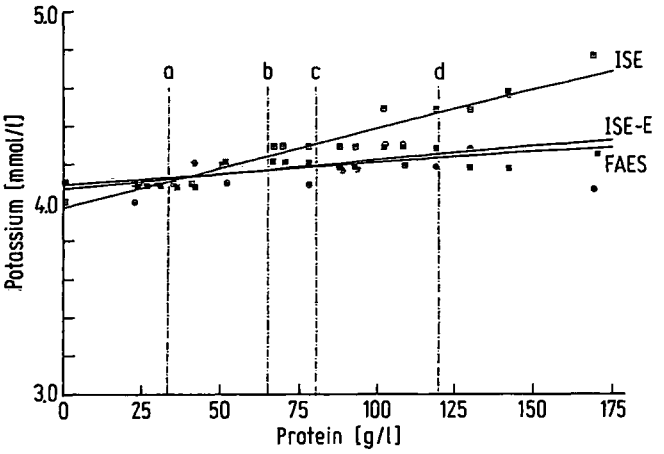

Fig. 3. Determination of potassium in serum preparations (pH 7.6) of different protein concentrations (prepared by ultracentrifugation, see "Methods") by ion-selective electrode (ISE ⊞), flame atomic emission spectrometry (FAES ■), and Ektachem DT 60 (ISE-E ⊕).
a) 33 g/l protein; b) 65 g/l protein; c) 80 g/l protein; d) 120 g/l protein.

3. Chloride

3.1 *Precision*

The relative standard deviation calculated from single measurements on 21 working days was 1.0% (tab. 1).

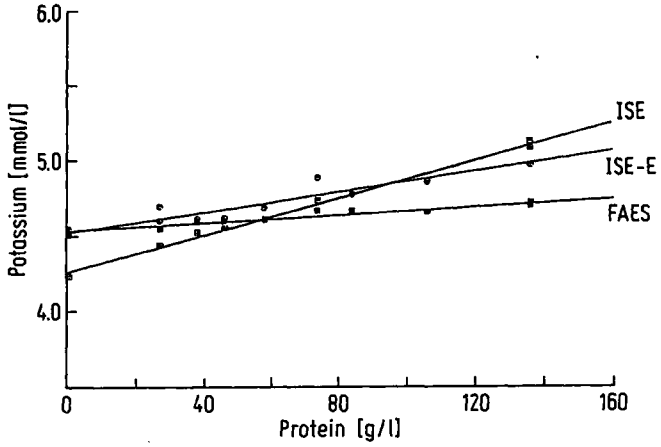

Fig. 4. Determination of potassium in preparations of paraproteinaemic sera (pH 7.4) of different protein concentrations (prepared by ultracentrifugation, see "Methods") by ion-selective electrode (ISE ⊞), flame atomic emission spectrometry (FAES ◘), and Ektachem DT 60 (ISE-E ⊕).

3.2 *Accuracy*

All measurements of *control sera* had a positive bias as compared with the definitive value, the reference method value, or the method-dependent assigned value; at least two results were even outside the allowable limits of ± 6% bias (4) (tab. 2).

Patient sera seemed to have a significantly higher chloride concentration, when measurements were performed with slides instead of coulometry (+6.0%) (tab. 3).

At the high *protein concentrations* (120 g/l) these differences were more evident (+10.9%) than at low protein concentrations (tab. 4). In extreme hypoproteinaemia, chloride determinations with slides yielded even lower results (fig. 5). The question of whether paraproteinaemic sera have a different impact on the Ektachem system could not be investigated, because an ion-selective electrode was not available for comparison.

The influence of pH on the chloride determination by slides or coulometry was negligible (tab. 5).

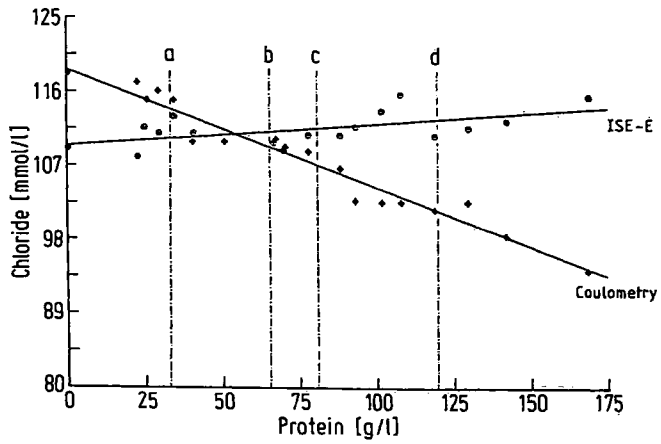

Fig. 5. Determination of chloride in serum preparations (pH 7.6) of different protein concentrations (prepared by ultracentrifugation, see "Methods") by Ektachem DT 60 (ISE-E ⊕) and coulometry (◆).
a) 33 g/l protein; b) 65 g/l protein; c) 80 g/l protein; d) 120 g/l protein.

Discussion

1. Precision and accuracy of the Ektachem DT60/DTE module

The determination of sodium, potassium and chloride by use of the Ektachem was precise and met the requirements of the new German guidelines (4) as well as those of the United Kingdom External Quality Assessment Scheme (5). It must be emphasized, however, that good precision is only obtained by skilled laboratory staff (6). If the determinations are performed by nurses as bedside tests, imprecision may be greater (the applicability of the Ektachem slides on the wards is restricted, because serum instead of blood is used as the test material).

The accuracy of the electrolyte measurements by Ektachem was evaluated with 15 control sera. The bias between the potassium results and the target values was adequately small. On the whole, the same was true for sodium. The target value of "SRM 909", however, was missed by 5.9%, which may be due to interference of the matrix with the Ektachem method. The same holds true for "QCS Abnormal", whose target value was confirmed by flame atomic emission spectrometry, but was repeatedly widely missed by the Ektachem. Chloride measurements showed a mean systematic error of + 4.4%, compared with the reference method values. The Ektachem results for patient sera also differed by + 6.0% from those obtained by coulometry, whereas values for sodium and potassium agreed satisfactorily. (In further experiments, using the Ektachem 700 but the same slides, the bias of the chloride measurements was no greater, i.e. the deviation was presumably due to an instrumental calibration error). One may conclude that commercial control sera are on the whole adequate for the accuracy control of this new type of electrolyte measurement, as they yield results comparable to those obtained with native sera.

2. Influence of protein

The *influence of protein* on the different methods for electrolyte measurement was studied by analyses of serum fractions prepared by ultracentrifugation (see "Material and Methods"). This technique quickly yields samples of a wide range of protein concentra-

tions without changes in protein composition (hyperproteinaemia due to a monoclonal gammopathy may have a different effect on electrolyte determination by ion-selective electrodes from that of polyclonal hypergammaglobulinaemia (see above)). However, one has to consider:

(1) that lipids floating at the surface are discarded, so that volume displacement effects are a little less pronounced in this experimental design, depending on the proportion of lipid in the sample;

(2) that sodium and potassium are attracted by the protein fraction due to their positive electric charge, whereas chloride will be enriched in the supernatant due to repulsion by the negative charge of the protein.

These phenomena are pH-dependent and more evident at a pH far removed from the isoelectric point of the protein. The findings, obtained by analysis of the samples prepared by ultracentrifugation, were confirmed by electrolyte measurements of native sera (fig. 6) and pool serum, which had been diluted stepwise with a sodium chloride solution (fig. 7). Very similar results were obtained by extrapolating results of a previous study (7): at a protein concentration of 33 g/l, the results from the ion-selective electrode were 1.4% lower than those from flame atomic emission spectrometry. This experimental design is another approach to the investigation of the influence of protein on ion-selective electrodes. Similar experiments were performed by l. c. (8) by use of dialysed bovine serum pools, which were spiked afterwards with sodium and potassium chloride.

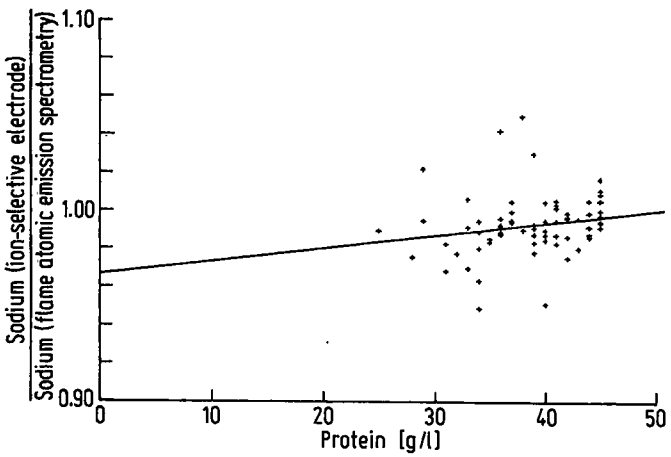

Fig. 6. Determination of sodium in native hypoproteinaemic sera (n = 72).
$y = 0.001\,x + 0.967$

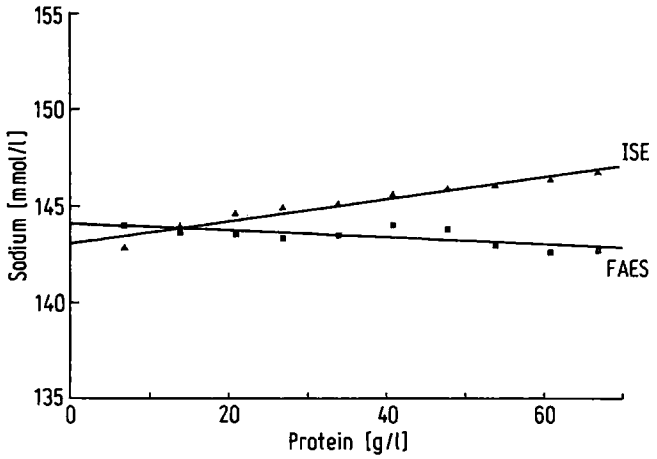

Fig. 7. Determination of sodium in preparations of a pool serum that were obtained by adding different amounts of an isotoic sodium chloride solution.
FAES: Flame atomic emission spectrometry (□).
ISE: Ion-selective electrode (▲).

2.1 *Ion-selective electrode*

The results from the ion-selective electrode clearly demonstrate (fig. 1) the increase of the sodium concentration in the serum water with the increase of the protein concentration. It is remarkable that the sodium concentration in the protein-free supernatant, as determined with the ion-selective electrode, is lower than that determined by flame atomic emission spectrometry. As pure sodium chloride solutions were measured accurately within the range 50 to 200 mmol/l by the ion-selective electrode, it may be assumed that the difference is caused by the fact that the activity coefficient of the supernatant is lower than the activity coefficient of the calibration solution. The supernatant contains additional ions, which are absent from the calibration solution, so that the supernatant has a higher ionic strength (this may be a further reason why the discrepancies shown by the ion-selective electrode values are less than expected on theoretical grounds). In this case, it might be proposed that the calibration of the ion-selective electrode should be performed at a higher ionic strength to obtain the concentration in serum water. The measurements of protein-free serum preparations should then yield the same accurate electrolyte results irrespective of the instrument, be it an ion-selective electrode or a flame atomic emission spectrometer. The difference between the results ("direct" potentiometry and flame atomic emission spectrometry) would increase continuously with increasing protein (or lipid) concentration starting from a difference of "zero". Molal concentration is a clearly defined physicochemical quantity. It can be established by using ultracentrifugation, and it can be measured accurately by flame atomic emission spectrometry according to the reference method (1) in two ways:

a) determination of the sodium content of the protein-free supernatant after ultracentrifugation, and

b) determination of the sodium concentration in serum and its corresponding water content (9).

In the latter case (b), electrolyte composition is not altered, in contrast to the shifts that occur in ultracentrifugation (a). Primary standard solutions can be used for calibration of the ion selective electrodes. Changes in protein and hydrogen carbonate binding, as well as changes of the activity coefficient are not taken into account, but they are known to be very small in blood. Determination of the molal concentration of chloride by ion selective electrodes can be performed on the same basis by use of the corresponding reference method (3).

Molal concentration is preferred to activity because molal activity of serum is difficult to establish and calculate. It cannot be measured with certainty by ion-selective electrodes, which are susceptible to many influences. A reference method is lacking. Molal activity is a difficult quantity to handle for therapeutic purposes. The determination of molal concentration, however, can help to investigate the controversial question of the influence of protein on the reference liquid junction potential (10–13). From our data, one can conclude that its influence is of minor importance. If the increase of protein were to lead to erroneously high sodium values as determined by ion-selective electrodes, then the sodium content of total serum as determined simultaneously by flame atomic emission spectroscopy should fall continuously due to volume displacement. However, it does not fall, but remains constant, due to a compensatory increase in sodium, i. e. the ion-selective electrode measures a real increase in free sodium content.

If, however, the ion-selective electrode is calibrated against flame atomic emission spectrometry for normoproteinaemic sera, the ion-selective electrode values will be lower than those from flame atomic emission spectrometry already in the presence of slight hypoproteinaemia, a condition that is most common in the hospital. At 33 g/l protein which, according to our files, is observed in every thousandth patient, the difference is about 5 mmol/l. Table 6 lists some points to be taken into consideration when introducing the new quantity, "adjusted active substance concentration" (14) in order to obtain results from the ion-selective electrodes comparable to those from flame atomic emission spectrometry in normoproteinaemia:

1) This new quantity is neither a measure of a serum concentration nor of a serum water concentration nor activity. It is a quantity just outside the clearly defined physico-chemical quantities.

2) As different quantities are determined by the ion-selective electrode and flame atomic emission spectrometry, it is not necessary that the reference intervals be identical.

3) Calibration is performed with standard specimens, which is the most unsatisfactory method of a calibration (15).

4) The accuracy control is hampered, because ion-selective electrode values agree exactly with those from flame atomic emission spectrometry values only at a single protein concentration.

5) It is not easily appreciated that sometimes values from the ion-selective electrode are higher and sometimes lower than those from flame atomic emission spectrometry.

When ion-selective electrode calibration is performed with solutions containing only sodium and potassium chloride, the results for the sodium and potassium concentrations of serum water are rather too low, but they are always higher than those from flame atomic emission spectrometry, with the exception of extreme hypoproteinaemia.

Tab. 6. Comparison of adjusted active substance concentration and substance concentration in serum water.

	Adjusted active substance concentration (l. c. (14))	Substance concentration in serum water
System:	not defined	serum water
Analyte:	sodium	sodium
Measuring unit:	mmol/l	mmol/l
Calibration:	standard specimen	primary standard solution
Comparison with sodium concentration in serum:		
Hyperproteinaemia:	higher	much higher
Normoproteinaemia:	in agreement as adjusted	higher
Hypoproteinaemia:	lower	slightly higher
Reference interval:	identical	higher
Accuracy control by reference method values:	not possible	reference method (l. c. 1, 2, 3) 1) applied to the supernatant of the ultracentrifugate of the corresponding serum. 2) applied to the serum and – additionally – determination of its water content (l. c. 9).

2.2 *Flame atomic emission spectrometry*

As the protein concentration increases due to ultracentrifugation, the sodium and potassium concentration increases. The molar concentration of sodium and potassium in the total sample that is measured by flame atomic emission spectrometry remains nearly constant. The increase due to electrostatic forces is counterbalanced by the volume displacement effect.

2.3 *Coulometry*

The chloride concentration in the sera decreases with increasing protein concentration due to volume displacement and repulsion of the negatively charged chloride by protein.

2.4 *Ektachem*

The Ektachem is calibrated with standard specimens to give values comparable to those from flame atomic emission spectrometry and coulometry within normal protein concentrations, although the Ektachem measures the electrolytes by ion-selective electrodes without predilution. As far as sodium and potassium are concerned it is remarkable that the values correspond to those from flame atomic emission spectrometry, even in hypo- and hyperproteinaemia. It may be assumed that this phenomenon is caused by an increase in viscosity which counterbalances the increase in electrolyte concentration in the serum water. It is not yet known, however, whether the different results obtained for paraproteinaemic sera are due to a different viscosity. Similar observations have been reported, using native sera of different protein composition (16). In hyperproteinaemia, the chloride values from the Ektachem are higher than those from coulometry, whereas in hypoproteinaemia the Ektachem values are lower. This is to be expected, if an ion-selective electrode, calibrated with standard specimens, is used without predilution. Unfortunately another ion-selective electrode for chloride measurement without predilution was not available.

3. Flame atomic emission spectrometry and ion-selective electrode after predilution

Determinations of the sodium concentration in total serum can be performed accurately by flame atomic emission spectrometry and ion-selective electrodes after predilution of the sample. In "pseudohyponatraemia" the accurately determined sodium concentration in total serum differs from the sodium concentration in serum water more than usually, due to volume displacement by e. g. excessive protein. As "pseudohyponatraemia" can be present at any sodium concentration and need not be confined to results below the reference interval, the term is inadequate and misleading. In "pseudohypernatraemia" the difference between the sodium concentration in total serum and the concentration in serum water is less than normal, because the volume displacement by proteins is less than normal. This may occur at any sodium concentration, in hyper-, normo- or hyponatraemia. The interpretation of the (analytically accurate) results will be difficult in these cases. Without knowledge of protein and lipoprotein concentrations, the clinician might not even be aware of a possible misinterpretation. (Some past results showing hypernatraemia in the presence of hypoproteinaemia have to be questioned and must be reassessed by ion-selective electrodes). In doubtful cases the sample should be measured by ion-selective electrodes (without predilution) to determine the concentration in serum water, instead of correcting the flame atomic emission spectrometry value with respect to the protein and "lipid" concentrations. The influence of the protein and lipid concentrations on sodium in serum and on its reference interval (fig. 1, fig. 8) was calculated by *Levy* (17). The correction is more expensive, more time consuming and less accurate than the direct ion-selective electrode measurement. Ion-selective electrode values and the corresponding reference interval are not dependent on the protein and "lipid" concentrations. They mainly depend on calibration. If ion-selective electrodes are calibrated with standard

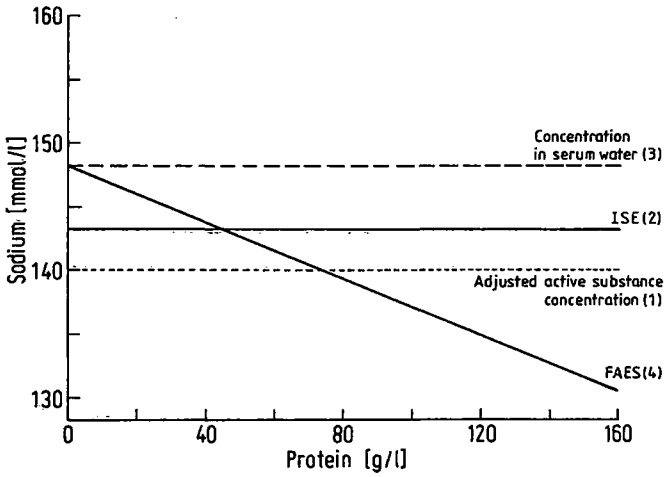

Fig. 8. Dependency of the sodium concentration in serum water at various protein concentrations as determined by ISE on the calibration of the ISE:
1) Calibration of the ISE by normoproteinaemic sera: "adjusted active substance concentration".
2) Calibration of the ISE with primary standard solutions free of protein.
3) Calibration of the ISE with primary standard solutions free of protein but adjusted to concentration in serum water.
4) Dependency of the sodium concentration in serum as determined by FAES on the protein concentration.
ISE: Ion-selective electrode.
FAES: Flame atomic emission spectrometry.

specimens to match normoproteinaemic flame atomic emission spectrometry results, the difference between the ion-selective electrode and the flame atomic emission spectrometry result is a direct measure of the extent to which the volume displacement differs from normality. If ion-selective electrodes are calibrated with primary standard solutions, the results are higher than by flame atomic emission spectrometry in normoproteinaemia. Volume displacement deviates from the "normal" extent when the discrepancy between results is different from the discrepancy between the corresponding reference intervals. Irrespective of the calibration procedure, however, ion-selective electrodes and flame atomic emission spectrometry always determine different quantities, which must be clearly documented in the laboratory report.

Although the effect of the protein concentration on the measured concentration of low molecular constituents of the serum is quite universal, it plays a decisive role only for quantities with relatively narrow reference intervals e. g. for chloride or sodium, but not for potassium or glucose.

4. Quality assessment

It was notable that the Ektachem was rather reliable when evaluated with control sera. The study clearly demonstrates, however, that control sera should include not only the measuring range of the analyte, but also a wide concentration range of those components of the matrix that may have an influence on the measurement, such as protein. In the measurement of sodium, the different behaviour of the Ektachem towards polyclonal and monoclonal globulins is a drawback, which can apparently be avoided by using a different reference fluid GEN 04 (16).

References

1. Velapoldi, R. A., Paule, R. C., Schaffer, R., Mandel, J. & Moody, J. R. (1978) Standard Reference Materials: A reference method for the determination of sodium in serum. NBS Spec. Public. 260-60.
2. Velapoldi, R. A., Paule, R. C., Schaffer, R., Mandel, J., Machlan, L. A. & Gramlich, J. W. (1979) Standard Reference Materials: A reference method for the determination of potassium in serum. NBS Spec. Public. 260-63.
3. Velapoldi, R. A., Paule, R. C., Schaffer, R., Mandel, J., Murphy, T. J. & Gramlich, J. W. (1979) Standard Reference Materials: A reference method for the determination of chloride in serum, NBS Spec. Public. 260-67.
4. Anonymous (1988) Qualitätssicherung der quantitativen Bestimmungen im Laboratorium. Dt. Ärztebl. *85*, B517–B532.
5. United Kingdom External Quality Assessment Scheme for General Clinical Chemistry. Distribution Number 353 6th June 1988.
6. Hicks, J. M. & Josefsohn, M. (1986) Hemoglobin, electrolytes, and other major clinical laboratory analytes as measured with a physician's office analyzer, the Kodak DT 60. Clin. Chem. *32*, 2201–2203.
7. Külpmann, W. R., Kannengießer, A. & Passing, H. (1986) Determination of sodium and potassium by ion selective electrodes in undiluted serum, plasma, and blood and by flame photometry in serum and plasma. In: *Methodology and clinical applications of ion-selective electrodes* (Maas, A. H. J., Buckley, B. M., Marsoner, H. J., Saris, N.-E. L. & Sprokholt, R., eds.) pp. 159–170. Elinkwijk Printing Co., Utrecht.
8. Truchaud, A. & Gourmelin, Y. (1986) A protein test to classify ISE instruments for Na^+ measurements. In: *Methodology and clinical applications of ion-selective electrodes* (Maas, A. H. J., Buckley, B. M., Marsoner, H. J., Saris, N.-E. L. & Sprokholt, R., eds.) pp. 315–323. Elinkwijk Printing Co., Utrecht.
9. Boink, A. B. T. J., Maas, A. H. J. & Sprokholt, R. (1985) Quick determination of the water content of serum. In: *Methodology and clinical applications of ion-selective electrodes* (Maas, A. H. J., Boink, A. B. T. J., Saris, N.-E. L., Sprokholt, R. & Wimberley, P. D., eds.) pp. 119–122. Radiometer, Copenhagen.
10. Payne, R. B. & Jones, D. P. (1987) Protein interferes with ionised calcium measurement at the reference electrode liquid junction. Ann. Clin. Biochem. *24*, 400–407.
11. Payne, R. B., Rawson, K. & Buckley, B. M. (1988) Investigations on possible protein effects at liquid junctions. In: *Methodology and clinical applications of ion-selective electrodes* (Maas, A. H. J., Buckley, B. M., Manzoni, A., Moran, R. F., Siggard-Andersen, O. & Sprokholt, R., eds.) pp. 321–322. Elinkwijk Printing Co., Utrecht.
12. Saris, N.-E. L. (1988) On the error of ISE-measurements. A study of possible protein interference with measurement of Na^+, Cl^- and K^+ using gelfiltrated albumin solution. In: *Methodology and clinical applications of ion-selective electrodes* (Maas, A. H. J., Buckley, B. M., Manzoni, A., Moran, R. F., Siggaard-Andersen, O. & Sprokholt, R., eds.) pp. 327–333. Elinkwijk Printing Co., Utrecht.
13. Saris, N.-E. L. (1988) Protein does not interfere with the ion-selective electrode determination of calcium, sodium or potassium ions. J. Clin. Chem. Clin. Biochem. *26*, 101–104.
14. Boink, A. B. T. J., Buckley, B. M., Christiansen, T. F., Covington, A. K., Maas, A. H. J., Müller-Plathe, O., Sachs, C. & Siggard-Andersen, O. (1986) Recommendations on the expression of results of ion-selective electrode measurement of sodium and potassium ion activities in undiluted serum, plasma or whole blood in clinical practice. In: *Methodology and clinical applications of ion-selective electrodes* (Maas, A. H. J., Buckley, B., Marsoner, H. J., Saris, N.-E. L. & Sprokholt, R., eds.) pp. 137–149. Elinkwijk Printing Co., Utrecht.
15. Stamm, D. (1974) Calibration and quality control materials. Z. Klin. Chem. Klin. Biochem. (J. Clin. Chem. Clin. Biochem.) *12*, 137–145.
16. Burnett, D., Ayers, G. J., Rumjen, S. C. & Woods, T. F. (1988) Sodium measurements in the presence of paraproteins by four direct ISE methods and flame photometry compared. Ann. Clin. Biochem. *25*, 102–109.
17. Levy, G. B. (1981) Determination of sodium with ion-selective electrodes. Clin. Chem. *27*, 1435–1438.

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