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# Reference Methods for the Determination of Sodium, Potassium, pH and Blood Gases with Ion-Selective Electrodes[1])

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*In honour of Prof. Dr. Dr. J. Büttner on the occasion of his sixtieth birthday*

**Summary:** The determination of sodium and potassium in serum will be performed in the near future mainly by ion-selective electrodes. When the samples are highly *diluted* before measurement, a demonstrably accurate value of the electrolyte concentration in serum can be obtained. Accuracy control by using the pertinent reference methods of the National Institute of Standards and Technology (NIST) is well established for this purpose.

In *undiluted* samples a potential is measured by ion-selective electrodes, which is dependent on the relative molal activity of the electrolyte, from which free molal concentration can be estimated. Usually, the total molal concentration of e. g. sodium is about 1.5% higher than that of free sodium, as it includes portions bound to e. g. carbonate and proteins. Accuracy control is hampered, because reference methods for either relative molal acitivity or free molal concentration are not yet available. Reference method values for total molal concentration will differ systematically from an accurate value for free molal concentration. When ion-selective electrodes are calibrated by using "normal" sera, accuracy control can be based on the reference method of the NIST; but this is valid only for a very narrow, at best "normal", concentration range of proteins and lipids, assuming, of course, that binding is normal. The greater the variation of the concentration of macromolecules from normal (lower or higher), the greater the difference between the values obtained by the two methods. Calibration of ion-selective electrodes by using sera (this is the least desirable approach for calibration) requires the introduction of a new unit of measurement, which is not compatible with the rational system of quantities and units.

Reference methods for pH and tonometry are available. A detailed protocol for their unequivocal use, guaranteeing repeatability of the stated precision and accuracy, is still lacking.

## 1. Sodium and Potassium

### 1.1 Determinations with ion-selective electrodes after dilution of the sample

When serum is highly diluted prior to measurement with ion-selective electrodes, the macromolecule-containing compartment is dramatically reduced. Although the electrodes determine the properties of analytes in *serum water*, it is then valid to assume that so-called "indirect" potentiometry determines the molar concentration of e. g. (total) sodium in (total) *serum*, just as a flame atomic emission spectrometer does. This means that the accuracy of these measurement procedures can be controlled by reference method values for sodium in serum, which are determined by the pertinent reference method of the National Institute of Standards and Technology (NIST, formerly NBS) (1).

[1]) Based on a lecture given at the Symposium "Reference Methods in Clinical Chemistry – Objectives, Trends, Problems" of the Congress Biochemische Analytik 90, München, May 8, 1990

## 1.2 Determinations in undiluted samples, using ion-selective electrodes

The situation is quite different, when the serum is not diluted before measurement with ion-selective electrodes. (In the following, measurement with "ion-selective electrode(s)" refers to the measurement of sodium). Since a variety of quantities may be reported, depending on calibration and calculation of results, the quantity involved in any measurement and calculation of result must be carefully defined.

### 1.2.1 *Relative molal activity and active molality*

When a sodium-selective electrode measurement system is placed in serum, a potential is observed. Its size is dependent on the amount of active sodium ions, as described by the *Nernst* equation (equation 1):

$$E = E_0 \pm \frac{R \cdot T}{n \cdot F} \cdot \ln a \qquad \text{(Eq 1)}$$

E: Ion-selective membrane potential measured
$E_0$: Constant potential dependent on electrode system
R: Gas constant
T: Temperature (K)
n: Charge of the ion being measured
F: *Faraday*'s constant
a: Relative molal activity of the ion being measured

Only about three fourth of total sodium ions are active (fig. 1).

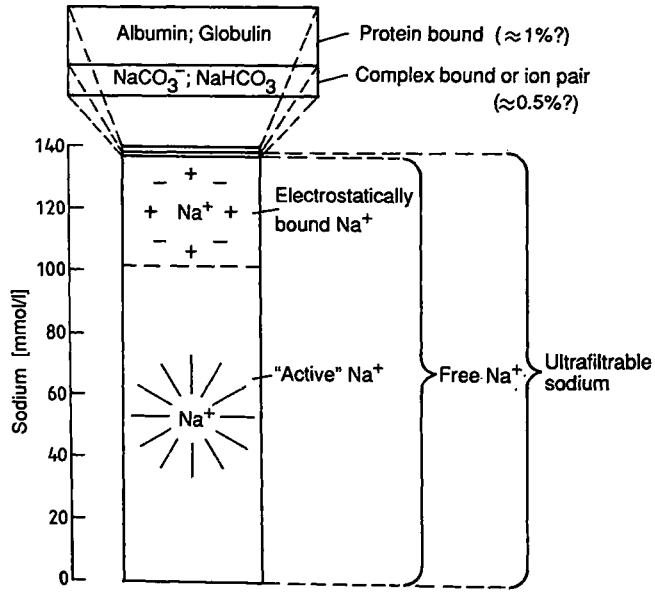


Fig. 1. Sodium fractions of blood plasma (modified from l.c. (2)).

When a sufficiently specific electrode is available and interference can be neglected, the potential is only proportional to pNa, the negative decadic logarithm of the relative molal activity of sodium, $a_{Na}$ (Equation 2 & 3).

$$-\log a_{Na} = pNa \qquad \text{(Eq 2)}$$

$$a_{Na} = \frac{\tilde{m}_{Na}}{mol \cdot kg^{-1}} \qquad \text{(Eq 3)}$$

$a_{Na}$: Relative molal activity of sodium in serum water (S(W))
$\tilde{m}_{Na}$: Active molality of sodium in serum water (S(W))

Reference intervals:

S(W) – pNa: 0.973 – 0.942

S(W) – $a_{Na}$: 0.106 – 0.114

Calibration can be performed with primary standard solutions made from Standard Reference Materials (2201 and 2202) of the National Institute of Standards and Technology (NIST) intended for use in the calibration of ion-selective electrodes. Certified values of activity, activity coefficients, pNa, pK and pCl are given.

Unfortunately, a reference method for the determination of the relative molal activity or active molality of sodium in serum is not yet available, but could be developed in the future, based on the experience of the pH reference method (3). This seems to be the only approach that requires no assumptions concerning the activity coefficient, sodium binding and water content of the sample. It could improve interlaboratory comparability, but its accuracy could not be proven conclusively, as a definitive method for this purpose is not available and can scarcely be imagined.

From a biochemical point of view it is generally accepted that active molality in serum water is probably the best parameter of sodium for describing ongoing biochemical processes. It is, however, also almost universally assumed that physicians will not accept this quantity.

Therefore, in the future, one could envisage accuracy assessment of ion-selective electrodes with a reference method (to be developed) for the determination of active molality that requires no assumptions. Patient results would then be reported in a form more familiar to the physician. This, however, implies assumptions concerning certain variables.

### 1.2.2 *Free molal concentration*

Free molal concentration can be calculated from active molality (equation 4):

$$m_{Na} = \frac{\tilde{m}_{Na}}{\gamma_{Na}} \qquad \text{(Eq 4)}$$

$m_{Na}$: Free molal concentration of sodium in serum water
$\gamma_{Na}$: Molal activity coefficient of sodium (0.747)

Reference interval:

$$S(W) - m_{Na}: 142.5 - 153.1 \text{ mmol/kg}$$

For this purpose one supposes that the sodium activity coefficient of a serum sample is identical to the activity coefficient of the corresponding calibration solution. A deviation of the actual activity coefficient of the individual serum from the activity coefficient of the calibration solution means an erroneous calculation of free molal concentration. Many factors influence the activity coefficient (equation 5):

$$\ln \gamma_{Na} = \frac{-\ln 10 \cdot A \cdot z^2_{Na^+} \cdot \sqrt{I}}{1 + å \cdot B \cdot \sqrt{I}} + h_{Na^+} \cdot M_{H_2O} \cdot \hat{m} - \ln \{1 + M_{H_2O} \cdot \Sigma [(1 - h_i) \cdot m_i]\} \quad \text{(Eq 5)}$$

z: Charge number
h: Hydration number of sodium (Na) or other ions (i)
$\hat{m}$: Osmolality
å: Ion size parameter
$M_{H_2O}$: Molar mass of water (18 g/mol)
A: Temperature-dependent constant
B: Temperature-dependent constant
I: Ionic strength

Nevertheless it is said to vary only within narrow limits in serum. If efforts to establish a reference method for ionized calcium (4) should be successful, one could imagine that a reference method for the determination of the free molal concentration of sodium could also be developed.

### 1.2.3 *Total molal concentration*

The total molal concentration of sodium is defined by equation 6:

$$\hat{m}_{tNa} = \frac{\tilde{m}_{Na}}{\gamma_{Na}} + \hat{m}_{NaProt} + \hat{m}_{NaCO_3^-} + \hat{m}_{NaHCO_3} + \hat{m}_{NaX} \quad \text{(Eq 6)}$$

$m_{tNa}$: Total molal concentration of sodium in serum water
$m_{NaProt}$: Molal concentration of sodium bound to protein
$m_{NaCO_3^-}$: Molal concentration of sodium bound to carbonate
$m_{NaHCO_3}$: Molal concentration of sodium bound to hydrogen carbonate
$m_{NaX}$: Molal concentration of sodium bound to other anions

Reference interval:

$$S(W) - m_{tNa}: 144.7 - 155.4 \text{ mmol/kg}$$

The free molal concentration of sodium is a quantity difficult to substantiate, as sodium binds to e. g. proteins and carbonate. Usually, the total amount of bound sodium is, however, only about 1.5% of total. That is why methods that determine total molal concentration may nevertheless be useful tools for the accuracy assessment of measurements of free molal concentration by ion-selective electrodes.

Two different approaches can be envisaged for this purpose:

Firstly, by neglecting sodium binding completely, the sodium concentration in serum water can be calculated from the concentration of sodium in (total) serum (determined by the established reference method (1)) and the water content of the serum, which can be measured according to l. c. (5).

As a second approach, it is proposed (6) that proteins and lipids are removed by ultracentrifugation. The concentrations of sodium in the supernatant, as determined by the NIST reference method (1) and by ion-selective electrodes, should agree closely, even if the value from ion-selective electrodes is slightly lower, as it does not comprise sodium bound to carbonate or to other anions not separated by ultracentrifugation. It must, however, be remembered that the electrolyte composition of the supernatant differs from the electrolyte composition of the serum water of the pertinent serum. Therefore this approach can be proposed only for the accuracy control of ion-selective electrodes, but not for the accuracy control of measurements of the total molal concentration of sodium in native serum water. A possible influence of proteins on the electrode is not monitored.

The sodium concentration in serum water will perhaps be more easily accepted by physicians than the active molality of sodium. Sodium concentration in serum water is, nevertheless, an independent quantity with a reference interval of its own, and it must be distinguished clearly from sodium concentration in (total) serum.

### 1.2.4. *Adjusted active substance concentration*

To remove all these difficulties, a new quantity has been proposed for use in the measurement of sodium by ion-selective electrodes: "Adjusted active substance concentration" (7). By calibration of the ion-selective electrode with so-called "normal" sera, one can achieve a situation, in which the values obtained by ion-selective electrodes for sodium agree with results obtained by flame atomic emission spectrometry, i. e. the sodium concentration of "normal" sera. In this meaning, a "normal" serum is characterised by a mass concentration of water of 0.933 kg/l, serum hydrogen carbonate concentration of 24 mmol/l, pH 7.40 and concentration of albumin, total protein, cholesterol and triacylglycerols within the reference range for healthy subjects. It is evident that this calibration procedure is rather tedious and time-consuming. Sera are needed, which are all "normal" as defined, but cover a wide range of sodium concentrations. This type of calibration material, i. e. secondary standard

specimens instead of primary standard solutions, is the least desirable. Which such calibration material, accuracy control on the basis of reference method values is hampered; it can only be performed with sera that match all the characteristics previously described, i.e. "normal" activity coefficient, "normal" binding and, most important, "normal" water concentration (equation 7):

$$c_{t\,Na} = \left(\frac{\tilde{m}_{Na}}{\gamma_{Na}} + m_{Na\,Prot} + m_{NaCO_3^-} + m_{Na\,HCO_3} + m_{Na\,X}\right) \cdot \varrho_{H_2O} \quad \text{(Eq 7)}$$

$c_{t\,Na}$: Total molar concentration of sodium in serum
$\varrho_{H_2O}$: Mass concentration of water in serum (0.933 kg/l)

Reference interval:

$$S - c_{t\,Na}: 135-145 \text{ mmol/l}$$

The NIST tries to produce calibration sera for this purpose (8). The sodium concentration of these sera is determined by the reference method (1). One main target is, however, not attained. Thus, the concentration of (total) sodium in serum will often unpredictably differ to a considerable extent from the "adjusted active substance concentration", because the concentration of serum water shows wide variations, e.g. due to hyperlipidaemia and hypo- or hyperproteinaemia. Such variations influence the sodium concentration in serum, but not its "adjusted active substance concentration" (fig. 2). These two values must therefore be reported separately. Identical reference intervals for both quantities are not advantageous, but may be deceiving. Indeed they agree only when the sera contain at least a "normal" amount of macromolecules. In hypoproteinaemia, the reference interval of sodium concentration in serum is higher, in hyperproteinaemia lower, whereas the reference interval of the "adjusted active substance concentration" is unaffected. Furthermore one is used to thinking of an active substance concentration as a fraction of the total, i.e. a value always lower than the total.

In addition to sodium, these considerations concerning macromolecules are also fully applicable to chloride. Even though macromolecules have a similar analytical influence in the determination of potassium, the clinical importance is less, as the reference interval is relatively large.

### 1.2.5 *Conclusions*

Summing up, one can say that depending on the calibration and calculation of results, different quantities are reported for measurements by so-called "direct" potentiometry, be it sodium or potassium. None

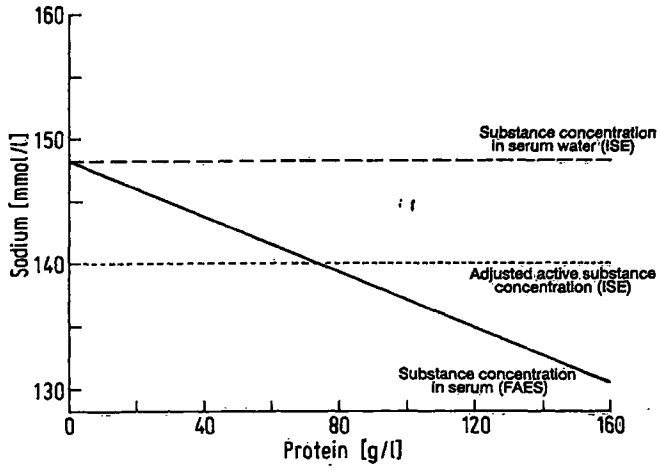


Fig. 2. Sodium determination with ion-selective electrodes and flame atomic emission spectrometry in serum.
Dependency of the sodium concentration in serum water at various protein concentrations as determined by ISE on the calibration of the ISE:
1) Calibration of the ISE by normoproteinaemic sera: "adjusted active substance concentration".
2) Calibration of the ISE with primary standard solutions free of protein adjusted to concentration in serum water: total molal concentration in serum water.
Dependency of the sodium concentration in serum as determined by FAES on the protein concentration: total molar concentration in serum.
ISE: Ion-selective electrode.
FAES: Flame atomic emission spectrometry.
From l. c. (6).

of the available reference methods is fully satisfactory for the assessment of accuracy, as they require assumptions, and assumptions are incompatible with stringent accuracy control. Now, as ion-selective electrode measurements for other analytes become more and more popular, a deliberate decision concerning what is measured and what should be reported is necessary. Any protocol should not impede the applicability of accuracy control with reference methods, and it should be applicable for all other analytes that may be measured with ion-selective electrodes in the near future, conforming to the rational system of quantities and units (9).

## 2. pH and Blood Gases

The assessment of accuracy of pH, $pCO_2$ and $pO_2$ measurements according to the new concept of quality assessment based on reference method values could be realized soon, because reference methods for pH and tonometry are available (3, 10). However, a detailed protocol, which is of utmost importance for attaining and guaranteeing precision, accuracy and transferability of the reference method according to the basic experiments of *Cali* et al. (11), is still lacking for these methods.

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