

Determination of Sodium with Ion-Selective Electrodes

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The advent of ion-selective electrodes made possible the potentiometry of sodium in serum and plasma. These methods were based on dilution of serum, as done in flame photometry, and the results were identical. Analysis of whole blood precludes dilution and so "direct" potentiometry was developed. Results by this technique are variable but tend to compensate for the spurious hyponatremias found by the "indirect" dilution methods due to displacement of volume by lipids and protein. However, there is no unambiguous theoretical basis on which to choose between the various direct ion-selective-electrode techniques and instruments. As an alternative, I propose use of current indirect methods, with numerical correction for the shift in normal sodium values in the presence of abnormal lipid and (or) protein. A table was constructed for making such corrections.

Traditionally, sodium in plasma or serum is determined by flame photometry. The development of electrodes that are selective for sodium (1) opened the possibility of potentiometric determination, which offers advantages. When we designed the first commercial instrument (2), we devised a scheme to track the results of flame-photometric determinations despite the fact that the two methods are inherently different. This method of potentiometric analysis is referred to as "indirect," and it involves automatic dilution of the sample. Similar schemes were subsequently used in several clinical analyzers in which ion-selective electrodes (ISE) are used.

More recently, a different approach was taken in some potentiometric instruments that permit analysis of whole blood. This "direct" method does not involve dilution. It became evident that the results differed from those obtained by flame photometry for clinical samples and, markedly, for controls (3). There were also differences between the results obtained with instruments of various manufacturers (4, 5), although the instruments in which the "indirect" ISE method were used were consistent with flame-photometric results (4).

Controversy prompted by these facts resulted in several Letters to the Editor of this journal (6-9), and the controversy rose to a crescendo at the last national meeting of the AACC in Boston, where five papers were presented on this topic (10-14). Nor does this controversy abate; four more Letters to the Editor were carried by this journal since then (15-18).

Evidently it would be appropriate to summarize the facts, which are not nearly as complex as many other clinical analytical problems. This is the aim of the present paper.

The Theory

Potentiometry follows clear physical chemical principles. The potential measured is proportional to the activity of the ion in the sample according to the Nernst equation: $E = \text{const.} + (RT/nF) \cdot \ln a_i$. However, for good and valid reasons, ISE instruments are used as comparators rather than "absolute"

instruments, despite the fact that most electrodes are largely "Nernstian"—i.e., their output corresponds to the theoretical equation. The equation is then modified: $\Delta E = (RT/nF) \cdot \ln(a_i/a_{i0})$, where a_{i0} represents the activity of the ion in a standard and RT/nF is essentially constant.

Ion-selective electrodes *do not measure concentration, but activity*, defined as the product of concentration and activity coefficient ($a = \gamma \cdot c$). The activity coefficient is 1 at infinite dilution, and its value depends on the concentration and valence of all ions present. According to the Debye-Hückel expressions for dilute aqueous solutions (19): $\log \gamma = -0.509 z^2 \sqrt{I}$, where z is the valence of the ion and I the ionic strength as defined by Lewis. It is a sum: $I = \frac{1}{2} \sum (m_i z_i^2)$, representing the concentration and valence of all ions present. In blood, plasma, or serum the value of the activity coefficient will vary from sample to sample, but will certainly not exceed 0.7. Concentration and activity are grossly dissimilar quantities. In "indirect" potentiometry, the sample is diluted with a relatively large volume of buffer of high ionic strength, so that the activity coefficient is controlled and virtually constant. Thus, even though the electrode responds to activity, when the signal is transformed the method yields a very good estimate of the concentration. $E = \text{const.} + (RT/nF) \ln (c_i \gamma / c_{i0} \gamma_0)$ but since $\gamma \approx \gamma_0$, the ratio becomes a concentration ratio: $\ln (c_i / c_{i0})$. This was proved in literally thousands of assays (4).

In the "direct" method this is not possible. In these, the instruments are also used as comparators and the results are quasi-transformed by assuming constant activity coefficients for the standards, calibrators, and samples. However, the activity coefficients of the samples—and of the standards or calibrators—considerably influence the results, as discussed below.

There are contrasts with direct potentiometric blood-pH determinations. First, pH is defined on the basis of hydrogen ion *activity*, not concentration. Thus, the prime datum is actually measured. Second, the range of physiological hydrogen ion concentration is almost a full order of magnitude (one pH unit). For sodium ion, the range is very much narrower, 120-160 mmol/L or, in logarithmic terms, 2.08-2.20 for the entire domain. So this determination is much more exacting than that of blood pH.

Why should sodium ion concentration be measured directly in undiluted samples? Originally, this was probably motivated by the desire for rapid determination on whole blood in certain situations, for example, open-heart surgery. However, once instruments were available another rationale was advanced. It has been known for more than 20 years that extreme cases of hyperlipemia (20) and hyperproteinemia (21) will simulate hyponatremia, i.e., spuriously low sodium concentrations will be determined in sera that are markedly lactescent or for sera with the extremely high protein values that are typical in multiple myeloma. In these instances the direct method will give values for sodium that are nearer the normal. In a very comprehensive study on hyponatremia, Fuisz (22) refers to these pseudohyponatremias as a well-known matter. This was pointed out by Ladenson again recently (23), and it is an argument in favor of direct potentiometry.

For a rational decision in these matters, the physiology must be considered. Renal regulation of sodium is marvellously sophisticated. Johnson (24) estimates that the energy consumption of the kidney assignable to the entropy inherent in

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sorting out what to retain and what to excrete exceeds the osmotic work by 30-fold. The main function of the kidney is to maintain an appropriate osmotic pressure, and sodium (and chloride) is the major instrument to accomplish it. There is a consensus that a concomitant index is the sodium ion concentration in "plasma water."

There are really two somewhat disparate effects at work. Lipids are dispersed in blood (and plasma or serum) and form a separate phase (as do erythrocytes). They are outside the electrolyte solution, as it were, but are included in the total volume when samples are taken, e.g., for flame photometry. Conversely, proteins are in colloidal solution. However, they occupy some volume, and the concept of partial volume in a sample can be introduced. Mohan et al. (25) showed that potentiometric measurements of sodium in albumin solutions under controlled conditions indeed follow the theoretical sodium ion activity in partial volume of water. An early empirical formula for serum water in protein solutions was given by McLean and Hastings (26). A rather similar formulation that includes total lipids and total protein originated with Waugh (27), who also cites the preferred analytical methods. This equation seems to enjoy general acceptance. The formulation, plasma water = 99.1 - 0.73 protein - 1.03 lipid, is admittedly an approximation, but it is shown to hold even for abnormal sera. It is used in the following to estimate "sodium in concentration in plasma water."

Discussion and Conclusions

For obtaining a diagnostically significant analytical result, five alternatives are apparent:

1. Separate the serum water and determine sodium ion concentration by indirect ISE methods or flame photometry.
2. Determine sodium ion activity with direct potentiometry and develop an activity scale.
3. Analyze by direct ISE potentiometry, but transform the results from activity to concentration units.
4. Determine concentration of sodium in total serum in the conventional way by flame photometry or indirect ISE methods and leave the interpretation to the clinician.
5. Carry out the determination as above, but aid the clinician with an estimate of the shift due to serum water level.

1. The first alternative of analyzing an ultrafiltrate by "indirect" methods is straightforward and rational. Tarail et al. (21) demonstrated its feasibility, using a centrifugal ultrafiltration apparatus designed by Toribara (28). However, even though there are practical commercial devices that seem to yield good ultrafiltrates (29), it is unlikely that this technique will find favor: it is not sufficiently rapid and economical for routine electrolyte analyses.

2. The determination of sodium activity is analogous to that of pH. In this context it must be appreciated that it took literally decades until a universally acceptable scale and standardizing buffers were evolved. The effort for a sodium-activity scale is simply overwhelming. More importantly, perhaps, there is no persuasive physiological argument in its favor, and it is doubtful that clinicians would ever accept numerical values that are totally alien to the accustomed concentration terms. On balance, this is the least-promising approach.

3. This alternative is essentially the system adopted by manufacturers of equipment in which "direct ISE" is used. It is beset by difficulties because of the previously stressed fact that the electrodes respond to *activity*, but the results are to be expressed in *concentration*. A simple, perhaps simplistic,

resolution is to use calibrators whose value is expressed in concentration terms. This is actually practiced, and the aim has been to arrive at the same analytical values as found by flame photometry for essentially normal sera (9). As it turns out, this is not exactly valid; theoretically, there should be a substantial difference when aqueous calibrators are compared with proteinaceous samples. With some instrument systems this difference is substantially less than the predicted shift. It has been opined (16) that this is due to junction potentials or to a mismatch of activity coefficients between calibrators and samples. Others argue that it is instead due to sodium binding by the protein (17). Although such sodium binding has been proposed by several authors (11, 15, 30), this is inconsistent with Mohan et al. (25), who found no such effects with albumin solutions. Mohan and Bates (31) discuss the role of junction potentials with clarity: the effect would tend to be in the direction of reducing the activity effects, as has been pointed out (16). One must consider that in practice we are dealing with solutions in the 0.2 mol/L range containing 50 to 100 g of colloids per liter, in solution. Such solutions are so far from ideal, and so remote from Debye-Hückel formulations, that theoretical considerations do not really pertain. Mohan and Bates (25) point out that "... additional effects in electrode potential may occur in whole blood, plasma, or serum." One must also consider that the total potential range for the clinical range of sodium is less than ± 4 mV within which the CV for analytical results must be less than 1%. If one considers that liquid junction potentials may reach hundreds of microvolts, in addition to more esoteric effects such as streaming potentials not even discussed here, the polemics on "theory" take on an aura of unreality.

Nevertheless, direct ISE methods transformed to measure sodium concentration in plasma water do, in fact, make corrections for pseudonatremias caused by extremes of lipid and protein values. However, the correction, while in the proper direction, will vary in magnitude almost as much as the effect itself, depending on the nature of the calibrators and of the equipment used. In the absence of a good theory, widespread disagreement among practitioners had developed, as pointed out by Fleischer and Schwartz (14).

4. This approach is taken at present by most laboratories, for good and valid reasons. Indirect ISE methods and flame photometry are almost ideal as standard methods. The calibrators are exactly defined and traceable to NBS materials. The indirect ISE method faces up to the problems inherent in non-ideal solutions and the fact that the transducer responds to activity. They are solved, as evident by the agreement with the entirely independent flame method. The only drawback is that physiology surely does not respond to sodium values in total serum—although the same criticism can be applied to all electrolytes determined in the clinical laboratory, including chloride. Still, the disadvantage is the well-known pseudohyponatremias—i.e., falsely low sodium values from the point of view of physiology. At present, the profession is satisfied to let the physician make a mental correction in cases of multiple myeloma and the like.

5. As an alternative it is proposed to give some quantitative guidance for this correction, which is now done more or less intuitively. Using the correction proposed by Waugh (27) I constructed a two-dimensional table (Table 1). It is laid out in a manner to conform with current clinical practice. The values for Na⁺, total lipid, and protein are median to the normal clinical range (32): 75 g of total protein, 7.50 g of total lipid, and 145 mmol of Na⁺ per liter. Table 1 is not according to theory in the sense that the basis is not zero percent protein and lipid but rather the accepted normal. It could be made to conform to any point for any parameter. However, the Table preserves presently accepted normal values and at the same

Table 1. Shift of Median of Normal Sodium Values with Abnormal Protein and Lipid

Protein g/dL	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5
Lipid g/dL														
0.25	149.2	148.1	146.9	145.8	144.7	143.5	142.4	141.2	140.1	139.0	137.8	136.7	135.5	134.4
0.75	148.4	147.3	146.1	145	143.9	142.7	141.6	140.4	139.3	138.2	137.0	135.9	134.7	133.6
1.25	147.6	146.5	145.3	144.2	143.1	141.9	140.8	139.6	138.5	137.4	136.2	135.1	133.9	132.8
1.75	146.8	145.7	144.5	143.4	142.3	141.1	140.0	138.8	137.7	136.6	135.4	134.3	133.1	132.0
2.25	146.0	144.9	143.7	142.6	141.5	140.3	139.2	138.0	136.9	135.8	134.6	133.5	132.2	131.2
2.75	145.2	144.1	142.9	141.8	140.6	139.5	138.4	137.2	136.1	134.9	133.8	132.7	131.5	130.4
3.25	144.4	143.3	142.1	141.0	139.8	138.7	137.6	136.4	135.3	134.1	133.0	131.9	130.7	129.6
3.75	143.6	142.5	141.3	140.2	139.0	137.9	136.8	135.6	134.5	133.3	132.2	131.1	129.9	128.8
4.25	142.8	141.7	140.5	139.4	138.2	137.1	136.0	134.8	133.7	132.5	131.4	130.3	129.1	128.0
4.75	142.0	140.9	139.7	138.6	137.4	136.3	135.2	134.0	132.9	131.7	130.6	129.5	128.3	127.2
5.25	141.2	140.0	138.9	137.8	136.6	135.5	134.3	133.2	132.1	130.9	129.8	128.6	127.5	126.4
5.75	140.4	139.2	138.1	137.0	135.8	134.7	133.5	132.4	131.3	130.1	129.0	127.8	126.7	125.6

time gives a estimate of the shift due to "serum water effect."

In conclusion, I strongly believe that the present methods for measuring sodium concentration in serum by flame photometry or indirect ISE potentiometry should be maintained, but as a practical guide to the physician to aid diagnosis of hyper- to hyponatremias, an estimated shift of the accepted normals can be furnished by the laboratory.

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Automated Determination of Copper in Undiluted Serum by Atomic Absorption Spectroscopy

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An injection method has been adapted for the determination of copper concentration in untreated, undiluted serum by flame atomic absorption spectroscopy. Serum, 50 or 100 μL , is automatically injected by a commercial microprobe system into a plastic cone connected to the capillary tube of the burner, at a rate of 240 samples per hour. The required sample volume is considerably decreased, and sensitivity is increased 20- to 40-fold. After 500 measurements we observed no memory effects, carryover, or clogging of the burner. We discuss common difficulties with calibration standards due to viscosity and other physicochemical interferences, and suggest the use of pooled human serum as a secondary standard. Within-run CV was 1.8%, the day-to-day CV 2.2%. Comparison with a dilution method gave a correlation coefficient exceeding 0.98.

Additional Keyphrase: trace elements

Several flame atomic absorption methods have been described for determining copper and other trace elements, all but one (1) of which necessitate pretreatment of the serum or plasma (2). A currently used procedure includes precipitation and extraction of samples (2-5), but is influenced by volume errors, errors caused by incomplete extraction, and contamination problems (6). Furthermore, this method is time consuming and expensive, and consequently not convenient for a routine laboratory. Dilution techniques are widely used, being easy to implement. Dilutions by (e.g.) 20-fold (7), 10-fold (8-10), twofold (3, 11) have been described. At high dilution, interactions related to the serum matrix are diminished, but so are sensitivity and precision. Moreover, the risk for con-

tamination increases. At low dilution, burner clogging and adjustment of standards may cause problems. In some cases, contradictory results reported concerning the influence of the matrix on trace-metal determination may be related to the preparation of standards. Thus the physicochemical properties of the standards should be as similar as possible to those of the samples.

We have therefore developed a simple, rapid, and fully automated micromethod without sample pretreatment that allows 240 measurements per hour.

Materials and Methods

Instrumentation

We used atomic absorption spectrophotometers (Models 400 or 432; these and all accessories are from Bodenseewerk Perkin-Elmer Co. GmbH, Überlingen, F.R.G.) equipped with a Perkin-Elmer Intensitron single-element hollow-cathode lamp (15 mA) and a single-slot burner (0.6-mm slit) with premix pneumatic nebulizer. The wavelength setting was 324.8 nm, the slit setting 0.7 nm.

Pressure for acetylene was 55 kPa and for compressed air 207 kPa. Concentrations or absorbances were printed out (Models UP-1 or PRS-10) or recorded (Model 56). Samples are injected with the autosampling system AS-3, with the aspiration tube kept as short as possible.

Reagents and Materials

Glassware was soaked in dilute (6 mol/L) HCl, then rinsed 10 times with de-ionized redistilled water. Plastic syringes, polypropylene tubes, and polythene stoppers (Walter Sartstedt, Nürnberg, F.R.G.) were found to be free from copper contamination, as already reported for zinc (13). Interferences from coated tubes have been reported (14). Standard solutions were either prepared from Titrisol^R (E. Merck, Darmstadt, F.R.G.) element standard solution (1 g/L) or alternatively from reagent-grade compounds of this manufacturer.

For additional measurements 20-, 50-, and 100- μL "Accupette" pipets (Dade Diagnostic, Inc., Miami, FL 33152) were

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