An All Solid-State Electrochemical Electrolyte Analyzer

Melvin D. Smith, Robert W. Rogers, Marvin A. Genshaw, and Jerome Greyson

Ion-selective electrodes have been applied to analysis of blood electrolytes with some success. However, currently available ion-selective electrode systems tend to be elaborate and expensive. We describe a clinical electrolyte analyzer in which inexpensive ion-selective electrodes are used. The electrodes are in the configuration of thin wires and are all solid in construction. They may be dipped directly into undiluted 250-μL samples of serum or plasma, are nondestructive of the sample, and may be used with an expanded-scale pH meter, although a more sensitive electrometer is preferred. The complete electrolyte analyzer consists of an electrode holder, into which the electrodes are plugged, and a solid-state digital electrometer that displays units of electrolyte concentration. A discussion of the thermodynamics essential to the construction of reversible electrodes is also presented, as well as the results of a clinical study in which it is shown that data from a flame photometer and the electrochemical analyzer compare favorably.

Additional Keyphrases: sodium and potassium ion determination • ion-selective electrodes • emergency measurements • blood electrolytes

Over the past few years, there has been a very large research effort involved in development of ion-selective electrodes (1, 2); and within the past six months, two electrolyte analyzers have been introduced to the clinical chemistry market in which electrodes are used for the measurements (3). To date there are no field returns in on these instruments, although it is certain that their manufacturer has clinically evaluated them on his own. One does know about the instruments, though, that they are automated, elaborate, and expensive.

The point of view in this laboratory has been that the beauty of electrode measurement, especially in a clinical environment, is its potential simplicity and economy. Electrodes lend themselves to simple, straightforward measurements with very basic instrumentation, similar in every respect to a pH meter. Furthermore, surveys of clinical laboratories have indicated no real need for new technology to do electrolyte measurements automatically (the need, apparently, being well satisfied by flame photometer technology), so efforts in this laboratory were directed to development of an electrolyte analyzer that could serve as a simple device for back-up and emergency use. In particular, we tried to develop miniaturized ion-specific electrodes that, in combination with simple instrumentation, could be used in a dip-and-read fashion in small sample volumes. As will be seen, the electrodes that were developed were built around a thin silver wire, small enough to use with 250-μL samples of undiluted serum or plasma.

It is helpful, before describing the electrodes developed, to discuss the necessary components of ion-selective electrodes, so as to dispel some misconceptions. One cannot, as some current reports suggest (4, 5), simply coat a wire with an ion-selective matrix and expect that such a combination will serve reliably as a measurement tool. Reliability in an equilibrium electrode measurement, which the use of ion-selective electrodes implies, requires a thermodynamically defined system. By thermodynamic definition, one means that all of the reactions and transport processes underway in the total electrochemical cell in which the electrode resides can be specified. As has been emphasized by Gibbs, one can measure the electromotive force only between the two leads extending from an electrochemical cell (6). To relate that measurement, unequivocally, to some cell parameter such as species activity or concentration, one should be able to define the total cell process. It can, of course, be argued that practical application of a concentration-measuring electrode requires only an exhibition of reproducibility in a few model systems. However, one should feel less than comfortable about accepting such an argument in clinical application.

A thermodynamically definable potassium-selective electrode might appear as in the cell configuration

\[ \text{Cu/AgCl/KCl (aq) / Sample solution} \]
\[ \text{KCl (aq)/HgCl}_2/Hg/Cu} \] (Cell I)

Potassium-selective electrode Reference electrode

______________________________

From the Physical Sciences Section, Ames Research Laboratory, Mars Co., Division Miles Laboratories, Inc., Elkhart, Ind. 46514.

Received Mar. 23, 1973; accepted May 18, 1973.
where each of the symbols has its conventional meaning. The single slashes are junctions between the components, and the double slash indicates a potassium-selective membrane. At each interface, an unequivocal process may be defined: electron transfer at the Cu/Ag and Cu/Hg interfaces, redox at the Ag/AgCl and Hg/Hg₂Cl₂ interfaces, and transport of ions between the various solutions at the membrane interface and the liquid junction. For each interface, one could write a mathematical expression relating that portion of the total cell emf to the process underway at that interface. However, in practice, this is not necessary because each of the interfaces, except the membrane-sample solution interface, gives rise to an emf contribution independent of the sample solution concentration.¹ With all other factors held constant, measurements from one sample solution to the next will yield a Nernst-like relationship between cell emf and solution concentration (7).

The electrodes described in this paper are based on such thermodynamic definitions.

Construction of the Wire Electrodes

All commercial ion-selective electrodes currently available are similar to the left-hand side of the above Cell I schematic, with differences only in the nature of the membrane, or, perhaps, in the nature of the internal reference electrode. All, however, are about the size of a standard pH electrode. One goal of this laboratory was to prepare electrodes that are more directly useful in small, clinically important samples. In addition, we wanted to use a solid-state configuration, so that the intermediate reference electrolyte solution shown in Cell I would not be needed.

The wire electrodes are fabricated by layering a hydrated gel containing KCl over a silver-silver chloride element, and then layering over the gel a membrane that imparts either ion-selective or reference-electrode properties to the structure. These components correspond to each of the components specified in Cell I.

Clearly, with such structures one must be concerned with osmotic effects. With an interior gel containing KCl bounded by an external membrane, water must travel back and forth across the bounding membrane, at a rate and in a quantity that depends on the relative concentration of salt in the gel and sample solution. If water is transported into the interior, the outer membrane will burst because of osmotic pressure. If water is transported out of the interior, the outer membrane will creenate, and electrode function will degenerate. Learning how to select the interior gel material in such a way that osmotic water transport was minimized represented the major development problem.

¹ With the help of the Henderson equation for the liquid junction potential (10), the salt bridge may be selected to yield an interface emf essentially independent of the sample solution concentration.

The exact formulations and procedures used to construct the wire electrodes are presented in detail in a forthcoming publication (8). Suffice it to say here that a silver wire anodized to silver chloride was coated with an inner layer made from a hydrophilic polymer containing potassium chloride. The outer membrane for the potassium electrode was made from a poly(vinyl chloride) formulation of valinomycin (an antibiotic substance). A neutral outer membrane was made for the reference electrode by incorporating a neutral carrier into a poly(vinyl chloride) matrix. As explained in reference 8, although water can pass through the outer membranes by osmosis, the interior formulations minimize the expansion and crenation effects that high osmotic flows might cause.

We were unable to formulate a sodium-specific electrode in the wire configuration. A number of different complexing agents were tried in the outer membrane, including many of the antibiotics and some of the macrocyclic ethers, even a couple of rather obscure compounds suggested by the literature (9). However, none was found that had sufficient selectivity over potassium to yield a useful device for measurements in clinical samples. As a result, a Corning NAS-11-18 sodium-selective glass capillary was used for the sodium electrode, with an anodized silver wire and an aqueous NaCl-KCl mixed solution sealed inside.

Figure 1 illustrates all the electrodes. Grouped as shown, the three fit easily into a 250-µl sample cup. At the tops of the electrodes are small electrical pin jacks to which the base silver wire has been soldered. These pin jacks are used to plug the electrodes into a handle, the bottom of which is shown. They are also the extreme copper elements shown in the Cell I schematic.

These electrodes are sensitive to the depth to which they are immersed in sample solution, so it is necessary to put a nonactive layer over the outer membrane surface so that an active area can be precisely defined. The two extreme electrodes have wax on them. The active element is the little round section at the bottom of each and the remainder is covered by the wax. Depth sensitivity was controlled with the glass electrode by a Pyrex glass collar, ringsealed upon it so as to leave only the spherical tip seen at the bottom as the active element.

All of these electrodes equilibrate rapidly in sample solution, usually in less than 30 s. It was found, incidentally, that the response time of the glass electrode was extremely sensitive to the thickness of the glass in the active area, the thinner glass yielding more rapid equilibration.

The sodium electrode yields a 59 mV per concentration decade response when opposed to a calomel electrode. The potassium electrode varies from 55 to 59 mV per decade when opposed to a calomel electrode. When opposed to the wire reference electrode, the concentration reproducibility in clinical samples was very good, as is seen in the next section.
Clinical Study

Electrodes similar to those illustrated in Figure 1 were used in a study comparing side-by-side sodium and potassium analyses via flame photometry and via electrode measurements. A Model 105 Beckman Flame Photometer was used. For the electrode measurements, a digital electrometer, which automatically converted the emf measurements to units of concentration, was constructed.

It is possible to make electrode measurements with an ordinary expanded-scale pH meter. However, this limits accuracy. The entire range of clinical sodium values corresponds to less than a 10 mV change in potential. For potassium, the range is about 25 mV. One pH unit corresponds to 59 mV, so it is seen that one would be working within a relatively narrow range on a typical pH meter. Construction of a more sensitive electrometer seemed the more desirable course and, with integrated circuits, was actually considerably less expensive than is a high-quality expanded-scale pH meter.

The details of the digital circuitry are not approp-riate to this paper. Furthermore, the digital aspects served more for measurement convenience than necessity. Suffice it to say here that the high input impedance requirements of the electrometer \(10^{11} \text{ohms} \) compared to \(10^6 \text{ohms} \) for the electrodes were met by feeding the electrodes output to a Siliconix U225 junction field effect transistor (FET). The FET was operated in a voltage follower mode with constant current provided to it at its zero temperature coefficient operating point. The output of the FET was amplified by a Motorola MC1437L operational amplifier, the output from which was used to drive a Signetics NE555 voltage controlled oscillator to convert the electrodes analog signals to digital form. Figure 2 is a circuit diagram for the critical portions of the electrometer circuit. The instrument yielded readability to 0.1 mmol of potassium and 1.0 mmol of sodium per liter, and its stability exceeded the reproducibility of the electrolyte measurements.

Experimental Procedure

Materials. Samples of serum and plasma (containing sodium heparin as the anticoagulant) were stored at 0°C until used. Beckman Flame Photometer Solutions No. 100394, 100390, and 100392 were used for the internal lithium standard, the zero standard, and the mid-scale standard, respectively. De-ionized water was used for the internal lithium standard preparation. Dry air and "Bernzomatic" propane were used for the burner gases. "Versatol" control sera (General Diagnostics Div., Warner-Chilcott Laboratories) were used for calibration and/or standardization.

Lithium internal standard was prepared in bulk, stored in a polyethylene container, and used throughout the study. All Versatol control sera and clinical samples were allowed to reach room temperature and then were diluted in a ratio of 200:1 with lithium internal standard as the diluent.

Flame photometer procedure. The flame photometer was calibrated with aqueous zero and mid-scale standards. Three Versatol control sera (low, normal, and high) were measured as a calibration check on both the sodium and potassium channels. Clinical samples were then analyzed with a mid-scale calibration check made after every third sample and a complete recalibration made after every 10 samples.
Electrode procedure. Versatol-A and Versatol-A Alternate control sera were used for electrode calibration. The sodium and potassium measurement channels were set with the manufacturer's stated sodium values of 124 and 153 mmol/liter and potassium values of 3.0 and 7.3 mmol/liter for the low and high sodium and potassium values, respectively. Versatol normal control serum was used as a midscale calibration check with a stated sodium and potassium values of 138 and 4.8 mmol/liter, respectively. Clinical samples, at room temperature, were analyzed, with a midscale check every third sample and complete recalibration after every 10 samples.

The digital electrometer was constructed with an automatic 1-min timing cycle, which was activated when the electrodes were immersed into each sample. The potassium display was read after the 60-s equilibration interval. The sodium value was read 4 s later. Thus, the total analysis time was about 70 s, including data recording time. The electrodes were simply blotted dry between samples.

Results

Results for serum sodium and potassium by flame and electrode are illustrated in Figures 3 and 4. Similar correlations were obtained with plasma samples. As can be seen, the correlations are excellent. The dotted lines bounding the least-square lines in the graph are two standard deviations apart. For potassium, the standard deviation from the least-square line is 0.27 mmol/liter (CV, 1.8%). For sodium, the standard deviation is 2.1 mmol/liter (CV, 1.5%). Some bias may be noted in the correlation, with the flame photometer yielding slightly higher values than the electrodes. The bias was attributed to electrode calibration with the Versatol controls, which yielded somewhat low electrode values. When preanalyzed pooled sera were used for electrode calibration in later work, much of the observed bias disappeared.

References