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New In Vitro Analytical Approaches for Clinical Chemistry Measurements in Critical Care

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The effective management of patients in intensive care units, operating rooms, and emergency rooms requires frequent measurement of a select group of analytes, preferably at or near the patient's bedside. Tests recognized as being essential for such management include blood gases and related variables (pH, p_{O_2}, p_{CO_2}, HCO_3^-, hematocrit/hemoglobin, O_2 saturation), electrolytes (Na^+, K^+, Ca^{2+}, Cl^-), and in some cases, certain metabolites (glucose, lactate, urea, creatinine). This report describes the measurement principles, practical instrumental designs, analytical performance, and limitations of several newer electrochemical sensor-based approaches useful for in vitro determination of these species in undiluted whole-blood samples. Considerable attention is given to the most recent advances in ion-selective electrode technology as they relate to blood gas and electrolyte determinations. Similar attention is given to modern enzyme-electrode techniques, which are useful for direct measurements of metabolites in whole blood. The challenges of integrating these new analytical methods into convenient, multi-analyte, user-friendly, bedside or stat-lab instruments are also discussed.

Additional Keyphrases: *blood gases · electrolytes · ion-selective electrodes · enzymic methods*

The effective management of critically ill patients often requires frequent measurement of a select group of analytes in blood. Tests recognized as being essential for such management include blood gases and related variables (pH, p_{O_2}, p_{CO_2}, HCO_3^-, hematocrit/hemoglobin, O_2 saturation), electrolytes (Na^+, K^+, Ca^{2+}, Cl^-), and in some cases, certain metabolites (glucose, lactate, urea, creatinine). Historically, such tests have been performed in centralized laboratories remote from the patient, but recent years have seen increased demands for measurement technologies that enable such tests to be carried out rapidly at or near the patient's bedside. The availability of instruments with such capabilities can provide clinicians with essentially "real time" diagnostic information, and this can result in more timely and proper therapeutic intervention.

Ideally, instrumentation designed for in vitro bedside or nearby stat-lab testing should be capable of measuring several of these "critical-care" analytes simultaneously in a small sample of undiluted whole blood. The more analytes that can be measured directly or calculated via appropriate algorithms, the more diagnostic information available to the clinician. However, because the accurate measurement of blood gases is often the most crucial factor in assessing the status of critically ill patients, the desire to design instruments capable of determining other important related analytes along with the blood gases mandates that the analytical methodology used for these other tests also be whole-blood compatible. Consequently, classical atomic spectroscopic techniques for electrolyte determinations (i.e., flame emission and atomic absorption) as well as conventional reagent-based photometric methods involving dilution for metabolite testing cannot be utilized in new-generation multi-analyte test instruments. However, modern ion-selective and enzyme-electrode chemical sensor technologies are suitable for such purposes, and the most recent advances in these areas as they relate to the design of new analyzers for critical-care situations will be the focus of this report.

It is also highly desirable that multi-analyte bedside and stat-lab instrumentation be "user friendly," such that reliable test results can be obtained even when the instruments are operated by personnel with little or no training in clinical chemistry. Such performance requires low- or zero-maintenance-type equipment that is convenient to use (e.g., with autocalibration, etc.) for both analyses of samples and quality-control purposes. The approaches taken to integrate the latest chemical sensing analytical methods into instruments that have these desirable features will also be described.

Blood Gas and Electrolyte Measurement Technologies for Whole Blood: Principles and Practice

Although the classical Clark-style polarographic oxygen sensor (*1*) remains the dominant method for quantifying arterial and venous p_{O_2} values, significant advances in ion-selective membrane electrode (ISE) technology over the last decade have had a major impact on the design of instruments that can measure Na^+, K^+, Ca^{2+}, Cl^-, HCO_3^-, pH, and p_{CO_2} accurately in undiluted whole blood (*2*, *3*). One of the key advantages of ISE technology is the ability to measure free ion activities, not total concentrations. In the case of calcium determinations, this capability has dramatically increased the number of requests for ionized calcium tests for diagnostic purposes. The fact that ISEs measure ionic activities in the plasma-water phase of whole blood also provides a significant advantage in instances where patients' samples have increased concentrations of solids (e.g., hyperproteinemia or hyperlipidemia) (*4*, *5*). In such cases, conventional diluted-sample atomic spectroscopy techniques and even diluted-sample ISE methods will yield values significantly lower than those obtained by ISE methods for undiluted samples, owing to the dilution error induced by the presence of excess solids. Because the electrolyte activity in the plasma-water phase of whole blood is the physiologically important variable, ISE measurements of electrolytes in undiluted samples provide the most clinically relevant information.

Note that, because of the effect of plasma solids, the normal range of values for Na^+ and other electrolytes determined by undiluted whole-blood ISE methods should be about 5–7% higher than corresponding values obtained by conventional diluted-sample techniques (e.g., flame methods). However, to avoid confusion, most instrument manufacturers offset ISE electrolyte determinations so that the concentration values reported are equivalent to those

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that would be obtained via diluted-sample flame-emission methods, with the assumption that the samples contain a normal concentration of dissolved solids. Thus, for samples with normal lipid and protein contents, the newer undiluted whole-blood ISE-based instruments will provide values that correlate well with flame-emission determinations in plasma taken from the same whole-blood samples.

To better understand the factors that influence the accuracy and reproducibility of ISE determinations in whole blood, and the operating principles of some of the newer polymeric membrane electrode-based sensing configurations suitable for determining electrolyte, pH, p_{CO_2}, and HCO_3^- values, one needs some basic knowledge of how ISE-based electrochemical cells function. Figure 1 illustrates a typical electrochemical cell, which includes a working ion-selective membrane electrode and an external reference electrode. The specific electrode geometries (both reference and working ISE) vary greatly, depending on instrument design factors. In some instances, the ISEs are in tubular flow-through designs, with the wall of the tubing serving as the ion-selective membrane. In any case, the key component of the cell is this membrane, which for most whole-blood measurements is composed of either glass (for pH and Na^+) or a polymeric material, usually poly(vinyl-chloride) doped with an appropriate ionophore (useful for pH, Na^+, K^+, Ca^{2+}, HCO_3^-, Cl^-, and p_{CO_2} measurements). In the polymer membrane systems, which are particularly attractive for fabricating disposable ion and gas sensors, the ionophore serves as a reversible and re-usable binding reagent that selectively extracts the analyte ion into the organic membrane phase, thereby creating a charge separation or phase boundary potential at the membrane/sample interface. A similar potential develops at the inner surface of the membrane, which in most instances is in contact with a constant activity of the detected ion. The electrical potential difference across the membrane, the membrane potential (E_m), is logarithmically related to the ratio of the analyte ion activities outside (sample) and inside, within the internal reference electrolyte (see Figure 1). An analogous situation exists for glass membranes. The overall cell potential (E_{cell}) can be expressed as

$$E_{cell}\,(mV) = K + (59/z_i)\log\,(a_{i(sample)}/a_{i(internal)}) + E_j \qquad (1)$$

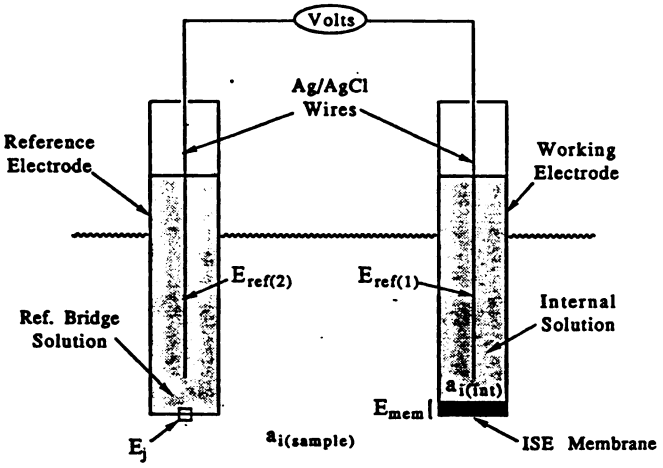

Fig. 1. Schematic showing components of typical ion-selective membrane electrode-based electrochemical cell

where K is the sum of all constant sources of potential in the cell (i.e., internal and external reference electrodes), z_i is the charge on the measured ion, and E_j is the junction potential that develops at the interface between the sample solution and the bridge solution of the external reference half-cell, due to differences in the mobilities and concentrations of ions in the two solutions. If the activity $a_{i(internal)}$ is in fact held constant (not the case in new p_{CO_2} and bicarbonate sensors described below), then equation 1 can be further simplified to

$$E_{cell}\,(mV) = K' + (59/z_i)\log a_{i(sample)} + E_j \qquad (2)$$

The logarithmic response of ISE devices creates some accuracy and precision limitations, because very small changes or uncertainties in the measured cell potential can result in relatively large changes or uncertainties in the determined ion activities or concentrations. In practice, E_j is usually considered a constant value and it is taken into account during the electrode calibration within a given instrument. However, the magnitude of this junction potential will change when switching from the aqueous calibrants to whole-blood samples. Moreover, variations in the composition of the blood, particularly in the hematocrit, can influence the liquid-junction potential and cause differences in the measured electrolyte concentrations in whole blood vs those in the corresponding plasma (*6*, *7*). This entire issue is complicated by the fact that the specific reference electrode design (i.e., free flowing vs restrained junctions) as well as the nature and concentration of the bridge solution can each influence the magnitude of liquid-junction potential effects observed.

Other factors that can limit the accuracy of certain ISE systems in whole-blood samples are the degree of ion-selectivity of the membrane and the adsorption of proteins or erythrocytes onto the surface of the membrane. Both of these effects are more pronounced with electrodes made with polymeric membranes doped with ionophores than with glass electrodes. Adsorption of blood components can also cause asymmetry potentials, which are not present when aqueous calibrants are introduced into the analyzers. This can lead to small errors in blood electrolyte and pH values, particularly for the first blood sample introduced into the instruments after a prolonged period in which the electrodes have been bathed in aqueous calibrant solutions. Fortunately, decreasing the hydrophobicity of the polymeric membrane phases, to reduce protein/erythrocyte absorption (via the use of alternative polymeric matrices), diminishes the magnitude of this asymmetry potential problem (*8*).

The selectivity of the ion carrier doped into the polymeric membrane is another key element that dictates the ultimate accuracy of a given ISE measurement of whole blood. Nonideal selectivity of an ISE system can be quantified via an expanded version of equation 2:

$$E_{cell}\,(mV) = K'' + (59/z_i)\log\,[a_{i(sample)} + \sum_j K_{i,j}\,a_{j(sample)}] \qquad (3)$$

where a_j is the activity of a potential interferent ion, and $K_{i,j}$ is the potentiometric selectivity coefficient. Thus, for the cell potential to reflect accurately the concentration of the desired electrolyte ion i, the concentrations of potential interferent ions and (or) the selectivity coefficients must be

small. This is a particular concern for certain Na^+ electrodes that do not exhibit very high selectivity over lipophilic cationic bacteriostat agents, which may be present in catheter sampling lines. Lack of adequate selectivity for Na^+ over endogenous K^+ can also pose a problem for these sensors, although this error can be eliminated by simultaneous measurement of blood potassium with a highly selective K^+ electrode. A similar type of probem can arise with certain polymeric ISE systems that are used to measure Cl^- in whole blood. Indeed, lack of selectivity for chloride over lipophilic anions, such as salicylate, can lead to significant measurement errors in samples from patients who have previously ingested aspirin (*9*).

Although the use of polymer membrane ISEs for the measurement of K^+, Ca^{2+}, Na^+, and Cl^- is now commonplace in many instruments, similar ISEs are now also used for accurate determinations of pH, p_{CO_2}, and HCO_3^-. All three of these new sensors are based on pH-sensitive polymeric membranes doped with a lipophilic tertiary amine compound (e.g., tridodecylamine), which serves as a highly selective proton ionophore within the membrane (see Figure 2). As shown in Figure 2*a*, if the internal reference solution of such a sensor is buffered, then the electrode functions as an excellent pH sensor, with selectivity equivalent to that of a classical glass-membrane electrode (*10*). Recent clinical evaluations of this type of electrode within new-generation blood gas/electrolyte analyzers have shown good correlation with pH values obtained by conventional blood gas analyzers equipped with standard glass-membrane pH electrodes (*11–14*).

The use of pH-selective polymeric membranes can be further extended to the design of novel p_{CO_2} and HCO_3^- electrochemical sensors. The development of these new sensing designs relies on the fact that carbon dioxide readily permeates the pH-sensitive polymeric membranes. In direct pH sensing, such diffusion of sample carbon dioxide does not interfere in the measurement of blood pH because a strong buffer is used as the internal reference electrolyte. However, as shown in Figure 2*b*, if two polymer membrane pH electrodes are used in a differential measurement arrangement, then the overall cell potential can be related to the logarithm of p_{CO_2} content in the blood sample. In this design, the internal reference electrolyte buffer of one of the pH sensors is replaced by a nonbuffered bicarbonate electrolyte (e.g., $NaHCO_3$/NaCl). The pH of this internal solution changes as a function of sample p_{CO_2}, whereas that of the second sensor does not. If both pH-sensitive membranes are identical in their response to sample pH, then variations in sample pH go undetected due to the differential measurement configuration. This type of differential p_{CO_2} sensor has been shown to function reliably within modern blood gas/electrolyte instruments for accurate measurement of p_{CO_2} in whole-blood samples, e.g., with commercial GEM-Stat and GEM-6 instruments (Mallinckrodt Sensor Systems) (*11–14*).

As illustrated in Figure 2*c*, the bicarbonate concentration in blood can be measured merely by using pH sensor-1 of the above differential p_{CO_2} scheme (see Figure 2*b*) and measuring its potential vs a standard external reference electrode (*15, 16*). In effect, this single sensor monitors the p_{CO_2} and pH of the blood simultaneously, thereby providing an output voltage that is proportional to the logarithm of the concentration of HCO_3^- in the sample. Such a relationship can readily be derived by utilizing appropriate CO_2/HCO_3^- equilibrium expressions to substitute for detected

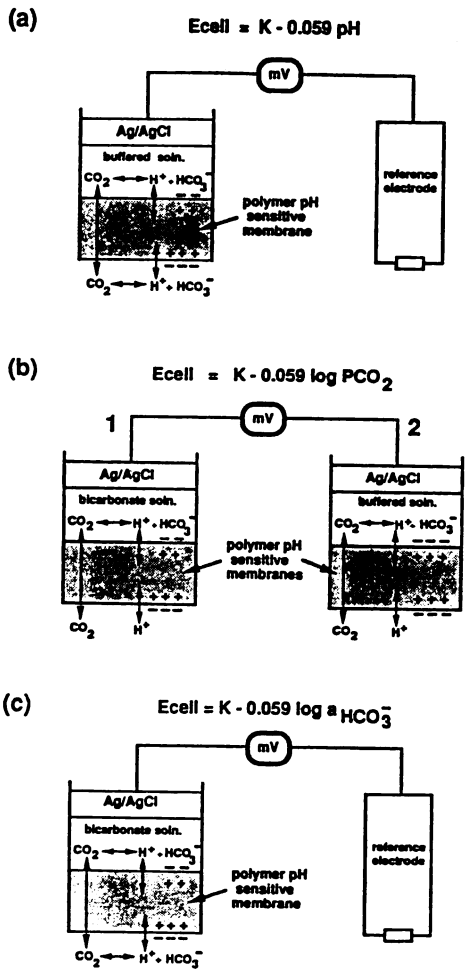

Fig. 2. Various new sensor configurations, based on pH-sensitive polymeric membranes, that can be used to determine pH (*a*), p_{CO_2} (*b*), and bicarbonate (*c*) in whole blood

Derivation of the response equation for each configuration:

The potential (in volts) of each polymer-membrane pH electrode may be written as:

$$E_{pH\ elect.} = K + 0.059 \log a_{H^+\ (sample)}/a_{H^+\ (internal)}$$

Therefore, for the arrangement in *a*: $E_{cell} = E_{pH\ elect.} - E_{ref.\ elect.}$

But $E_{ref\ elect.}$ and $a_{H^+\ (internal)}$ are constant; thus, $E_{cell} = K + 0.059 \log a_{H^+\ (sample)}$, or

$$E_{cell} = K - 0.059\ pH$$

For the P_{O_2} sensing arrangement (*b*):

$E_{cell} = E_{pH\ elect.\ 1} - E_{pH\ elect.\ 2}$, or $E_{cell} = K + 0.059 \log a_{H^+\ (sample)}/a_{H^+\ (internal)} - K - 0.059 \log a_{H^+\ (sample)}/a_{H^+\ (internal)}$

But $a_{H^+\ (internal)}$ of electrode 2 is constant because of the internal buffer; therefore,

$$E_{cell} = K + 0.059 \log (1/a_{H^+\ (internal)})$$

The internal solution of electrode 1 contains a bicarbonate electrolyte; therefore, when in equilibrium with a sample containing CO_2,

$$a_{H^+\ (internal)} = K_a \cdot p_{CO_2}/a_{HCO_3^-\ (internal)}$$

Thus, $E_{cell} = K + 0.059 \log (a_{HCO_3^-\ (internal)}/K_a \cdot p_{CO_2})$

Or, because $a_{HCO_3^-\ (internal)}$ is fixed and K_a is the first dissociation constant for H_2CO_3,

$$E_{cell} = K - 0.059 \log p_{CO_2}$$

For the HCO_3^- sensing configuration (*c*):

$E_{cell} = E_{pH\ elect.} - E_{ref.\ elect.}$, or $E_{cell} = K + 0.059 \log a_{H^+\ (sample)}/a_{H^+\ (internal)}$

But $a_{H^+\ (sample)} = K_a \cdot p_{CO_2}/a_{HCO_3^-\ (sample)}$ and $a_{H^+\ (internal)} = K_a \cdot p_{CO_2}/a_{HCO_3^-\ (internal)}$

Then $E_{cell} = K + 0.059 \log (1/a_{HCO_3^-\ (sample)})/(1/a_{HCO_3^-\ (internal)})$

Because $a_{HCO_3^-\ (internal)}$ is fixed, therefore

$$E_{cell} = K - 0.059 \log a_{HCO_3^-\ (sample)}$$

H^+ activities on both sides of the polymer membrane (see legend to Figure 2). As an indirect bicarbonate sensor, this electrode will determine bicarbonate concentrations that correlate with calculated values derived from individual sensor measurements of sample p_{CO_2} and pH (as in conventional blood gas analysis instruments). Indeed, measured or calculated concentrations of bicarbonate may differ from true blood values because of the uncertainty in assigning a correct constant for the CO_2/HCO_3^- equilibrium reaction (*17*). This historical problem will be solved only with the development of an ionophore-based membrane electrode that responds directly to bicarbonate activity.

Hematocrit by Conductivity

Conventional microcentrifuge and modern cell-counting cytometric methods for measuring blood hematocrit are not amenable to the design of convenient bedside/stat-lab multi-analyte test systems. The simplest method for monitoring hematocrit in whole blood involves the measurement of blood conductivity (1/resistance, Ω^{-1}). This is generally accomplished by using two platinum black electrodes in a standard ac impedance-type measurement arrangement. Because spherical erythrocytes are essentially nonconducting relative to the extracellular plasma electrolyte phase, an increase in the number of erythrocytes per unit volume of blood will decrease the number of charge-carrying ions and thus decrease the current in the circuit. Several models and equations have been proposed in the literature (*18–20*). Blood resistivity (in ohm-cm) of human blood is related to hematocrit as follows (*19*):

$$p = p_{plasma}\,[1+(f-1)\,H/100]/[1-(H/100)] \qquad (4)$$

where p is the whole-blood resistivity, H is the hematocrit, f is constant, and p_{plasma} is the resistivity of the plasma phase. In practice, variations in the electrolyte content of the plasma phase (particularly Na^+) can cause error in such hematocrit determinations. Consequently, the most nearly accurate hematocrit values are attained only when p_{plasma} is corrected for the Na^+ concentration in whole blood by simultaneous measurement of Na^+ activity via an appropriate ISE. At least two commercial instruments (the NOVA-Stat Profile series from Nova Biomedical, and the GEM series from Mallinckrodt Sensors) have conductivity sensors for determining hematocrit. Clinical evaluations of this method by comparison with conventional microcentrifuge procedures have yielded acceptable agreement between the two approaches (*11–14, 21, 22*).

Enzyme Electrodes for Measuring Metabolites in Whole Blood

Convenient methodologies for bedside testing of metabolites rely heavily on the use of specific immobilized enzyme reactions. Desired whole-blood measurements can be performed by detecting products of the enzymatic reactions electrochemically in a thin layer adjacent to the electrode surface (so-called enzyme-electrode configuration). For example, glucose may be determined by using glucose oxidase (EC 1.1.3.4) in accordance with the following reaction:

$$\text{glucose} + \text{electron acceptor (e.g., } O_2) \rightarrow$$
$$\text{gluconic acid} + \text{reduced acceptor (e.g., } H_2O_2)$$

If molecular oxygen is used as the electron acceptor, the steady-state concentration of hydrogen peroxide formed in the reaction layer can be measured to determine the concentration of glucose. In one approach, glucose oxidase is immobilized between two semipermeable membranes at the surface of a platinum or carbon anode (see Figure 3). The liberated peroxide formed in the enzyme layer is quantified amperometrically via its oxidation to molecular oxygen when endogenous oxygen in the blood is used as the electron acceptor. Other easily oxidized species normally present in blood (e.g., uric acid, acetaminophen, ascorbic acid, etc.) are prevented from reaching the electrode surface by the inner semipermeable membrane and thus do not interfere. To extend the linearity of the standard curve to high concentrations of glucose in the presence of low concentrations of dissolved oxygen in blood, the permeability of glucose through the outer membrane must be significantly lower than that of oxygen (*23, 24*). This enzyme-electrode concept is now used in the NOVA Stat-Profile instruments to provide results for glucose as well as blood gases, electrolytes, and hematocrit for a single sample of whole blood (*22*). Results correlate highly with plasma glucose concentrations, as determined by diluted-sample methodologies (*22*). A similar enzyme electrode for detection of lactate in whole blood also will be available shortly. The lactate sensor is configured the same as the glucose probe except that the detected peroxide is generated by the action of lactate oxidase, which catalyzes the conversion of lactate to pyruvate and hydrogen peroxide in the presence of molecular oxygen.

Alternatively, electron acceptors other than oxygen, such as ferrocene derivatives, may be co-immobilized with the oxidase enzymes, thereby eliminating the need to control relative rates of O_2 and substrate permeability through the outer membrane (*24, 25*). In addition, because the electrochemical signals generated from these alternative electron acceptors can be obtained at less anodic voltages, no inner semipermeable membrane is required (i.e., the endogenous redox species do not oxidize at these potentials). This latter

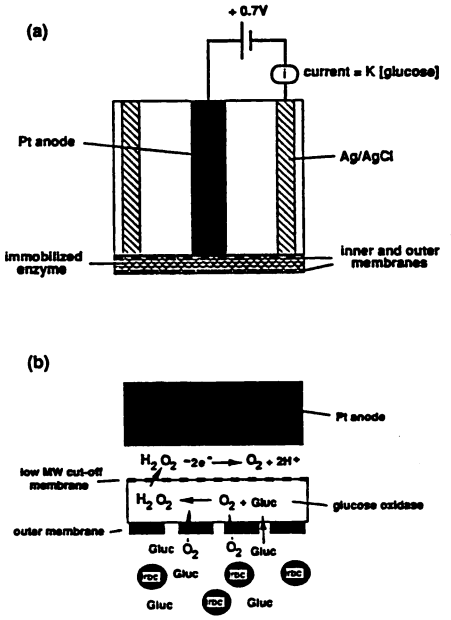

Fig. 3. (*a*) Schematic of amperometric sensor for whole-blood glucose measurements; (*b*) expanded view of sensing region illustrating the chemistry and membranes required for whole-blood glucose (gluc) measurements
rbc, erythrocytes

approach has been implemented in at least one commercial single-use glucose sensing system.

The development of sensors for other metabolites also involves the marriage of electrochemical detectors and immobilized enzymes. For example, urea in whole blood can be measured via the enzyme urease (EC 3.5.1.5) immobilized at the surface of a potentiometric ammonium ion-selective membrane electrode (*26*). Urease catalyzes the following reaction: urea $\rightarrow$ 2 NH_4^+ + CO_2. Thus, the steady-state concentration of ammonium ions generated in a thin reaction layer at the surface of the electrode is proportional to the urea concentration in the sample. However, the ionophore used to fabricate ammonium-selective membranes (nonactin) is not totally selective; such membranes also exhibit a significant response to potassium. Therefore, to obtain accurate urea values in undiluted whole-blood samples, one must correct for endogenous potassium concentrations in the samples via a separate measurement with a potassium ion-selective electrode.

The measurement of creatinine in undiluted whole blood remains a significant challenge. Two enzyme-electrode-based approaches appear to be feasible, one potentiometric, with ammonium ion or ammonia gas detection, and the second involving a multiple enzyme sequence that ultimately produces hydrogen peroxide. In practice, however, the low concentrations of creatinine relative to other blood components, particularly species that can interfere with the electrochemical sensors, pose a major problem.

Integrating Sensors into User-Friendly Bedside and Stat-Lab Instrumentation

Multi-analyte blood gas/electrolyte/metabolite instruments generally involve use of an array of the above electrochemical sensors in a flow-through channel. In most instances, the measurement signals are acquired with the sample or calibrant solution being stagnant (static) in the channel. After the sample is measured, the entire array is flushed with a calibrating solution; this allows the instruments to continually compensate for any drift in the sensors' output signal. Periodic two-point calibrations allow instruments to update the sensors' calibration sensitivities (slopes) to help assure accuracy for each analyte measured. Several manufacturers (Nova Biomedical, Corning Medical, Instrumentation Laboratory, Radiometer, etc.) have designed systems that enable simultaneous measurement of blood gases and electrolytes in a single sample (100–500 μL) of undiluted whole blood, with sensor arrays of conventional blood-gas and ion-selective electrodes. However, such systems tend to require periodic maintenance of the electrodes and assorted pump tubings. One manufacturer (Mallinckrodt Sensors) has designed a bedside multi-analyte test system that requires minimal operator interaction and no routine maintenance. The appropriate blood gas and electrolyte sensor arrays are packaged with the calibrating and flush solutions all in a single disposable cartridge (see Figure 4). Once the cartridge is placed in the instrument, the instrument can analyze 50 samples over a period of up to 72 h. Unlike other blood gas analyzers that calibrate by tonometering solutions with tanks of gas or by using the gas tanks to calibrate in the gas phase, calibration of the disposable blood gas sensors in the Mallinckrodt system is achieved with pre-tonometered solutions stored in disposable foil pouches. This eliminates the need for gas tanks and reduces the complexity and size of the bedside instrument.

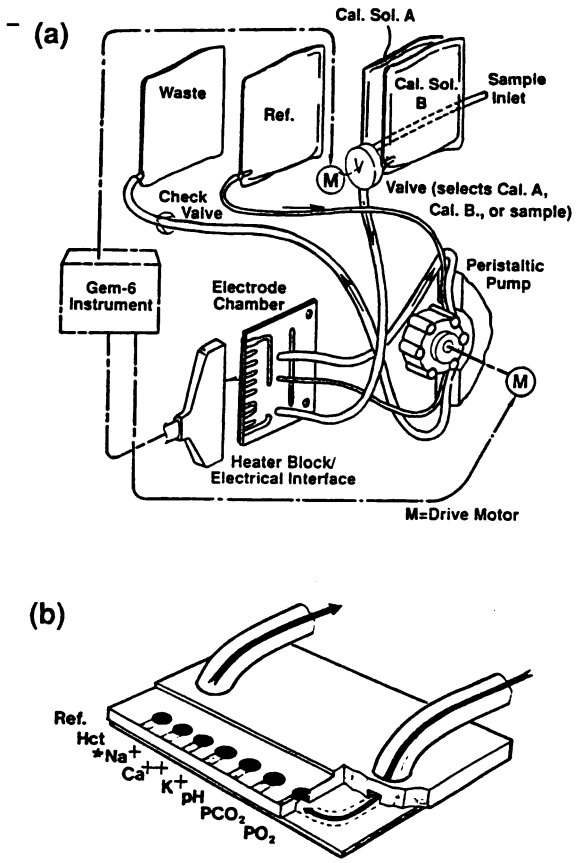

Fig. 4. (*a*) Components of the GEM blood gas and electrolyte system's disposable reagent/sensor cartridge; (*b*) expanded view of the GEM system's disposable, flow-through electrochemical sensor card

Ref., reference electrode

Future Prospects

Future efforts relating to the development of in vitro test instrumentation for critical care will likely focus on increasing the menu of analytes available for a single blood-sample test by expanding the number of chemical sensors incorporated within a given array. This will require the development of completely new optical and (or) electrochemical devices (e.g., for creatinine) and of immunosensors for rapid detection of diagnostic enzymes such as creatine kinase MB isoenzyme, plus further improvement in the performance of prototype sensors already devised. In addition, considerable attention will be given to implementing the various sensors in instrumental arrangements that are convenient to use, preferably by individuals with little or no training in the field of clinical chemistry. The development of blood gas/electrolyte/metabolite sensor arrays that can be mass-produced into low-cost disposable units will play a major role in this effort.

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