

# Critical care analyzer with fluorescent optical chemosensors for blood analytes

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This article summarizes the application of fluorescent sensors to *in vitro* diagnostic systems measuring critical care analytes in whole blood. We discuss recent advances in fluorescent chemosensor technologies employing fluorescence indicators used for this application. This paper touches on key materials, structure–property relations, and design considerations for maximum utility in low-cost portable systems. In this application the optical sensor technology demonstrates precision and stability comparable or better than that of the best-available conventional technologies employing electrochemical sensors.

## Introduction

The concept of an optical sensor, where a change in light properties is the transduction process, has been known for several decades. Such sensors using optical fibers were devised in the 1960's for blood oximetry, and two decades later appeared in the form of colorimetric glucose strips and fluorescent blood gas monitoring optodes.<sup>1</sup> However, the development of optical sensors, or 'optodes', has been facilitated in recent years by advances in the communications industry, optical switching components, and the development of high-quality optical fibers or optical waveguides.

Optical sensing was initially confined to substances that had intrinsic absorbance or fluorescence but the discovery of certain key indicator compounds and coupling mechanisms to designed receptors extended the technique to parameters such as blood gases (oxygen, carbon dioxide, and pH) and more recently to electrolytes and metabolites, such as glucose, lactate, urea and creatinine.<sup>1</sup> In many cases the measurement process is fluorescence, which this paper will focus on.

The processes associated with fluorescence are shown in Fig. 1. Certain compounds may be excited to a higher electronic energy state and in descending back to the ground

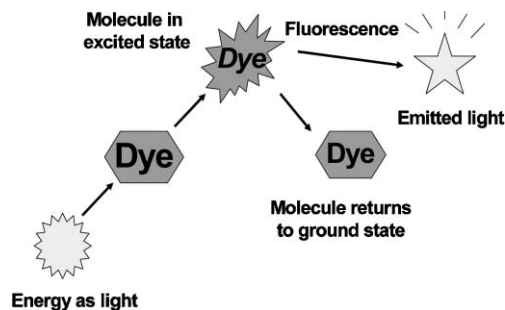


Fig. 1 Emission of light or fluorescence due to excitation of dye molecule by light.

state they emit light. This general process is termed luminescence but when light is used to provide the excitation energy (rather than chemicals or electricity), it is termed fluorescence. Alternative types of luminescence include, for example, chemiluminescence, bioluminescence, electrochemiluminescence, and phosphorescence.

With proper molecular design and control of the molecular environment, the emission process may be modulated by the presence of certain blood analytes. For example, when oxygen



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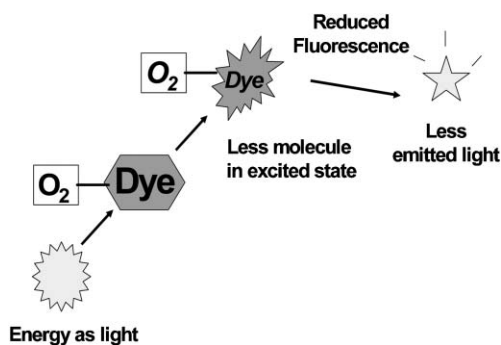
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is present, the fluorescence of certain indicator molecules with a long excited-state lifetime is quenched or reduced in proportion to the amount of oxygen present in the sample thereby providing a means of measurement (see Fig. 2). In this case one may ‘tune’ the sensor for measurement at very low partial pressures of oxygen by placing a long-lived fluorophore into a molecular environment with high oxygen solubility, *e.g.* polydimethylsiloxane. For measurement of pH a fluorescent pH indicator dye is immobilized in a hydrophilic matrix such as modified polyurethane—the dye reversibly binds with hydrogen ions and changes in fluorescence intensity roughly proportional to pH. For measurement of CO<sub>2</sub> this system is placed behind a gas-permeable membrane, through which CO<sub>2</sub> diffuses and causes a local pH drop.

A key feature of optodes distinguishing them from electrodes is that optodes do not require reference electrodes, giving them the advantage of robust operation and inexpensive manufacture necessary for reliable and economic single-use application. Also, optodes typically do not consume any analyte molecules directly measured, *e.g.* oxygen molecules. (The exception to this includes indirect optical biosensors, *e.g.* optical sensors coupled with enzymes and designed to measure concentration changes in the enzyme’s co-reactants such as oxygen or reaction products such as hydrogen peroxide). Hence with no reference electrode and reversible, non-consumptive operation, optodes are generally considered more stable in blood than electrochemical sensors, evidenced by their performance in continuous or near-continuous blood gas monitoring with very infrequent recalibration. Optodes have two disadvantages: they often require more complex and expensive instrumentation than electrochemical; and they require high R & D expenditure to synthesize and immobilize the dye systems necessary for robust manufacture and operation.

Two major configurations of optodes have been developed for medical applications. The first type has the sensor chemistry placed on the tip of an optical fiber and this format has been used for *in vivo* applications, for example intravascular blood gas monitoring systems residing several days within the human radial artery. For *in vitro* applications, including the Osmetech/Roche OPTI systems described herein, the sensor chemistry is placed on a flat, rigid and optically transparent support and is termed a planar optode.



**Fig. 2** Reduction of fluorescence due to binding of oxygen into dye molecule.

## Basic requirements and physical properties for clinical utility

Analysis of quickly-changing blood analytes is crucial to adequately assess, stabilize and manage critical care patients. In view of the considerable spontaneous variability in blood gases and other quickly-changing analytes that occurs even in apparently stable patients, it has been argued that clinical decisions should be made on the basis of trends observed with continuous, on-line monitoring. However, over the last few decades it has become apparent that, despite tremendous technological breakthroughs, truly continuous intravascular measurements are not sufficiently reliable and economic to be used routinely in critically ill patients. This has left the door open for growth of what might be considered the next-best clinical alternative, frequent discrete ‘‘point of care’’ measurements made near the bedside, which minimize the therapeutic decision time, defined as the time elapsed between a blood-measurement order and an action based on its result. In addition these systems must provide laboratory-quality results without complicating the day-to-day activities of the busy critical care unit with maintenance and reliability problems.

The so called ‘critical care’ analytes include blood gases (O<sub>2</sub>, CO<sub>2</sub>, pH), electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>), and certain metabolites (glucose, lactate, urea, creatinine). Traditionally, these analytes were determined in large benchtop analyzers with multiple on-board liquid calibration reagents, dictated by their electrodes requiring frequent recalibration. The sensor electrodes for measurement of blood gases and electrolytes were miniature electrodes typically measuring potentiometric differences relative to a reference electrode (except for amperometric measurement of P<sub>O<sub>2</sub></sub> with the ‘Clark electrode’); the metabolites were measured using so-called biosensors employing enzymes, typically immobilized onto an electrode capable of measuring a product or reactant of the enzyme reaction, such as hydrogen peroxide. The needs and demand for point of care testing near the patient’s bedside have continually driven the development for portable systems utilizing small disposable sensors capable of quick but accurate whole-blood measurements. Since the core of any analyzer is its sensors, one must turn to new, improved sensor technologies to meet these new requirements for small size, high stability, economy, reliability, and freedom from maintenance. Consequently, the development of practical and inexpensive sensors and systems for the clinical determination of these analytes in whole blood remains an important area of research.

## General sensing principles of recognition and transduction

A sensor may be defined as a device that, without the addition of a reagent, interacts predictably with a chemical or biological analyte and yields a measurable change in signal response. Many scientists and clinicians to date have considered sensors for critical blood analytes like blood gases, electrolytes, and metabolites, as *macroscopic* devices, for example the pH electrode. However with increasing advances in nanotechnology and host–guest molecular recognition, there remains little reason not to view suitably engineered or harvested individual

*molecules* as sensors. At this microscopic level most sensors contain a receptor or recognition element to chemically bind or ‘recognize’ the analyte with adequate selectivity at physiologic concentrations, and a transduction element to help convert this binding process into a measurable signal change, be it optical, electrochemical, gravimetric, or other (see Fig. 3).

At the macroscopic level most sensors of critical blood analytes are categorized according to their transduction element as electrochemical or optical, and referred to as electrodes or “optodes” (sometimes “optrodes”), respectively. In some cases they employ a recognition process rather than element, for example the Clark  $P_{O_2}$  electrode has no recognition element but measures current generated by direct reduction of oxygen.

Recognition elements or ‘receptors’ vary in their binding strength and reversibility. Most receptors, like the potassium ionophore valinomycin and the chloride ion exchanger tridodecylmethylammonium (chloride), both of which are commonly employed in ion selective electrodes (ISEs), bind their target analytes relatively weakly and reversibly. Other recognition elements such as antibodies targeting very low concentration cardiac markers, or DNA probes targeting trace nucleic acid oligomers from infectious agents, typically bind strongly and irreversibly; if employed in re-useable configurations they must be specially “coaxed” or regenerated to release their target. Molecular recognition chemists use the term ‘chemosensor’ for sensors employing abiotic, man-made recognition elements, and ‘biosensor’ for sensors employing biotic, naturally-derived recognition elements such as enzymes. Such biosensors employing enzymes usually selectively bind the analyte and catalyze its conversion to a different molecule detectable by a conventional chemosensor, for example the oxidase enzymes such as glucose oxidase consume oxygen and generate hydrogen peroxide while oxidizing their target analyte. Enzyme-based biosensors typically have better selectivity than chemosensors, however some enzyme systems such as those used to measure lactate and creatinine, have rather poor stability and rapidly lose activity while stored wet or kept in-use within the analyzer, relative to chemosensor systems.

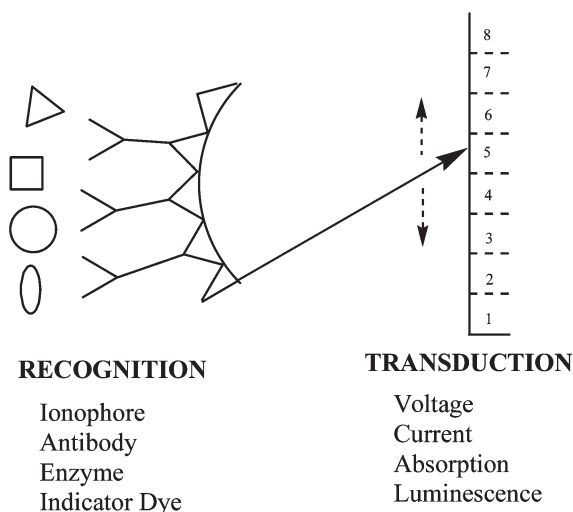


Fig. 3 Elements of a sensor, recognition and transduction.

Receptors are sometimes immobilized within a membrane matrix to extend reusability and lifetime by reducing leaching into hydrophobic components of blood.

A sensor needs more than a receptor; the target may be bound but go undetected without some type of transduction system. Transduction elements or processes provide a way to quantify the amount or rate of chemical binding that has taken place, for example a change in the measurable electrical or optical properties of the sensor material. Electrochemical sensors of critical care analytes are typically potentiometric, measuring analyte dependent potentials of the electrode *versus* a reference electrode potential, or amperometric, measuring current caused by an electrochemical reaction. Example transduction systems include potentiometric ISE’s which measure an electrical potential generated at the sensor surface by ions bound by ionophore molecules, the amperometric glucose electrode which measures current due to oxidation of hydrogen peroxide generated as the enzyme turns over glucose, the oxygen optodes which measure a reduction or ‘quenching’ of fluorescence as oxygen molecules diffuse nearby dye molecules and induce non-radiative transitions, and the colorimetric glucose test strips which generate a color change of a dye molecule acted-on by the hydrogen peroxide generated as the enzyme turns over glucose. In general the transduction system determines the sensor system’s “gain”, *i.e.* its amplification of the measurable output related to the number or rate of recognition events, as will be described below.

Sensor systems are typically characterized by their four “S characteristics”, namely sensitivity, selectivity, speed, and stability. The sensitivity or responsiveness is a function of the binding constant of the recognition element and of the transduction element’s conversion and amplification of the binding process (signal) *versus* extraneous uncorrelated processes (noise). Although the sensor must show response across the entire pathologic range of analyte concentration, responsiveness or “slope” in units of percent change in signal per change in analyte concentration, quantified at mid-physiologic concentrations, is usually a more useful figure of merit for sensitivity to non-trace critical care analytes than is “minimum detectable quantity”. A sensor’s selectivity is a measure of its sensitivity to the desired analyte relative to that of other analytes or interfering species within the matrix, often expressed as a unitless log ratio for the sensitivity pair. Selectivity is usually a function of the recognition element alone, and very often the selectivity challenges are driven by just one interfering analyte. For example a potassium ionophore must be at least 300 times less sensitive to sodium changes than to potassium changes, otherwise pathologic variations of  $\pm 30 \text{ mmol L}^{-1} \text{ Na}$  (centered near  $140 \text{ mmol L}^{-1}$ ) could cause an apparent potassium change greater than  $\pm 0.1 \text{ mmol L}^{-1}$  (centered near  $4.5 \text{ mmol L}^{-1}$ ), which begins to appear as a clinically significant change. In some cases the sensor simply has inadequate selectivity against other, measurable interferents, and is mathematically corrected for this particular interferent. For example, sensors for lithium are typically corrected for measured sodium, and sensors for ionized magnesium are typically corrected for measured ionized calcium. Speed of the raw sensor response is often quantified as the 0–95% response time to a step function

change, and the response function is usually fitted to a single or double exponential function. Speed of sensors is mostly determined by the thickness of the sensor membranes, due to diffusional processes within the sensing membrane, and is often a trade-off with the robustness of the sensor membrane. However, apparent speed is frequently improved by using endpoint detection/prediction algorithms, at the cost of small loss of precision. Finally sensor stability refers to the stability of a key calibration parameter of the sensor, such as its slope or fluorescence intensity in the absence of the measured analyte. Such stability of the sensor's response function is an important determinant of the type and frequency of calibration. Conventional benchtop analyzers employing electrodes perform 1-point calibrations frequently to correct for drift in their baseline (e.g. due to protein deposition), and 2-point calibrations less frequently to correct for degradation of responsivity or slope. Single-use disposable optical sensors such as those used in the Osmetech/Roche OPTI perform only a 1-point calibration because their slope is quite stable and batch-calibrated *via* bar codes. Single-use dry-stored disposables such as certain glucose test strips require no calibration, due to the stability of their responsivity and background signal.

There is frequent debate among scientists over the question of which sensor technology is "better": optical or electrochemical. The answer is: "it depends on the application, the configuration, and the cleverness of implementation". Optical quantitative transduction systems often require more complex and expensive instrumentation than electrochemical, due to difficulties in normalizing output of optical emitters and maintaining optical calibration in the midst of uncontrolled changes in background reflectance and fluorescence. However, electrochemical sensors are often more complex and expensive to manufacture than optical sensors due to their electrical contacts and reference electrodes, and are usually less stable due to protein deposition on the active or reference electrodes. Generally speaking, electrochemical sensors can be made faster in response than optical because they inherently rely on surface phenomena, for example self-limiting charge build-up on the electrode surface, whereas optical sensors usually rely on bulk diffusion throughout the sensor layer. Both types

have interference problems related to their transduction mechanism—optical sensors often require membranes to screen out colored materials in plasma such as bilirubin and free heme, whereas electrochemical sensors are sensitive to electro-active interferents such as ascorbate and acetaminophen. In general, optical sensing methods such as absorbance, luminescence and fluorescence have been predominant in larger clinical chemistry and immunoassay analyzers, whereas electrochemical sensors are more established in measurement of whole blood/serum critical care analytes such as blood gases, electrolytes and metabolites. New-generation bedside analyzers are emerging with miniaturized sensors of both types and it is not yet clear which will dominate. Table 1 summarizes the critical care analytes and their required measurement ranges, sensitivities, and selectivities.<sup>2</sup>

These "S characteristics" mentioned above play a large role in determining the key system characteristics of interest to the medical consumer: precision, accuracy, and cycle time. For example precision is largely determined by sensitivity and calibration stability, typically expressed as a day-to-day 1 SD or 1 RSD figure measuring a stable ampuled aqueous material over a period of 20 days, as set forth in guidelines published by the National Committee for Clinical Laboratory Standards (NCCLS).<sup>3c</sup> Typical competitive requirements are 1–3% ( $\pm 0.02$  for pH) and are shown in Table 1. Accuracy in whole blood or serum/plasma requires both precision as well as selectivity, that is, freedom from biases due to major and minor interferents within the complex matrix of blood, which vary considerably from patient to patient. To critically assess accuracy the new system must be compared side-by-side against a reference analyzer using split blood samples from hundreds of pathologic patient samples within a hospital. This is best repeated in multiple hospitals against different reference analyzers, using serum or plasma as well as whole blood, then the paired results are fitted using linear regression and the resultant slope and bias are quantitatively assessed. It is generally expected that regression slopes must fall within 10% of unity and mean biases (near mid-physiologic analyte levels) should fall below 5%. Cycle time is the minimal time between measurements, usually dictated by sensor response time (related to sensitivity and stability), as well as frequency of

**Table 1** Critical care analytes and their required measurement ranges, sensitivities, and selectivities

Analyte/units	Normal range	Precision target (1sd)	Responsivity needed	Pathologic range	Selectivity required	Correction allowed
<b>(A) Blood gases</b>						
pH	7.35–7.45	$\pm 0.005$	0.06%/mpH	6.7–7.7		Ionic strength
$P_{CO_2}$ /Torr	30–45	$\pm 0.5$	0.6%/Torr	10–200		
$P_{O_2}$ /Torr	70–100	$\pm 0.8$	0.3%/Torr	20–600		Hematocrit
<b>(B) Electrolytes</b>						
$Na^+$ /mM	135–145	$\pm 1.0$	0.3%/mM	100–180	pH, 4 : 1 by $K^+$ if measure K	
$K^+$ /mM	3.0–5.0	$\pm 0.06$	5%/mM	1–10	pH, 30 : 1 by $Na^+$ if measure $Na^+$	
$Cl^-$ /mM	95–115	$\pm 1.0$	0.3%/mM	60–160	$HCO_3^-$	
$Ca^{2+}$ /mM	1.0–1.4	$\pm 0.015$	20%/mM	0.3–2.5	60 : 1 if don't measure $Mg^{2+}$	
$Mg^{2+}$ /mM	0.40–0.65	$\pm 0.012$	25%/mM	0.2–2.0	3 : 1 by $Ca^{2+}$ if measure $Ca^{2+}$	
$Li^+$ /mM	0.5–1.5	$\pm 0.03$	10%/mM	0.2–5.0	pH, 30 : 1 by $Na^+$ if measure $Na^+$	
<b>(C) Metabolites</b>						
Glucose/mg dl <sup>-1</sup>	50–120	$\pm 3$	0.1%/mg dl <sup>-1</sup>	20–600		Hematocrit
Lactate/mM	0.4–2.2	$\pm 0.04$	8%/mM	0.2–15		pH
Urea/mM	2–8	$\pm 0.1$	3%/mM	0.5–40	pH, $K^+$ , $NH_3$	
Creatinine/ $\mu$ M	30–110	$\pm 3$	0.1%/ $\mu$ M	15–1000		Urea, creatine

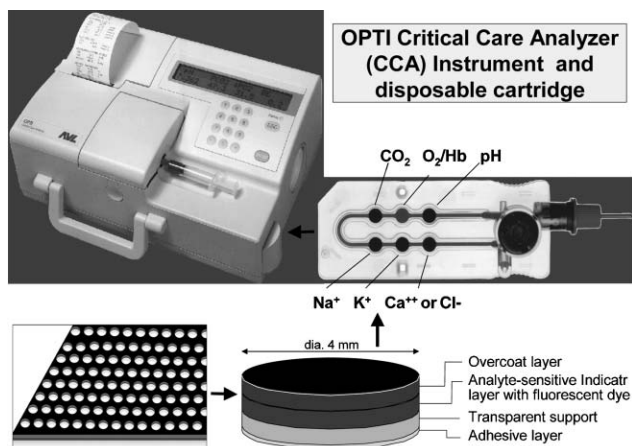
calibration dictated by sensor stability. Typical critical care systems perform a minimum of 15 whole blood measurements per hour and some are capable of 30–40 tests per hour.

## Optode technologies for diagnostic applications and their material requirements

Within the hospital environment the first panel of critical care analytes measured optically were blood gases, using the CDI “Gas-Stat” system, introduced in 1983.<sup>1b</sup> The system’s performance was sufficient for continuous ‘trend monitoring’, however when initially introduced several clinicians suggested it was insufficiently accurate and stable to replace periodic *in vitro* measurements. Its sensors employed the same principles used in all subsequent fluorescent optode systems. Nine years later, in 1994, AVL Scientific Corporation based in Graz, Austria and Roswell, GA, USA introduced the OPTI line of portable *in vitro* diagnostics tests. This system consists of a single-use or reusable measurement cassette described below in detail, together with a small, portable, micro-processor-based instrument that measures optical fluorescence or reflectance (see Fig. 4). The OPTI system measures eight analytes in whole blood, performing the measurement within 2 min using 120  $\mu\text{L}$  of whole blood. This system was acquired by Roche Diagnostics in 2000 and later by Osmetech Inc. in 2003.

### OPTI cassette

The sensors or optodes used in the OPTI instruments are incorporated into a disposable cassette. Fig. 3 (lower right) shows the cartridge (cassette) with the six optodes for measurement of pH,  $P_{\text{CO}_2}$ ,  $P_{\text{O}_2}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ , all by fluorescence. Additional cassettes are available with optodes to measure  $\text{Cl}^-$ , glucose and urea. In addition the  $P_{\text{O}_2}$  sensor optode provides measurement of total hemoglobin ( $t_{\text{Hb}}$ ) and calculated oxygen saturation ( $S_{\text{O}_2}$ ) by reflectance measurements at three wavelengths. After blood is aspirated into the cassette it is held immediately above each of the optodes allowing time for its small aqueous constituents to diffuse



**Fig. 4** Picture of OPTI CCA instrument (upper left), disposable cartridge (cassette, upper right), sensor foil (lower left) and sensor disc (lower right).

through an optical isolation layer into the sensor layer. The purpose of the isolation layer is to prevent potential interferences and ambient light from reaching the sensor.

## Measurements of hydrogen ion (pH) and $P_{\text{CO}_2}$

For pH measurement the hydrogen ions in the blood bind to a pH sensitive fluorescent indicator dye which is immobilized in the sensing layer of the optode. The indicator dye, 8-hydroxyppyrene-1,3,6-trisulfonic acid (HPTS) exists in two states, protonated and deprotonated, in relative amounts determined by the pH of its local environment (in equilibrium with the blood sample). The deprotonated form of the indicator dye fluoresces in an aqueous environment; the protonated form does not, so the lower the pH (higher  $\text{H}^+$  ion concentration), the higher the proportion of protonated form, and the lower the fluorescence. This is a reversible equilibrium-based process of binding, hence the higher the pH, the greater the fluorescence.

The  $P_{\text{CO}_2}$  optode is a modified pH optode, using the same indicator dye but with a membrane covering the sensing layer. The membrane blocks the passage of  $\text{H}^+$  and other ions into the sensor but allows  $\text{CO}_2$  through, where it forms carbonic acid, increasing the local  $\text{H}^+$  ion concentration (reducing the pH) and reducing the fluorescence. Thus increased  $P_{\text{CO}_2}$  is associated with low fluorescence and decreased  $P_{\text{CO}_2}$  with high fluorescence. This method of indirectly measuring pH is called the Severinghaus construction and is employed in all conventional blood gas analyzers in conjunction with electrochemical pH electrodes.

### Measurement of oxygen

The  $\text{O}_2$  optode is combined with a measurement system for calculated oxygen saturation or functional saturation ( $S_{\text{O}_2}$ ) and total hemoglobin ( $t_{\text{Hb}}$ ). Oxygen from the blood rapidly diffuses down into the sensor and interacts with an immobilized dye that is specific for oxygen, where it quenches or reduces the fluorescence of the dye. The relationship between the partial pressure of oxygen ( $P_{\text{O}_2}$ ) and fluorescence intensity is defined by the Stern–Volmer equation ( $I_0/I = 1 + kP_{\text{O}_2}$ ), where  $I_0$  is the maximum intensity seen at 0 Torr oxygen and  $k$  is the (inverse) Stern–Volmer quenching constant related to the oxygen diffusion rate and quenching cross-section. The relationship is an inverse one, like  $\text{CO}_2$ , leading to reduced fluorescence intensity with higher  $P_{\text{O}_2}$ .

### Measurements of total hemoglobin ( $t_{\text{Hb}}$ ) and oxygen saturation ( $S_{\text{O}_2}$ )

A key component of the measurement system is a pink or reflective overcoat that lies on the top of the  $P_{\text{O}_2}$  optode. Red and infrared light from one LED and two laser diodes is directed onto the unhemolyzed blood that lies over the  $P_{\text{O}_2}$  optode. The light is partially absorbed and reflected by the red blood cells in proportion to the hemoglobin concentration. At low hemoglobin levels the unabsorbed photons strike the pink overcoat and are reflected back up through the blood for a second time. A portion of the reflected light exits the top of the

cassette and is measured by the detector in the instrument. From a combination of readings at the two infrared wavelengths and the red wavelength it is possible to obtain optimum measurements of the two hemoglobin species, oxy- and deoxyhemoglobin. The sum of these is equivalent to the  $t_{\text{Hb}}$  and the proportion of oxyhemoglobin as a percentage of the both oxy- and deoxyhemoglobin is the  $S_{\text{O}_2}$ . Sensitivity of the measurement to erythrocyte aggregation (rouleaux formation) is minimized by maintaining high shear force just prior to measurement.

## Measurements of common cationic electrolytes

The optodes for sodium, potassium and calcium have been discussed previously.<sup>3</sup> Their measurements are similar to the electrodes described above in that they also use ion selective recognition elements (ionophores), however the ionophores are linked to fluorescent dyes instead of electrodes, and are termed fluoroionophores. Increasing ion concentrations lead to increased amounts of the ion bound to the ionophore which increases the fluorescence intensity. As mentioned earlier there is no need for a reference electrode but some ion optodes do exhibit a small pH sensitivity which may be compensated in OPTI devices by measurements of the pH. The key components to these optode are fluoroionophores shown in Fig. 5.

The design principle is based on photo-induced electron transfer (PET). Those types of fluoroionophores have proven highly successful as direct fluorescent cation sensing molecules, utilizing the switchable intramolecular self-quenching mechanism associated with PET.<sup>4</sup> The sensing layer for each contains the immobilized fluoroionophore and is attached on top of a transparent polyester foil. The sensing layer is covered with a black hydrophilic overcoat to separate the sensing layer from optical interferences within the sample. Both the sensing and overcoat layers are hydrogels allowing the free and rapid diffusion of ions throughout the sensor, and restrict the passage of optically interfering blood constituents during the measurement time. Each of the three fluoroionophores is synthesized to have a reactive linker for covalent

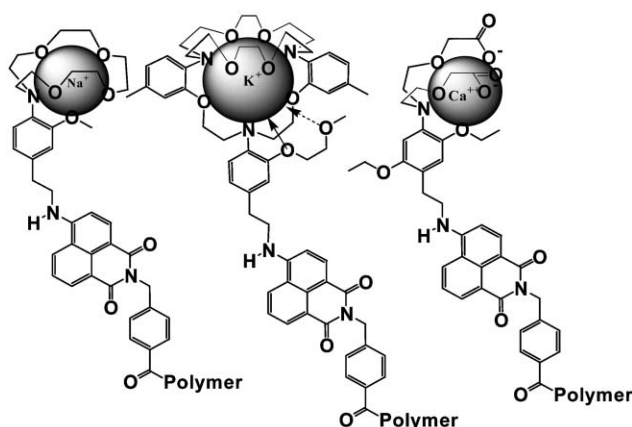
immobilization to hydrophilic polymers without loss of ionophore or fluorophore function. In contact with aqueous solution or the aqueous phase of blood, the ionophore part of the three fluoroionophores reversibly bind  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ , with respective dissociation constants of  $119 \text{ mmol L}^{-1}$ ,  $17 \text{ mmol L}^{-1}$ ,  $1.09 \text{ mmol L}^{-1}$ , respectively, in neutral aqueous solutions at  $37 \text{ }^\circ\text{C}$ ,  $\text{pH } 7.4$  and  $160 \text{ mmol L}^{-1}$  ionic strength. All three utilize 4-aminonaphthalimide as fluorophore, stable against hydrolysis and photo-degradation, and showing strong fluorescence characterized by the product of the extinction coefficient and the fluorescent quantum yield, and is excitable using blue LED's. Reversible binding of the cation to each of the ionophore moieties triggers an increase in green fluorescence from an adjacent 4-aminonaphthalimide fluorophore *via* PET. Unlike most ion selective measurement mechanisms, binding and signal transduction take place in a hydrophilic environment. All three of the new fluoroionophores satisfy the system requirements for selectivity, sensitivity and spectral accessibility.

## Measurement of chloride

Chloride optode is constructed by photo-activated immobilization of a proprietary chloride sensitive indicator based on acridinium, onto a hydrophilic polymer. Chloride from the blood diffuses down into the sensor and quenches or reduces the fluorescence of the dye. Being a collisional quenching mechanism like that governing the above-mentioned oxygen sensor, the relationship between chloride and fluorescence intensity is characterized by the Stern–Volmer equation. The relationship is an inverse one, like  $\text{CO}_2$  and  $\text{O}_2$ , leading to reduced fluorescence intensity with higher concentration of chloride.

## OPTI instrumentation

The main components of the measurement system in the OPTI analyzer are shown in Fig. 4 as well. Light emitting diodes (LEDs) supply excitation energy to the optodes and laser diodes are utilized for the reflectance-based oximetry measurements. The LED light is directed to the sensors *via* a dichroic beam splitter. The fluorescence emission from the sensor is directed back through the dichroic beam splitter and filters to isolate specific wavelengths, then to the photodiode detector which converts the light photons into a measurable electrical current. Each single-use disposable cartridge measures up to eight blood analytes following a single-point intensity calibration at its respective mid-physiologic analyte level, combined with a lot-specific factory barcode with calibration and expiration data. They are used in a portable analyzer which heats them to  $37 \text{ }^\circ\text{C}$ , performs an automatic calibration using six blue LED's for optical interrogation, and automatically aspirates and measures a  $120 \text{ } \mu\text{L}$  blood or serum sample from either syringe or capillary. The measurement takes less than 60 s after aspiration; the entire cycle takes approximately 150 s, including calibration and aspiration. The fluoroionophores have sufficient hydrolytic stability to maintain their calibration response characteristics through 8 months wet-storage at room temperature ( $4\text{--}30 \text{ }^\circ\text{C}$ ).



**Fig. 5** Structure of fluoroionophores for sodium, potassium and calcium.

## Manufacture of disposable cartridge (cassette)

Optodes are easy to manufacture because of their simple planar structure that has no electrical connectors or reference electrodes. They are prepared as compound sheets or foils comprising the sensing layer sandwiched between the upper optical isolation layer and a lower optical transmissive adhesive layer. Individual sensor or optode discs are punched out and inserted into the plastic, disposable cassette. The latter is injection moulded as two halves which, after the insertion of the optode discs, is ultrasonically welded together to form the final cassette.

A key part of the optode manufacturing process is their factory calibration. A typical calibration curve for a batch of optodes is obtained by measuring the fluorescence intensity at six different analyte levels. The responses are fitted to a polynomial curve and multiple parameters are put into a bar code which accompanies the cassette and is entered into the instrument software when the cassette is placed in the instrument. The factory calibration is checked once with precision buffers and gas mixtures immediately before use. The OPTI also uses runtime diagnostics and additional electronic QC procedures to check that the optics, fluidics, heating, and electronics of the instrument are operating optimally. All of the above features together with the fact that no reagents, other than a single gas cylinder, are required with the OPTI measurement system, represent considerable advantages over electrochemical systems. The optical sensors themselves are much less complex than their electrochemical analogs, as mentioned above, with fewer macroscopic components, no reference sensors, no surface-fouling by biologic proteins, and no leachable plasticizers or other chemicals. There are fewer elements to go wrong, leading to a robust sensor and system capable of repeated or continuous exposure to whole blood without washing or calibrations.

## Clinical performance

Clinical performance of the optode-based system was characterized at four field sites with 528 cartridges sampled from four different manufacturing lots. The clinical performance of electrolyte measurement shows superior precision and equivalent accuracy, compared to the day-to-day precision and accuracy available with modern electrode-based whole blood analyzers. Table 2 shows typical 20 day field precision (1 SD) using ampuled control materials is  $\pm 0.4$  mmol L<sup>-1</sup> for Na<sup>+</sup>,  $\pm 0.025$  mmol L<sup>-1</sup> for K<sup>+</sup>, and  $\pm 0.011$  mmol L<sup>-1</sup> for Ca<sup>2+</sup>. Typical field accuracy across the reportable ranges are: Na<sup>+</sup>  $\pm 2.1$  mmol L<sup>-1</sup> (1.4%) across 100–180 mmol L<sup>-1</sup>, K<sup>+</sup>  $\pm 0.10$  mmol L<sup>-1</sup> (2.2%) across 0.8–10 mmol L<sup>-1</sup>, and Ca<sup>2+</sup>  $\pm 0.029$  mmol L<sup>-1</sup> (2.4%) across 0.2–3 mmol L<sup>-1</sup>. The reported electrolyte values are independent of pH and common interferences. The combined incidence of problems requiring repeat measurements, due to clots, bubbles, cartridge calibration errors, user errors, outliers, *etc.*, was less than 1% during the course of over 500 measurements involving multiple lots of cassettes and multiple unskilled users.

Fig. 6 shows a typical correlation of OPTI CCA K<sup>+</sup> with a benchtop reference instrument.

**Table 2** Typical 20 day customer precision (at field site), material: ampuled aqueous controls

OPTI-check level 3 (Lot 918400, E-Ca)								
Morning run 1st replicate								
Day no.	pH	P <sub>CO<sub>2</sub></sub> / Torr	P <sub>O<sub>2</sub></sub> / Torr	Na <sup>+</sup> / mM	K <sup>+</sup> / mM	Ca <sup>2+</sup> / mM	t <sub>Hb</sub>	S <sub>O<sub>2</sub></sub>
1	7.600	22.8	143.5	161.0	6.22	0.85	9.3	100.0
2	7.598	22.8	145.1	161.8	6.20	0.85	9.3	99.8
3	7.598	22.8	141.9	162.1	6.23	0.85	9.1	100.0
4	7.600	23.0	143.9	161.6	6.26	0.85	9.0	100.0
5	7.602	23.0	139.5	161.6	6.21	0.85	9.0	99.9
6	7.599	23.0	141.6	161.2	6.21	0.85	8.9	99.9
7	7.600	22.9	138.2	161.6	6.28	0.85	9.0	100.0
8	7.598	22.9	140.6	161.6	6.24	0.87	9.0	100.0
9	7.587	23.9	141.3	161.2	6.17	0.85	9.1	100.0
10	7.594	23.1	144.9	161.7	6.21	0.85	9.2	100.0
11	7.588	24.0	143.4	160.8	6.14	0.85	9.3	99.7
12	7.597	23.0	142.3	161.9	6.21	0.87	9.0	100.0
13	7.596	23.1	146.4	161.7	6.20	0.85	9.1	99.9
14	7.598	23.3	141.8	161.8	6.21	0.86	9.1	100.0
15	7.596	23.4	143.2	161.9	6.20	0.85	9.0	99.9
16	7.599	23.2	141.9	162.0	6.22	0.87	9.1	99.8
17	7.590	23.9	137.9	161.7	6.19	0.86	9.2	100.0
18	7.595	23.4	142.2	161.6	6.18	0.85	9.3	100.0
19	7.594	22.8	141.1	162.7	6.21	0.87	9.0	99.8
20	7.594	23.4	143.2	162.0	6.18	0.85	9.3	99.8
Mean	7.596	23.19	142.20	161.68	6.21	0.86	9.12	99.93
Min:	7.587	22.8	137.9	160.8	6.14	0.85	8.9	99.7
Max:	7.602	24.0	146.4	162.7	6.28	0.87	9.3	100.0
1 sd:	0.004	0.38	2.16	0.42	0.03	0.008	0.13	0.10
CV%		1.6%	1.5%	0.3%	0.5%	1.0%		
OPTI-check level 1 (Lot 918400, E-Ca)								
Morning Run 2nd Replicate								
pH	P <sub>CO<sub>2</sub></sub> / Torr	P <sub>O<sub>2</sub></sub> / Torr	Na <sup>+</sup> / mM	K <sup>+</sup> / mM	Ca <sup>2+</sup> / mM	t <sub>Hb</sub>	S <sub>O<sub>2</sub></sub>	
7.166	73.1	65.3	125.5	2.36	1.61	16.8	88.1	
7.168	72.6	68.7	125.5	2.37	1.59	16.8	87.9	
7.170	72.6	63.2	124.9	2.33	1.61	16.7	88.1	
7.169	73.5	68.6	125.4	2.37	1.62	16.7	88.2	
7.169	72.7	66.8	125.6	2.40	1.62	16.7	88.0	
7.168	72.0	66.2	125.2	2.36	1.61	16.6	88.0	
7.170	71.4	64.3	125.5	2.41	1.65	16.7	88.0	
7.171	71.5	67.3	125.7	2.36	1.60	16.8	87.9	
7.168	72.2	66.7	125.4	2.45	1.62	16.7	87.9	
7.170	71.7	68.5	125.5	2.40	1.61	16.7	88.0	
7.165	72.6	68.4	125.1	2.36	1.62	16.7	88.0	
7.171	73.2	64.1	125.1	2.37	1.61	16.7	88.2	
7.172	72.3	65.7	125.7	2.39	1.62	16.8	88.0	
7.174	72.3	67.9	125.4	2.41	1.61	16.8	88.0	
7.173	72.1	65.8	125.4	2.39	1.62	16.8	88.1	
7.175	71.4	66.4	125.1	2.38	1.61	16.8	88.1	
7.173	72.0	66.0	125.3	2.41	1.61	16.7	88.1	
7.172	72.4	62.1	125.7	2.43	1.60	16.7	88.2	
7.173	73.3	62.6	125.2	2.40	1.62	16.8	88.2	
7.172	71.9	64.8	125.2	2.41	1.63	16.8	88.2	
7.170	72.34	65.97	125.37	2.39	1.61	16.7	88.1	
7.165	71.4	62.1	124.9	2.33	1.59	16.6	87.9	
7.175	73.5	68.7	125.7	2.45	1.65	16.8	88.2	
0.003	0.62	2.00	0.23	0.03	0.012	0.06	0.10	
	0.9%	3.0%	0.2%	1.2%	0.8%			

## Summary

The sensor is the basis of the medical diagnostic analyzer, and new sensor technologies are being developed to meet the emerging needs of critical care. No one sensor technology meets all needs. Although microelectrodes and thick film

### Whole Blood Correlation at Field Site

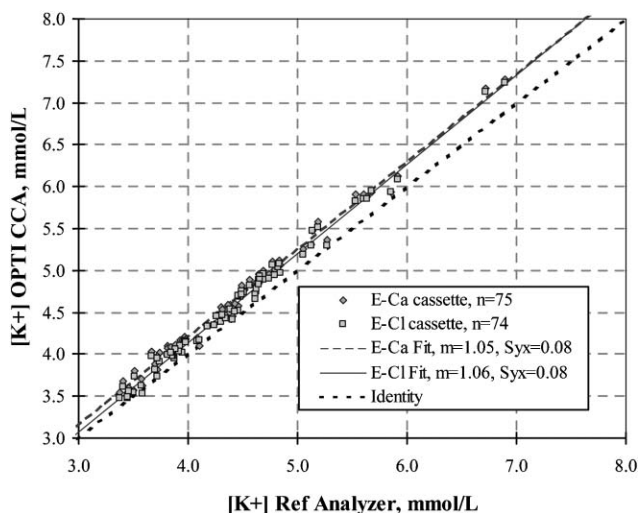


Fig. 6 Correlation of OPTI CCA with other instrument.

electrode arrays are firmly established in large benchtop analyzers incorporating frequent calibration, optical sensors provide an attractive, stable, and economic alternative for single-use disposables in portable applications near the bedside, or patient-dedicated monitoring applications. In its infancy, the optical sensors' inherent advantages of stability, simplicity, and economy have not yet been fully exploited in medical diagnostic systems. Thanks to recent advances in new host-guest interactions, optical materials, and practical telecommunications componentry, medical systems utilizing optical sensors are expected to advance at a rapid rate.

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