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Ion-selective field-effect transistors (ISFETS)

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Most microelectronic chemical sensors are based on the insulated-gate field-effect transistor (IGFET), where the insulator–semiconductor is silicon dioxide–silicon, overlain by the ion-blocker, silicon nitride, and an additional layer conferring alternative ion-sensitivity and selectivity. Materials used to confer ion-sensitivity include, besides silicon nitride (H^+), aluminium and tantalum oxides (H^+), special glasses (H^+, Na^+, K^+), valinomycin (K^+), tetraalkylammonium salts (Cl^-, NO_3^-), and various synthetic ionophores (Ca^{2+}, Na^+). The metal gate connection of the FET structure is replaced by a reference electrode in the solution containing the ion to be determined (analyte). The problems of design, construction and use of ion-selective FETS (ISFETS) as biosensors are surveyed and illustrated by work in Newcastle on *ex vivo* monitoring of blood during surgery for potassium and calcium ions and pH.

1. Introduction

The first report of an ion-selective field-effect transistor (ISFET) by Bergveld (1970) stimulated much research, over the past 15 years, on semiconductor-based sensors. These devices would seem to be particularly well-suited for biomedical applications, with advantages over the use of ion-selective electrodes (ISEs), in spite of very real practical difficulties (discussed below).

Although several types of chemical-sensitive semiconductor devices have been described, most are based on the field effect transistor (FET) or insulated gate field effect transistor (IGFET). The ISFET structure is very similar to that of the IGFET; a typical n-channel IGFET construction is shown in figure 1. It consists of a p-type silicon substrate with source and drain diffusions separated by a channel which is overlain by silica as insulator and a metal gate. The polarity and magnitude of the gate voltage (V_G) to be applied between the substrate and the gate is selected so that an n-type inversion layer forms in the channel between the source and drain regions. The magnitude of the drain current (I_D) is determined by the effective electrical resistance of this surface inversion layer and the source-drain voltage difference (V_D). It is an advantage to operate at low V_D in the unsaturated mode.

The ISFET (figure 2) differs from the IGFET in the following respects:

(i) The electrolyte solution is brought in direct contact with the gate insulator layer(s) and a reference electrode in the solution replaces the metal gate. Bergveld (1970, 1972) thought that a reference electrode was unnecessary, but it is now agreed that this is not so (Kelly 1977).

(ii) A layer of silicon nitride (Matsuo & Wise 1974) overlying the silica provides a charge-blocking interface, conferring improved pH sensitivity over that of the silica film.

(iii) Other films, such as glasses or valinomycin-polymer membranes, can be used to confer

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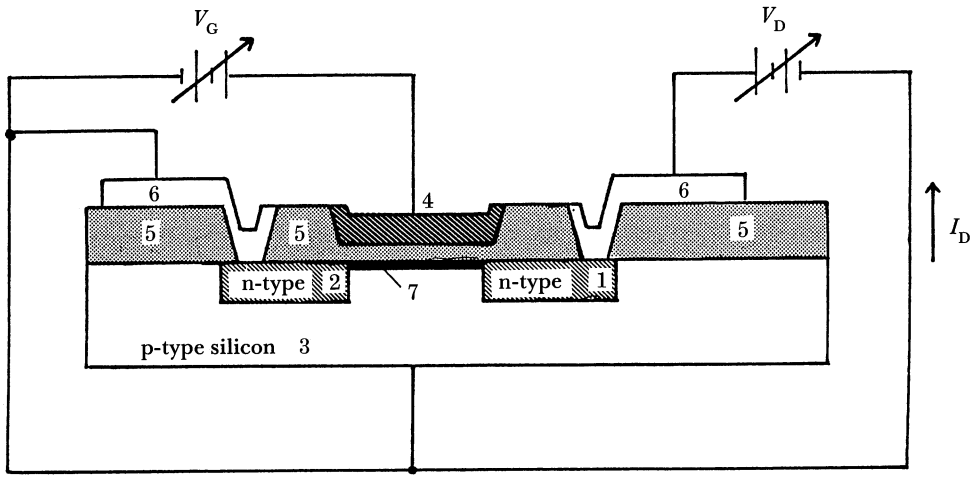

FIGURE 1. Schematic diagram of an IGFET. Numbers indicate: (1) drain, (2) source, (3) substrate, (4) gate, (5) insulator, (6) metal contacts, (7) conducting channel. The electric field at the semiconductor surface, due to the gate–substrate bias voltage (V_G) induces an inversion layer of electrons, forming a conducting channel between the source and drain.

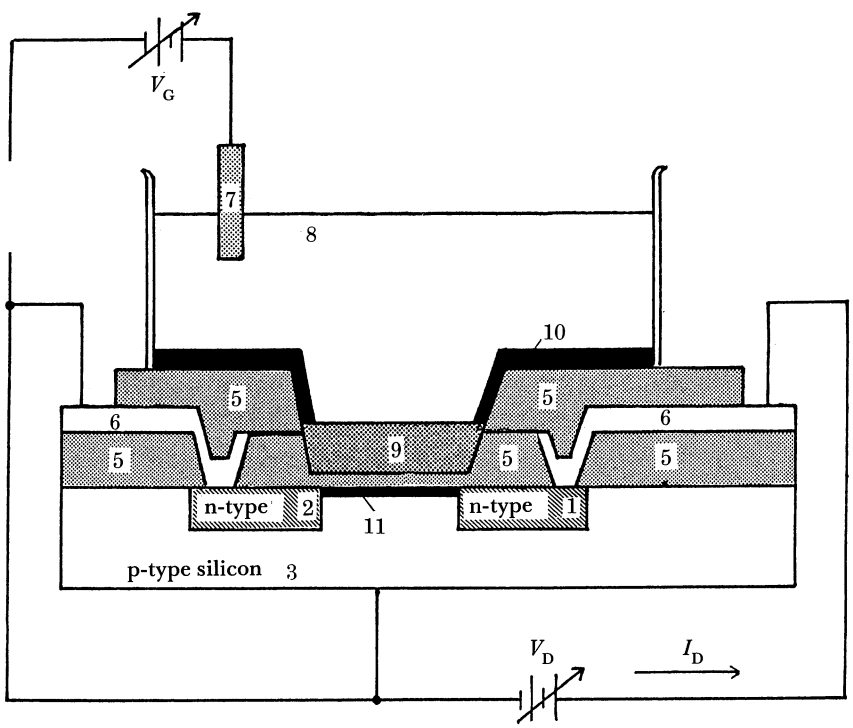

FIGURE 2. Schematic diagram of an ISFET. Numbers indicate: (1) drain, (2) source, (3) substrate, (5) insulator, (6) metal lead, (7) reference electrode, (8) solution, (9) electroactive membrane, (10) encapsulant, (11) conducting channel. The metal gate of the IGFET is replaced by the reference-electrode–solution–electroactive-membrane system. For constant V_D and reference-electrode–solution potential, the drain current (I_D) depends on the activity of the sensed ion in the solution.

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other ion-selectivities to the ISFET (Moss *et al.* 1975) by virtue of the ion exchange between solution and electroactive gate affecting the magnitude of the drain current. Successful encapsulation of all regions of the device other than the gate region(s) to be exposed to the electrolyte solution is mandatory (Sibbald *et al.* 1984). Highlights in ISFET development are given in table 1.

TABLE 1. HIGHLIGHTS IN ISFET RESEARCH, 1970–1986

year	development	author(s)
1970	First description of ion-sensitive field-effect transistor, responsive to Na^+ and H^+.	Bergveld
1972		
1974	Anisotropically etched 'probe'-type devices with Si_3N_4 as combined ion-barrier and H^+-responsive gate material.	Matsuo & Wise
1975	K^+-responsive ISFET with valinomycin-doped PVC as the electroactive gate material.	Moss, Janata & Johnson
1978	First dual-function (Na^+, H^+) device.	Esashi & Matsuo
1980	Penicillin-responsive enzyme FET.	Caras & Janata
1980	Adaptation of pH-responsive ISFET for p_{CO_2} measurement; *in vivo* application to monkeys.	Shimada *et al.*
1981	*In vivo* measurement of Ca^{2+} in dogs.	McKinley *et al.*
1981	*In vivo* measurement of K^+ in dogs.	McKinley *et al.*
1982	Online measurement of K^+ in man.	Sibbald, Covington & Carter
1983	First three-function (K^+, H^+, Ca^{2+}) ISFET.	Covington & Sibbald
1985	First four-function (K^+, H^+, Na^+, Ca^{2+}) ISFET applied to monitoring of blood during surgery.	Sibbald, Covington & Carter
1985	Chemically sensitive integrated circuit (Optran).	Sibbald
1986	Dual-function enzyme FET (glucose, urea).	Hanazato *et al.*
1986	First miniature liquid-junction reference electrode integrated on water with CMOS ISFET.	Smith & Scott

In 1981, the unsolved problems of ISFETs were listed (Janata & Huber 1981) as:

(i) automatic encapsulation and automatic membrane deposition;

(ii) vulnerability to static electricity

(iii) drift due to unknown origins.

To this list can be added:

(iv) short lifetimes, if the electroactive material leaches out from the thin coating covering the gate region;

(v) thermal sensitivity;

(vi) nonlinear response;

(vii) non-buffered device output, which is unsuitable for driving multiplexing circuitry.

Such problems have so far precluded the commercial availability of ISFETs, although some developmental designs have been available for research purposes from the Utah group and others.

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2. ISFET INTEGRATED CIRCUITS AT NEWCASTLE

A number of different ISFET designs have been developed in Newcastle, primarily for application to the online determination of the clinically important cations in whole blood. The use of electron-beam lithography has permitted the fabrication of several designs with dimensions in the 1–2.5 mm range on the same 75 mm diameter, p-type, 14–20 ohm cm (100) silicon wafers by using an n-channel, metal-gate process. Both enhancement-mode device structures and depletion-mode structures (made by using an arsenic implant and hence creating n^- channels, thus obviating the need for a polarizing, serial, gate-voltage source) are available. The gate insulator is a composite dielectric, 50 nm thick, of thermally grown silica overlain by 90 nm silicon nitride, prepared from dichlorosilane and ammonia by subatmospheric chemical vapour deposition (CVD) at 800 °C. Three basic types of device were used:

(i) rectangular designs, 1.25×2.00 mm, (Eμ 145, 146) similar in form to the well-known Utah chip (Moss *et al.* 1975) with either two or three ISFETS located separately from one or two IGFETS and the bonding pads. These designs were originally intended for catheter-tip applications (Janata & Huber 1981).

(ii) A square (2.52×2.52 mm) chip (Eμ 144), designed specifically for flow-cell application, comprising four centrally located, dual-dielectric ISFETS and four IGFETS, all transistors having 206×12 μm channels.

(iii) A rectangular chip (2.03×2.43 mm) chemical-sensing integrated circuit (Eμ 358A) which utilizes an ISFET as an integral element in an analogue electronic circuit to provide an operational transducer or Optran (Sibbald 1985).

3. ENCAPSULATION METHODOLOGY

ISFETS have been described which have been encapsulated with a variety of materials. The most extensively used is epoxy, although it is vital to choose a type which is not only suitable with regard to chemical inertness and low water absorption but also has the correct degree of thixotropicity for selective encapsulation.

Epoxy is adequate for small-scale laboratory work, but is not practicable for extensive usage, which needs a suitable mass-encapsulation technique. For this reason, a novel encapsulation procedure was developed, which makes use of materials common in integrated circuit fabrication processes. This procedure uses a thermally cured polyimide and negative photoresist and permits the selective exposure of the chemosensitive gate regions while ensuring adequate protection and electrical isolation of the remainder of the chip, the associated electrical connections and the printed-circuit board on which the chip is mounted. The composite encapsulant has an estimated overall thickness of 30–50 μm.

16-pin DIL (dual in-line) ceramic headers are used for mounting the devices before encapsulation. The cleanness of the chip and the header are of paramount importance for achieving successful encapsulation. A successive ultrasonic cleaning routine was adopted, consisting of washes in detergent, distilled water (twice) and then propan-2-ol. After cleaning, the IC connections are wire-bonded to the substrate. The IC is selectively encapsulated (see below) so that only the gate regions are exposed, and the header is soldered to a 16-pin DIL socket adaptor.

For encapsulation, the polyimide precursor is applied dropwise to the chip and surrounding header, and then partly cured. (In later designs, polyimide is also applied at the wafer

fabrication stage.) After cooling, the headers are dip-coated in negative photoresist and soft-baked. Optical masking of the chemosensitive gate regions is achieved by using carbon black drawing ink applied with a 0.15 mm drafting pen, and the photoresist is polymerized by UV light. The unexposed regions of the negative photoresist are developed with the appropriate developing solution; a mixture of hydrazine hydrate and diaminoethane is then used to etch the underlying polyimide (Sibbald *et al.* 1984). The small inevitable amount of undercutting of the polyimide by the etchant is advantageous because it acts as a 'key' for the polymer-based electroactive gate membranes, which would otherwise have relatively poor adherence to the silicon nitride surface of the chip. The devices are finally baked at 180 °C for 3 h to cure fully the composite encapsulant.

4. Electroactive gate materials

Ionophore-doped polymeric gates were used for the fabrication of the K^+, Ca^{2+} and Na^+ devices, whereas the silicon nitride surface of the composite gate dielectric of the remaining device was used directly as a pH-responsive material (Sibbald 1982; Moss *et al.* 1976; Vlasov 1981); the details are given below.

(*a*) *pH-responsive*

90 nm silicon nitride was etch-cleaned in buffered 5% hydrofluoric acid for 60 s immediately before bonding and encapsulation. In more recent work, pH glasses have been sputtered on top of the silicon nitride. Other workers have used metal oxides such as TaO_2 (Akiyama *et al.* 1982).

(*b*) *Potassium-ion-responsive*

This preparation contained 5 mg valinomycin, 165 mg dioctyl adipate, 330 mg PVC and 3 ml tetrahydrofuran (redistilled to remove stabilisers).

(*c*) *Calcium-ion-responsive*

There is a choice of electroactive material between calcium alkylphosphoric acid salts such as di (octylphenyl) phosphoric acid (t-HDOPP) (Ruzicka *et al.* 1973), and the neutral carrier material (ETH 1001) synthesised by Anker *et al.* (1981). The former material suffers from a higher Na^+ interference (Lanter *et al.* 1982). Commercially available electrode membranes (Pye–Unicam type IS561-Ca^{2+}) were redissolved in approximately 0.5 ml redistilled tetrahydrofuran, or ETH 1001 from Fluka was used in conjunction with plasticizer.

(*d*) *Sodium-ion-responsive*

Sodium-ion-responsive ISEs are usually based on aluminosilicate glasses, but neutral carriers such as monensin and synthetic ligands (Simon *et al.* 1975) have been used. Commercially available electrode membranes (Pye–Unicam type IS561-Na^+) were redissolved in approximately 0.5 ml redistilled tetrahydrofuran.

(*e*) *Chloride-ion-responsive*

Chloride-responsive ISEs are usually based on lipophilic quaternary ammonium chlorides dissolved in a suitable inert solvent (Baumgartner 1981). We have used tri(octylpropyl)-ammonium chloride in n-decanol but these ISFETs have not been used in clinical applications.

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(*f*) *Nitrate-ion-responsive*

Tetradodecyl ammonium nitrate (Nielsen & Hansen 1976) was prepared from the bromide (Fluka) by ion exchange with KNO_3 and recrystallized from ethanol + water. Plasticizer was dibutylphthalate (Aldrich).

(*g*) *Magnesium-ion-responsive*

Although Simon and co-workers (Lanter *et al.* 1980) have reported a synthetic ionophore (ETH 1117), selectivity over calcium is not high. Magnesium is of undoubted clinical interest. The ligand is available from Fluka but our attempts to use it on ISFETS were unsuccessful.

(*h*) *Bicarbonate- or carbonate-ion-responsive*

Bicarbonate- or carbonate-responsive systems have been reported (Herman & Rechnitz 1975; Greenberg & Meyerhoff 1982; Funck *et al.* 1982); although both ISES and ISFETS have been constructed by us, they have not yet been applied to clinical investigations.

Polymeric gates were formed by two-stage solvent-casting with an interval of 4 h between applications, and were estimated to be approximately 50 μm thick. Devices were stored at ambient temperature and humidity for 24 h before the flow-through cell cap was sealed in place, and were then re-stored for a minimum of 48 h before use.

5. Practical considerations

(i) *Devices: mode of deployment*

The small size of ISFET devices, together with other characteristics (solid state, low impedance, fast response) have led most principal researchers to believe that catheter-tip, *in vivo* usage would be the most appropriate mode of deployment. ISFETS are, we believe, most effectively used *ex vivo*, on line, in a flow-through cell connected directly to the patient by an indwelling cannula with minimal dead space. Devices are thus incorporated into a miniature cell through which blood samples can be drawn intermittently, analysed and discarded. This technique provides fresh, reliable physiological data minute by minute without the hazards and difficulties associated with *in vivo* monitoring. The principal advantages are as follows:

(*a*) Ethical problems and objections associated with use *in vivo* are virtually eliminated.

(*b*) Technical problems associated with use *in vivo* are eliminated (for example, the fact that intravascular sensors can be rendered ineffective by being swept to the blood-vessel wall and becoming effectively screened from the main stream.

(*c*) Online calibration is possible.

(*d*) There is no need for a reference electrode on the chip.

(*e*) Sterilization requirements are less stringent.

(*f*) Blood may be diluted and heparinized if necessary to prevent blood clotting and system failure.

(ii) *Devices: mode of operation*

The I_D–V_G characteristics of an ISFET device are essentially that of the FET substructure on which it is based and depend on device design, in particular the channel geometry and structure, chosen materials and processing conditions. If the applied gate bias potential is fixed, then

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changes at the solution–electroactive material interface are reflected in changes in I_D. However, if the drain current is maintained at a constant value by means of an operational amplifier which directly controls the applied gate bias potential by using a negative feedback loop (figure 3), then the output is a voltage which varies with change in activity of the sensed ion in accordance with the Nernst equation. The output is therefore effectively the same as that from an ISE, although inverted in polarity.

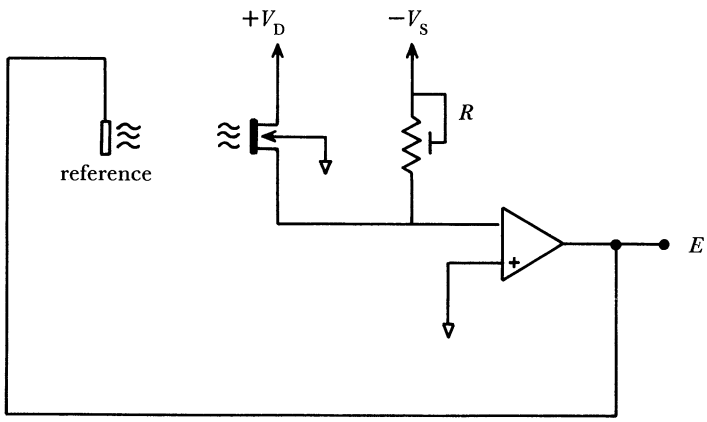

FIGURE 3. Constant drain-current operating mode with an operational amplifier in a feedback loop. The (constant) drain current is set by varying R or V_S since $I_D = V_S/R$.

The constant drain-current method has disadvantages if the circuit is broken when the analyte solution is changed. This can cause the feedback potential to assume large positive values, and lead, when it is re-established, to voltage transients which polarise the reference electrode and/or the electroactive gate, causing spurious offset potentials and even breakdown of encapsulation.

Alternatively, an equivalent gate voltage circuit may be used (Sibbald *et al.* 1984) in which the drain current is inverted, and undergoes I–V conversion and the resultant voltage is used to drive a further circuit incorporating a matching IGFET, so that the IGFET drain current mirrors that of the ISFET. This requires several operational amplifiers, voltage reference sources and a means of gain adjustment to compensate for slight differences between ISFET and IGFET.

A recent development is the operational transducer (Sibbald 1985) which incorporates a matched ISFET–IGFET pair, connected in source-coupled, dual-differential configuration, with the output voltages coupled to a differential–single-ended converter and then to several DC amplification stages and fed back to the IGFET gate, such that the ISFET is the non-inverting input device and the IGFET the inverting input device. The operational transducer successfully overcomes the inherent thermal sensitivity of ISFETS, provides a linearized output signal and does not require two stabilized power supplies.

(iii) *Devices: mode of evaluation*

A constant-dilution technique (Horvai *et al.* 1976; Covington & Whalley 1986) may be used to obtain calibration curves and estimate selectivity coefficients for the interferences on the primary ion-responses of devices. The design and configuration of the constant-dilution

apparatus is given schematically in figure 4. The electrical output from the device and reference electrode was monitored continuously with a digital multimeter and a chart recorder. Additionally, data acquisition and estimation of activities (by using a Debye–Huckel expression for activity coefficients) was by microcomputer, with output voltages recorded every 30 s during the dilution of the primary ion from 10^{-1} to 10^{-6} M. A constant flow rate between 4.5 and

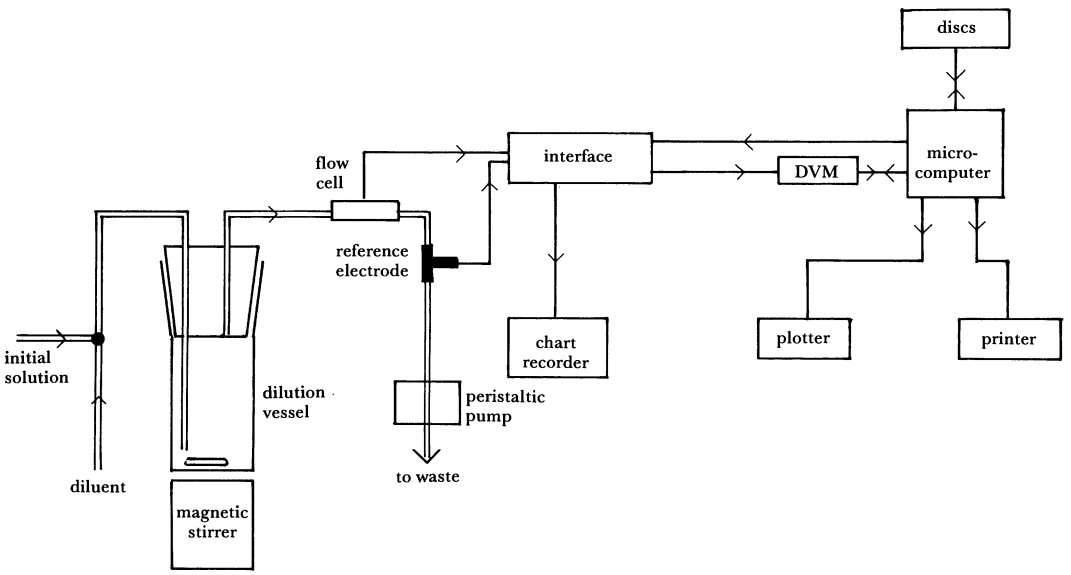

FIGURE 4. Computer-controlled constant-dilution apparatus.

5.5 cm^3 min^{-1} resulted in a complete calibration in approximately 50 min. The relation between flow rate (F), volume of the dilution vessel (V), initial concentration of the primary ion (c_0), and concentration (c_t) after a given time (t) is given by:

$$\lg c_t = \lg c_0 - (tF/V) \lg e.$$

For determination of the selectivity coefficient, the concentration of the interfering ion is kept constant throughout the dilution at, say, 10^{-1} M by including the interfering ion in both the initial solution and the diluent at 10^{-1} M (corresponding to the mixed-solution method for determining selectivity coefficients (Covington 1979, pp. 15–18)).

(iv) *Precision of measurement*

High-precision voltage measurement is necessary for clinical applications. For example, the change in electrical signal which corresponds to an increase in potassium concentration from 4.0 to 4.1 mM is only 0.63 mV. Ideally, 0.1 mV resolution is necessary, which requires that any offset signal drift (either of electrical or chemical origin) must be better than 0.5% h^{-1}, or less than 0.13 mV h^{-1}. This is difficult to achieve with ISEs, and although it is possible to achieve this level of stability with ISFETs, it is not usually observed during the first 12 h of usage while the electroactive gate is hydrating, nor apparently when anticoagulants are present (Sibbald *et al.* 1982).

(v) *Analyte-chopping analysis technique*

To ensure high reliability of measurements we have employed an analyte-chopping analysis (ACA) technique in which solution is continuously drawn through the analyser cell, alternating between a known 'baseline' standard solution and analyte (blood). The ISFET gate voltages are recorded at the base and peak of the resulting signal, the difference is computed and the sample concentration calculated. If the baseline:sample mark–space ratio is 105 s : 15 s, then, by using the previous criterion (0.5% error), even a gross drift of up to 31.2 mV h^{-1} can be tolerated if necessary. This chopping technique requires:

(*a*) a miniature solenoid valve capable of handling blood;
(*b*) rapid-response sensors;
(*c*) efficient flow-through cell design;
(*d*) computer control.

(vi) *Flow-through cell design*

The Utah group's ICS (Janata & Huber 1981) were mounted on to the tips of glass tubes and were then hand-encapsulated; no true flow-through ISFET cells have been reported in the literature other than our early design (Sibbald *et al.* 1982) but some practical difficulties were encountered, in later work, which necessitated the re-evaluation of the cell design.

A significant improvement was made by the use of a V-type cell cap. This was made by bending a Pyrex tube (1.5 mm bore, 4 mm outside diameter) through 90°, sectioning the tube at the elbow to expose the bore, and cutting both arms to approximately 10 mm in length. A stainless steel tube (1.5 mm diameter × 8 mm long) was glued into each arm with epoxy to provide push-fit tubing connections. Later versions have been made from Perspex (figure 5).

The V-shape directs a flow of analyte directly on to the ISFET surface, ensuring adequate flushing of the electroactive gate with fresh solution, and minimal bubble-trapping. The glass or Perspex is relatively blood-compatible and clean in use, and the chip surface is visible for inspection (although the cell is used with a clip-on blackened cover because of the slight photosensitivity of the devices). The V-cell's dead space is approximately 30 µl, and the cell's

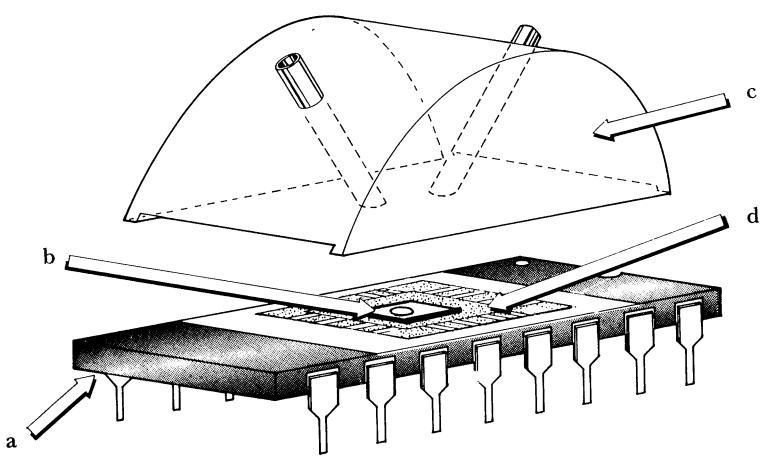

FIGURE 5. Exploded diagram of miniature ISFET flow through analysis cell. Letters indicate: a, 16-pin DIL header; b, ISFET; c, V-type plastic flowthrough cap; d, polyimide–photopolymer encapsulation.

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response time at a flow rate of 24.3 µl s^{-1} (1.46 ml min^{-1}) is between 15 and 20 s (plus 5 s delay) when used with a heat exchanger–F-piece–valve input arrangement (see below); the latter contributes a delay of several seconds to the response time because of analyte–carrier boundary mixing during transport from the valve to the sensor cell. A downstream, side-entry porous-plug reference electrode is used, connected directly the ISFET cell outlet port. An 'exploded' diagram of the complete ISFET V-cell is shown in figure 5.

(vii) *Remote sensor unit*

It is essential that the ISFET analysis cell (with its associated components) is deployed as closely as possible to the indwelling cannula to minimize the dead space of the sampling line, thereby ensuring minimum blood loss of the subject and a minimum time lag before the presentation of results. This hardware package (figure 6) comprises some items that merit further description. Commercially available miniature solenoid valves become rapidly contaminated by protein when exposed to blood. Accordingly, a small two-way actuator was designed to fit a miniature solenoid, which was arranged to compress either one of two 1.0 mm bore, 2.0 mm OD silicone rubber tubes, which were arranged to be a press-fit into the surface of the actuator so as to be easily changed after use. The two output fluid lines were joined by using glass F-piece with 1 mm bore to provide a single outlet stream.

ISFET devices, in common with all semiconductor sensors, exhibit some thermal sensitivity. It is possible to define an 'athermal' point (or, more strictly, a locus) on the I_D–V_G characteristic where the thermal coefficient of conductance is approximately zero. By operating at or near the athermal level a useful reduction in thermal sensitivity is achieved, but this alone is not sufficient to permit precision measurement (0.1 mV resolution), when the device is subjected to considerable fluctuations in analyte temperature, i.e. chopping between the baseline solution at ambient temperature (say 22 °C) and blood at body temperature (37 °C). This problem was overcome by taking advantage of the presence of the baseline solution at ambient temperature near the ISFET cell and using it to cool down the incoming blood with a small glass

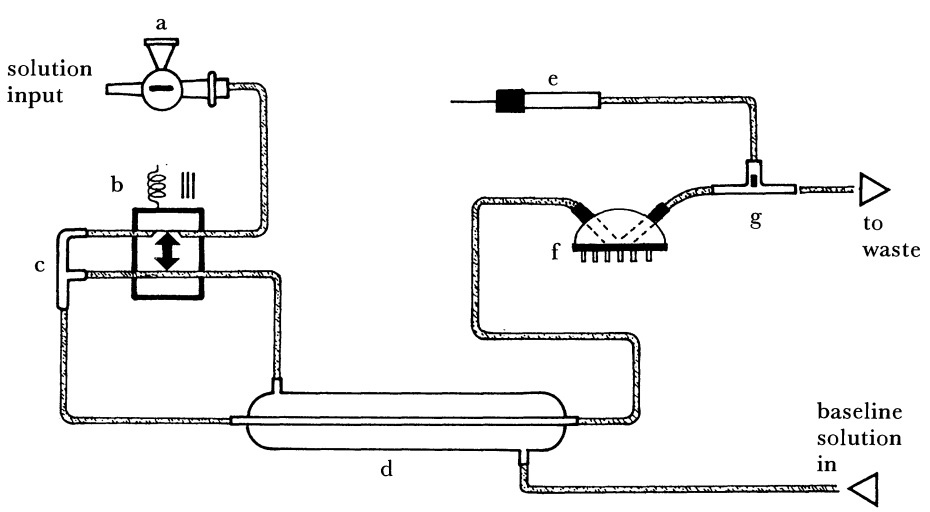

FIGURE 6. Schematic diagram of remote sensor unit. Letters indicate: a, three-way Luer input tap; b, pinch-tube solenoid valve, c, glass -piece; d, glass heat exchanger; e, reference electrode; f, flow-through cell; g, porous-plug reference junction.

heat exchanger. The blood passes down the narrow central tube, which is surrounded by a relatively large volume of baseline solution with which it equilibrates thermally; this also permits compensation for thermally induced pH changes in the sample.

The hardware is assembled on a 98 × 48 mm printed-circuit board, thus forming a small, robust remote sensor unit, with easy access to all fluid lines and incorporating a light-emitting diode (LED) indicator to show when the valve is being actuated. This unit is mounted on to the end of a spring-cantilevered arm, which is then attached to the instrumentation trolley such that the remote sensor unit can be swivelled and rotated freely, and can be positioned rapidly and easily wherever required by the clinician, thus allowing flexible access for use in a wide variety of applications, e.g. at operating-table height, with cardiopulmonary bypass equipment (where the sampling site is close to the ground) or at the bedside.

6. Clinical applications

The online analysis system has been tested extensively in the laboratory (Sibbald *et al.* 1983, 1984), before being used clinically, to ensure that numerous operating factors were adequate and optimal. Clinical situations were chosen where it was expected that marked fluctuations in blood electrolyte composition would occur: (i) in cardiac bypass surgery; (ii) in general surgery involving substantial blood transfusion; and (iii) in renal dialysis.

Example 1: *cardiopulmonary bypass surgery*

Figure 7 shows the data recorded during online blood analysis of a subject undergoing vein grafting for coronary-artery bypass surgery. After pre-usage calibration, the remote sensor unit was connected intraoperatively directly to the arterial line of the cardiopulmonary bypass machine; sampling was initiated on the priming solution (event 0) immediately before the initiation of bypass (event 1) and switched back to the precalibration solution (event 5) after

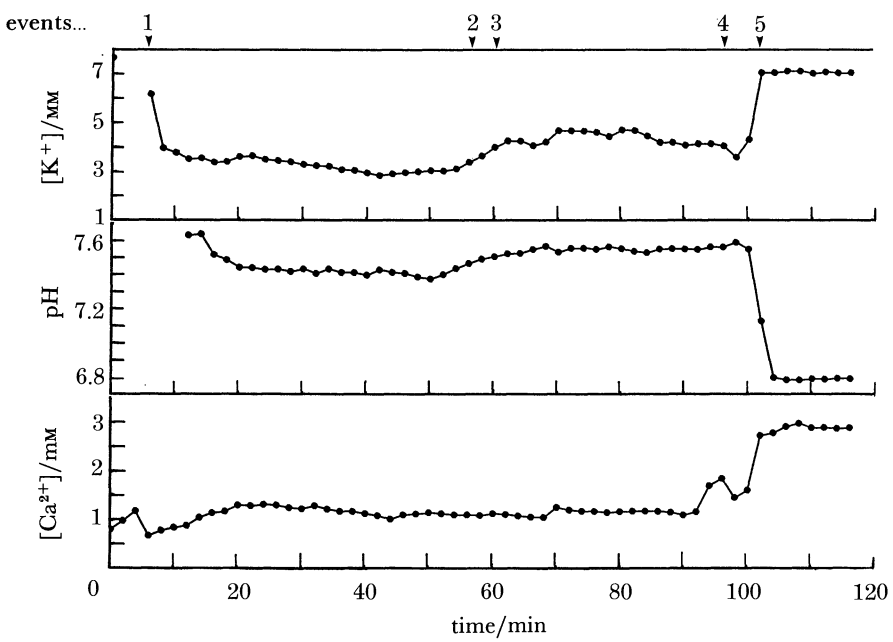

Figure 7. Online analysis, during cardiopulmonary bypass surgery, for K^+, pH and Ca^{2+}. (See text for details.)

termination of the bypass procedure (event 4) to check the long-term stability of the analysis system. As the subject was already (necessarily) heparinized (approximately 4.6 units ml^{-1} sodium heparin, initially) active heparinization was unnecessary.

For the first 55 min, when the vein graft was being inserted, the potassium concentration was stable, but on completion of the grafting (at event 2) the aortic clamp was removed. This allowed potassium-rich cardioplegic solution to mix with the blood in the circulation, causing a substantial elevation in potassium concentration from 3.0 mM (at 52 min) to 4.7 mM (at 70 min). Similarly, the pH level was stable at approximately 7.43 for the initial 55 min, and then rose to pH 7.55 over the following 12 min; this level was maintained until cardiopulmonary bypass was terminated. The ionized calcium concentration was relatively stable throughout the operation. Figure 8 shows results of K^+ and Ca^{2+} monitoring during a second operation, in which a potassium chloride bolus was given. K^+ rose from 3.3 to 4.2 mM over a 6 min period. Removal of the aortic clamps caused a transient, which decayed after 16 min.

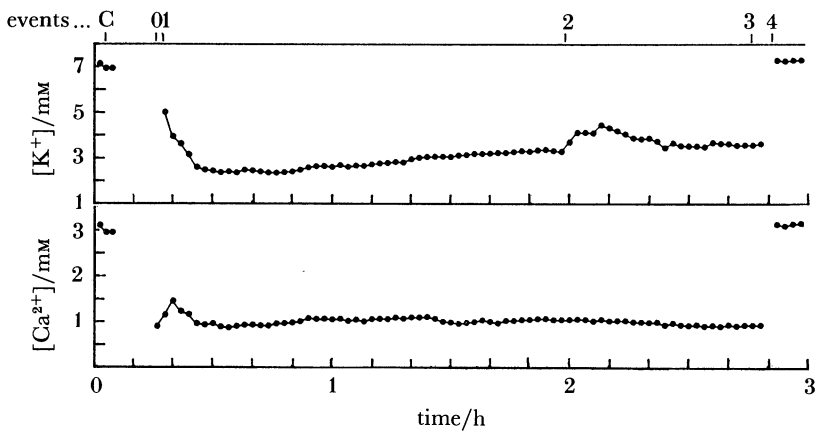

FIGURE 8. Online analysis during cardiac surgery during which a KCl bolus was given. (See text for details.)

We have previously reported (Covington & Sibbald 1983) considerable difficulties in the analysis of Na^+ in whole blood, related primarily to the quality of the electroactive materials (ionophore–solvent–polymer combination) and to the small signal (*ca.* 2.4 mV) associated with the normal physiological range of this species: these difficulties have not yet been satisfactorily resolved and no results are given in figure 7.

Example 2: general surgery

Figure 9 shows the K^+ and Ca^{2+} analysis of a subject undergoing liver resection (excision of tumour). This procedure involves substantial blood loss and therefore requires massive transfusion of (citrated) blood. The remote sensor unit was connected to the jugular vein by using a dual lumen cannula after surgery had been in progress for some 15 min.

There were no significant disturbances in K^+ concentration, but there was a massive decrease (60%) in Ca^{2+} concentration during the initial 28 min period, from 1.36 mM (first sample) to 0.55 mM (at 28 min), and this value remained depressed at the end of the operation (0.6 mM at 78 min). This is attributed to the chelating action of the sodium citrate present in the transfused blood, which ordinarily would be metabolized within 30–60 min. In this instance,

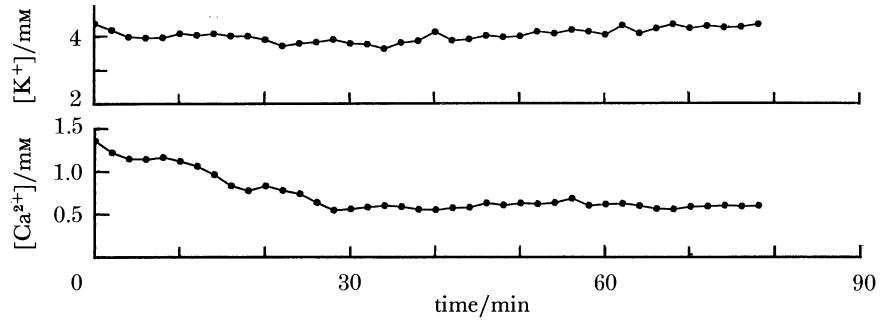

FIGURE 9. Online analysis of K^+ and Ca^{2+} during liver resection surgery. (See text for details.)

clearance of citrate from the blood was impaired by the nature of the surgical interference, and the Ca^{2+} concentration was depressed to a level which ordinarily might be 'considered at least undesirable', or, in this instance, where citrate metabolism is impaired, 'a real danger' (Ludbrook & Wynn 1958).

Example 3: renal dialysis

Figure 10 shows the results of online K^+ monitoring every 2 min during routine renal dialysis. The K^+ concentration falls as is known from discrete monitoring by ISE or flame photometry. Continuous monitoring on a routine basis, provided the additional cost of doing so was small, would be valuable if it led to economies in the management of renal dialysis patients.

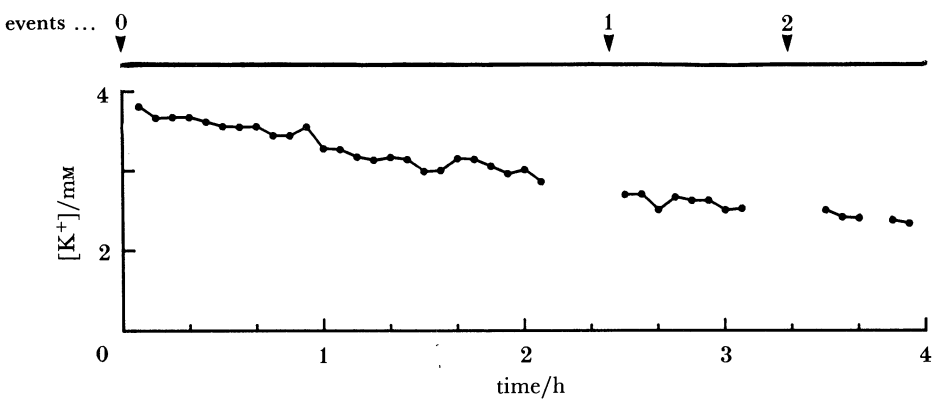

FIGURE 10. Blood K^+ monitoring during renal dialysis. Results are suppressed during two periods when bubbles formed in the ISFET cell. (See text for details.)

7. CONCLUSIONS

It has been shown that *ex vivo*, continuous monitoring of whole blood K^+ in humans by using ISFET biosensors is feasible. There is no reason why multi-functional devices for K^+, Na^+, Ca^{2+}, H^+, Cl^-, glucose, urea etc. cannot be similarly deployed to provide a comprehensive clinical, online, blood analyte monitoring system which could be used for continuous bedside monitoring or during surgery. Continuous monitoring of calcium during liver transplantation (Gray *et al.* 1986) would appear to be an important new clinical area of application.

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There are some important factors requiring further work:

(i) *Stability of devices*. Under laboratory conditions with aqueous solutions, the stability is excellent as shown from long term tests on K^+-valinomycin devices in figure 11. Sensors with improved selectivity for Na^+ are desirable. Their stability in aqueous solutions is excellent (figure 12) but there are problems in whole blood. The long-term stability of devices in contact with a variety of whole blood samples remains to be assessed.

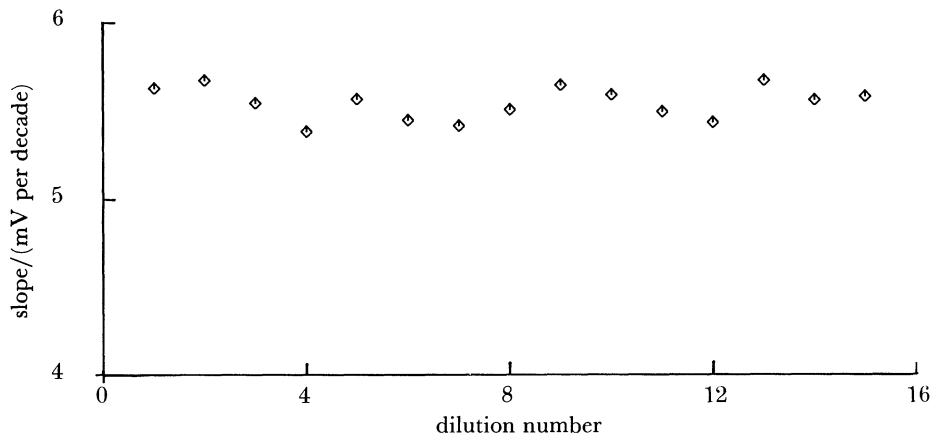

FIGURE 11. Long-term tests of slope calibration of K^+ ISFETs. Experiments were done with four devices over a period of six months.

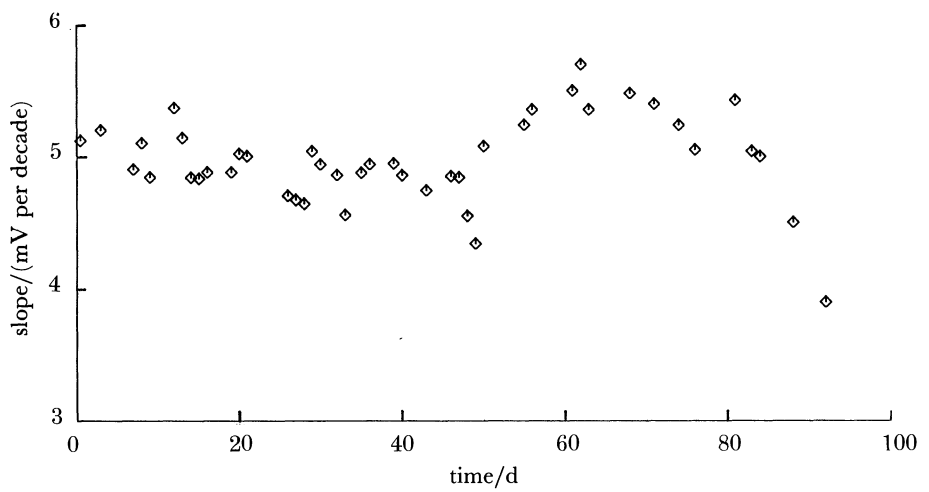

FIGURE 12. Daily long-term tests of slope calibration of Na^+ ISFET based on ETH 227. Failure after 80 d was due to membrane lift-off. The semiconductor device was reusable; this showed that failure was not due to encapsulation problems.

(ii) *Anticoagulation procedures*. Although in some instances the patient will be already heparinized, active heparinization will otherwise be necessary and can be achieved by the dual-lumen cannula system. Study of the effect of heparin on the cation content of standard solutions is in progress (A. K. Covington & R. Kataky, unpublished.)

(iii) *Clinical significance of* ISFET*-derived data*. The same problems exist about the clinical

significance of the data output from ISFET-based analysers as they do for discrete ISE-based analysers regarding matters such as the requirement for the presentation of ion concentration or ion activity, and the likely bias introduced by liquid junction, activity/complexing and temperature effects (Sibbald & Covington 1983; Covington 1985; Ferra & Covington 1986).

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