OPTOELECTRONICS AND ADVANCED MATERIALS – RAPID COMMUNICATIONS Vol. 2, No. 8, August 2008, p. 502 - 506

Sensitive device for ions of biological interest

E. EFTIMIE TOTU*, E. RUSE, I. GHICA, D. MANUC[a]
Department of Analytical and Instrumental Analysis, Faculty of Applied Chemistry and Materials Science, University Politehnica of Bucharest, Str. Polizu 1 -4, Bucharest, Romania
[a]*Department of Public Health, University of Medicine and Pharmacy Carol Davila, Eforie Str., no.4, 5 Bucharest, Romania*

The objective of this paper was to study the suitability of ion sensitive field effect transistors (ISFETs) for clinical work with special reference to a flow cell assembly. This was intended for use in the analysis of biological fluids, where the concentrations of specific ions, e.g. calcium, potassium, are closely monitored. Evaluations the new electrochemical assembly, for use in clinical chemistry, were made. Also, ETH 2041 (tetra - n – undecil 3, 3' ,4, 4' – benzophenone tetracarboxylate) and DOS (bis (2 –ethylhexyl) sebacate) plasticizers were used and their performances compared, proving that the proposed plasticizer ETH 2041 improves the electrochemical characteristics of the selective device finally obtained. Several points have arisen in this investigation with regard to the flow cell requirements and these are discussed as appropriate.

(Received June 25, 2008; accepted June 30, 2008)

Keywords: Dialysis flow-cell, ISFET, Ion selective membrane, Biological fluids

1. Introduction

There are various techniques applied in clinical analysis. Extensively used was the flame photometry. The important points to note are that the blood samples are diluted one part sample to 199 parts diluent (depending on the ion nature and ion concentration) and that it is the concentration of ions (mmol.dm^{-3} water) and not activity that is determined. In reality, activity is a more important parameter as this is the available species, e.g. 50% of calcium is protein bound [1], therefore unavailable, in serum. It is well known that proteins will bind to low molecular weight species [2], and that the degree of interaction is pH and temperature dependent (e.g. calcium and hydrogen ions will compete for binding sites). For sparingly soluble compounds this is a mean of transportation, e.g. calmodulin.

Another important method used in the clinical analysis is the potentiometry, when the activity is the parameter is measured while the devices are simple. Mini-electrodes are used in these analyzers, but due to the small membrane size, these electrodes can have a very high membrane resistance; this is a limiting factoring the construction of miniaturized ion selective electrodes (ISEs) [3]. Ion selective filed effect transistors (ISFETs) present certain advantages when compared to the classical ion selective electrodes (ISEs) due to its high input impedance, and corresponding low output impedance [4].

As the cell was intended for use in monitoring serum, blood and other biological fluids, it is essential that the complex composition of blood is appreciated. Buffering in blood plasma is maintained by hemoglobin, bicarbonate and phosphates. Although hemoglobin has better buffering action, bicarbonate is easier to determine. The concentration of bicarbonate is related to pH and pCO_2 by the Henderson – Hesselbalch equation:

$$pH = pK_a – \log \{[CO_2] / [HCO_3^-]\} \quad (1)$$

pH measurements alone are sufficient only for short term monitoring of respiratory conditions. Blood is composed of: plasmatic water that is water, ions and small hydrophilic constituents e.g. glucose; proteins, lipids and lipoproteins; corpuscles, erythrocytes, leucocytes and platelets.

Using direct potentiometry with undiluted samples, rather than direct dilution methods, 7% greater sodium and potassium ion concentrations were observed due to the volume displacement effects of proteins, lipids [5], and a positive bias of 4 – 5% arose for the concentration of calcium ions, [Ca^{2+}], measurements in whole blood compared with the true value in plasma phase. However, this bias could be reduced by creating a larger area of contact between blood and potassium chloride bridge solution and by correct choice of standards. Also, by taking into account variation in dissolved carbon dioxide content, avoiding calcium complexation and calcium / heparin binding [6]. The protein used in experiments, albumin as well as cellophane dialysis membrane were purchased from Sigma Chemicals.

There is much controversy as to the effect of proteins on ISE and ISFET experimental performance. Payne [7], an advocate of the belief that the proteins do interfere with ISEs, suggests that protein and other macro-molecules, surrounded by bound counterions, behave as neutral protein-water-in micelles in the water – ion phase, giving rise to a difference in the total ion concentration across the protein impermeable dialysis membrane. Payne and Jones

[7] state that in measurements of ionized calcium, protein interferes with measurement of the reference liquid junction potential. It was suggested [8] that interfacial changes at the membrane surface are produced by protein deposition; a change in physical nature of the interface leading to a shift in the standard electrode potential. This irreversible shift was not eliminated using albumin containing calibration solutions [9], but was eliminated using a cellophane dialysis membrane over the ion selective membrane. As drifting was not observed, it was suspected that the dialysis membrane had introduced another liquid junction.

2. Experimental

When ISEs or ISFETs are placed in biological samples, e.g. whole blood, plasma, which have lipophilic character (e.g. proteins), favorable extraction of lipophilic membrane components, processing only a moderate mobility, will occur, giving rise to a shift in the standard potential.

In experiments where the membrane is in contact with a flowing solution of blood serum, the lipophilicity, P (defined as the partition coefficient between water and octan-1-ol), of the plasticizer should be at least 12.8 [10]. In this context, we decided to use as plasticizer, a benzophenone (ETH 2041) which has high lipophilicity due to its long alkyl chain which decrease mobility and restrict diffusion of the ionophore in the membrane. An added advantage of this plasticizer is its favorable adhesion to PVC and silicone substrates making them particularly suitable for use with ISFETs. This component is also believed to be carbon dioxide impermeable. This has an important consequence for blood analyses. The buffering action in blood plasma is controlled by $NaHCO_3$ / H_2CO_3 and NaH_2PO_4 and NaH_2PO_4 / H_3PO_4. Although, as stated earlier, hemoglobin is a better buffer, the action of these buffering systems is also important.

ETH 2041 (tetra-n-undecil 3,3',4,4' – benzophenone tetracarboxylate) and DOS (bis (2 - ethylhexyl) sebacate) plasticizers were used and their performances compared. Valinomycin was used as electroactive component of the selective membrane. As polymeric matrix for the ion selective membrane was used PVC of high molecular weight and for dissolving the components it was used freshly distilled tetrahydrofuran (THF). All chemicals were specific for use in ISE work and were supplied by Fluka Chemicals Ltd.

All percentage compositions were by weight. The ionophore used, namely valinomycin, was 1.2%. The plasticizer, either ETH – 2041 or DOS, was 66.76% and the high molecular weight polyvinylchloride as 32.22%.

Before setting down the membranes, the ISFETs were thoroughly cleaned. The membranes were deposed by using the end of a plastic disposable pipette tip cut off at an oblique angle to form a shallow scoop. This tip was then dipped into the THF solution and the solution could be deposited easily over the gate of the device. Also, after casting the membrane over the dielectrical gate, devices were examined using a microscope to ensure that no air bubbles obscured the gate area. If air bubbles were found to be present, or if a new membrane was to be deposited on the device, the PVC membrane was removed from the chip using a scalpel and fine tweezers. The device was the washed again before the deposition of a fresh membrane. For this work autoclaving is recommended as a suitable means of sterilization.

The obtained devices were calibrated using the dip method with solutions in the range from 10^{-1} to 10^{-5} $mol.dm^{-3}$ potassium chloride and also using the constant volume dilution method using a flow rate of 3.58 $ml.min^{-1}$ and a dilution vessel of 38.37 ml. The selectivity coefficients, k_{ij}, of both membranes (ETH 2041 and DOS) were determined for calcium and pH using the constant volume dilution method. The reference electrode used for the determinations was a porous plug saturated calomel electrode – Russell pH Plc, with a 10^{-1} mol. dm^{-3} NH_4NO_3 salt bridge.

All devices were operated in the constant current mode [11] with I_D (drain current) set at 100 A and V_D (drain potential) set at 1V. The determinations were run on a digital multimeter – Thandar TM451 with precision of 0.1 V.

In order to fit into the flow dialysis cell, from Bellhouse Medical Products, Fig. 1, for the ISFET it was used an Eμ - 146 structure, Fig. 2.

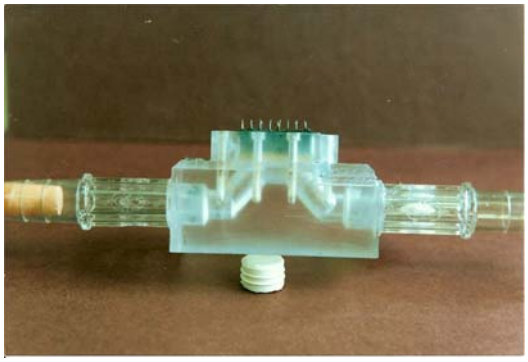

Fig. 1. The Bellhouse Dialysis Flow-Cell Assembly.

The Eμ146 chip features folded gate geometry which allows three active channels. This device presents 3 ChemFETs and a single IGFET on the 1.25 mm x 2.00 mm chip. In order to use the space efficiently, folded gated are used. The diffused source and drain regions have low aspect ratios and the metallization tracks extended as far to the channel as effective encapsulation allows, providing minimal serial parasitic resistance. A common drain connection for all devices minimizes the number of lead-out wires.

The characteristics of the device were identified, recording the bias voltage versus output voltage and the "switch on" potential noted.

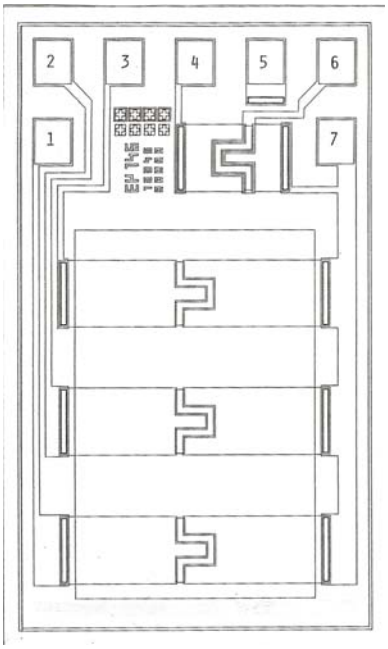

Fig. 2. Eμ 146 integrated circuit chip (x100). 1. A source; 2. B Source; 3. C Source; 4. IGFET Source; 5. Bulk connection; 6. IGFET gate; 7. Common drain.

Also, the devices were tested to ensure that none was liable to electrical leakage by connecting together the drain, source and substrate of the ISFET and polarizing from -3 V to +3 V against a low resistance (< 100 kΩ) reference electrode [11]. No leakage current should be observed as the bias potential is increased. Any device with a leakage current greater than 1 nA was rejected. These experiments were repeated when the ion selective membrane was set down.

For sensitive devices in flow through cell, 16 pin DIL ceramic headers – Shinko, SHK – CA-P160035, Dage Intersem Ltd., were used. These headers are compatible with 16 pin PIL socket adaptors – types 103 -218, Farnell Electronic Components Ltd.

Having satisfied these criteria, the drift, noise and optical sensitivity of the devices were determined. Between tests, the ISFETs were stored with calibration solution (usually 10^{-2} mol.dm^{-3} potassium chloride) over the membrane to prevent it drying out, and placed in specially designed electrostatic – free boxes (RS Components) [11].

Light on a reverse-biased p-n junction (here an ISFET) leads to the generation of electrons [12]. In the case of an ISFET, these are driven by the source to drain voltage and are therefore summed into the drain current, so that overall an increase in the drain current is observed. It was noted a typical 3mV optical sensitivity at constant drain current. Therefore, the characteristics and the drift of the devices were determined under normal laboratory conditions (daylight and fluorescent strip lightening)

The ISFET was sealed into a Perspex holder incorporating the dialysis membrane and seal, using silicone rubber 12 h curing. The assembly as presented in the Figure 3 was then screwed into the main body of the cell.

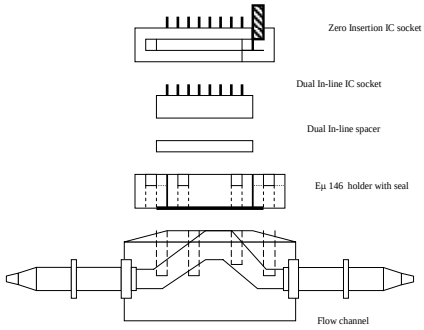

Fig.3. Schematic of the dialysis flow-cell assembly.

This was then connected via thin, flexible PVC tubing to the sample and the peristaltic pump. This cell was designed for use with Bellhouse's vortex mixing system.

3. Results and discussion

ISFETs based on a PVC / valinomycin membrane proved to operate satisfactorily in the presence of proteins, encouraging the development of ISFETs as clinical sensors [13, 14]. The research did show small negative drift over a three hour period which they believed arose due to desorption of protein from the membrane surface.

It was, therefore, extremely important that the drift characteristics of devices were studied before analysis, as a change from e.g. 4.0 mM to 4.1 mM potassium corresponds to a change of only 0.63 mV. Resolution of 0.1 mV is required of devices, i.e. a drift of less than 0.13 mV h^{-1} [11].

Tests carried out for potassium, in system using cellophane as dialysis membrane, showed that the dialysis membrane did affect the time response. An increase from 1s to 15s for 95% response was observed, however, this was considered acceptable as the membrane was now protecting the sensor.

Impedance studies carried out using as the interferent protein the albumin does not affect the diffusion of ions at the membrane surface. It would therefore be advisable to repeat tests initially carried out with proteins other than albumin e.g. globulin.

Utilizing all this information, following the example of commercial analyzers, it was considered advisable to use a cellophane dialysis membrane, integrated in to the flow cell, to separate the ISFET sensing membrane from the sample solution. The dialysis membrane, thereby, avoids sterilization problems associated with protein and anti-coagulant adhesion on the FET.

Calibration solutions for the examination of biological samples should contain protein, thus resembling the sample as closely as possible.

The ion-selective membranes were prepared as presented in previous works [14-16] from the cast membrane solution and the behavior of these membranes was investigated. It was found that DOS membrane showed good Nernst response in both dip tests and constant volume dilution calibrations (55.68 mV dec^{-1}, 57.31 mV dec^{-1} respectively), whereas ETH 2041 membrane showed sub-Nernstian responses using both calibration methods, this was most marked for the constant volume dilution calibration, see Table 1.

Table 1. Results of the calibration studies.

Calibration method	Slope (mV dec^{-1})	
	DOS Plasticizer	ETH 2041 Plasticizer
Dip test	55.68	51.98
Constant Volume Dilution (CVD)	57.31	48.84

The working range of the membranes extended to 10^{-4} mol dm^{-3} for ETH 2041 and 4.5 x 10^{-5} mol dm^{-3} for the DOS containing membrane. It would be prudent to repeat experiments with ETH 2041 in order to ascertain that these results were characteristic, although in one of our previous work the results obtained were similar [14]. Although the response and working range are acceptable, this plasticizer was reported [17] as having better behavior than was observed here.

The selectivity coefficients, k_{ij}, of the membrane for calcium and hydrogen were determined graphically from results of the mixed solution method of the constant volume dilution technique. The values of k_{ij} obtained are compared in Table 2 with the literature values.

Table 2. Selectivity coefficients for the ion selective membrane.

Interferent ion	Literature Value, Reference		Experimental value	
	[18]	[19]	DOS	ETH 2041
H^+	6.3 x 10^{-5}	-	32.2 x 10^{-3}	2.8 x 10^{-3}
Ca^{2+}	1.6 x 10^{-5}	4.9 x 10^{-5}	4.4 x 10^{-4}	2.1 x 10^{-4}

As a shift of 15 mV was noted for both electrodes (with DOS and ETH 2041 membranes) when immersed in mixed solutions containing 0.1 mol dm^{-3} HCl. In this case, it could be stated that they are pH sensitive, however, this was not considered to be a problem for the physiological working range of pH = 7. The membranes did not exhibit severe interference by calcium ions, and of the two membranes, the ETH 2041 membrane showed least interference. The experimental selectivity coefficients are, however, slightly higher than the literature values, Table 2.

If as mentioned before, ETH 2041 is carbon dioxide impermeable, then this plasticizer should replace DOS in potassium membranes for use in plasma and serum studies where changes in pCO_2 can occur [20].

The Bellhouse flow cell design has obvious pitfalls. Handling of devices should be minimized in order to reduce the likelihood of the static charging from the operator to the integrated circuit. The manipulation of the ISFET into the holder was awkward and there was a high probability of physical damage to the device if frequent insertion and removal should be necessary.

As the original design did not allow sufficient clearance for the locking arm of a zero integrated circuit socket to extend fully a channel was cut into the main body of the flow cell and a small section removed from the FET holder to overcome this problem.

The most serious flaw in the system is the location of the sealing gasket. In order to overcome this problem it was used a specific flow cap, designed in such way that only the gate region is exposed to the solution.

The internal diameter of the flow channel is too large, 16 mm, for a standard peristaltic pump to operate, as the internal diameter values are normally in the range of 1 mm. The large internal diameter initially used was to allow interfacing with the vortex mixing system.

4. Conclusions

As in all in direct potentiometry, ISFETs sense activity rather than concentration and that the devices are very small and easy to operate, and then it would seem practical to use these devices in preference to flame photometry, currently being used.

The behavior of the microelectronic devices used substantiated claims by our earlier work [14] that integrated circuits are suitable for ex-vivo clinical sensors.

The membrane compositions used in these experiments gave good Nernst response with valinomycin as ionophore, though repeat calibration of ETH 2041 should be carried out. The ETH 2041 plasticizer was found to yield devices which were more selective to potassium than the more commonly used DOS and it would therefore seem wise to convert to the use of ETH 2041 in future potassium selective membranes for clinical analysis, sustaining this way our previous conclusions regarding the usage of this plasticizer [14].

The cell design needs modifying with special regard to the large gap in the ISFET holder which allowed solution to come into contact with the device and therefore short circuit the electronics. As it was necessary to seal the device into the place, requiring 12 h drying, thus isolating the device, some method must be found to allow replacement of solution at the gate. This method should permit inspection of the gate under microscope to ensure that no air bubbles are obscuring the gate, thus preventing electrical contact (gate / solution) from being made.

If the cell is to be used in conjunction with a standard peristaltic pump, then the flow channel diameter should be reduced. The flow cell has many flaws and it is doubtful whether further time and financement should be expended on this design, instead of better to consider developing a smaller and more practical system.

References

[1] Michael T. Henze, John J. Tanner, Protein Science, **17**, 431 (2008).
[2] Yubin Zhou, Wen-Pin Tzeng, Wei Yang, Yumei Zhou, Yiming Ye, Hsiau-wei Lee, Teryl K. Frey, Jenny Yang, Journal of Virology, **81** (14), 7517 (2007).
[3] G. K. Budnikov, J. Anal. Chem., **55** (11), 1014 (2000).
[4] Totu Eugenia, Ruse Elena, Luca C., Rev. Roum. Chim., **51** (5), 331 (2000).
[5] Bernhard P.H. Schaffar, Heinz Kontschieder, Christoph Ritter, Hans Berger, Clin.Chem, **45**, 1678 (1999).
[6] Chenghua Shao, Fuming Zhang, Melissa M. Kemp, Robert J. Linhardt, David M. Waisman, James F. Head, Barbara A. Seaton, J. Biol. Chem., **281** (42), 31689 (2006).
[7] Payne R.B., Jones D.P., Ann. Clin. Biochem, **24**, 400 (1987).
[8] Míriam Gironès, Rob G.H. Lammertink, Matthias Wessling, J. Membr. Sci., Vol. **273** (1-2), 68 (2006).
[9] Cornelia Kullnig, Robert L. Mach, Matteo Lorito, Christian P. Kubicek, Appl Environ Microbiol., **66** (5), 2232 (2000).
[10] Takacs – Novak K., Jozan M., Szasz G., Int. J. of Pharm., **113** (1), 47 (1995).
[11] Totu Eugenia, Covington A.K., "Ion selective Membranes Based on Polyimide Polymeric Matrix", Proceedings The 6th European Conference on Electroanalysis, Durham, 1996.
[12] Grove A.S., "Physics and Technology of Semiconductor Devices", J.Wiley & Son, New York 1977, Chapter 12.
[13] Eugenia Totu, A. K. Covington, S. Picioreanu, Gh. Nechifor, C. Luca,, J. Rom. Coll.Surface Chemistry Assoc., **2**, 21 (1997)
[14] Totu Eugenia, Josceanu Ana Maria, Covington A.K., Mat. Sci Eng. C **18**, 87 (2001).
[15] Totu Eugenia, Covington A.K., Analyst, **121**, 1811 (1996).
[16] Eugenia Totu, Ana Maria Josceanu, Mihaela Emanuela Craciun, Elena Ruse, Science and Technology of Environmental Protection, **10** (1), 1 (2003).
[17] Janata J., McBride P.T., J. Bioeng., **2**, 459 (1989).
[18] E. Grygoowicz-Pawlak, K. Pachecka, B. Wolanin, E. Malinowska, Int.Agrophysics, **20**, 101 (2006).
[19] Pungor E., Toth K., Hrabececzy – Pall A., Pure & Appl. Chem., **51**, 1915 (1989).
[20] Horacio J. Adrogué, Nicolaos E. Madias, J. Am. Soc. Nephrol., **15**, 1667-1668 (2004).

*Corresponding author: eugenia.eftimie@et-consulting.ro