PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Semiconductor Biosensors [and Discussion]

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Semiconductor biosensors

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Hydrogen- and ammonia-sensitive metal-oxide semiconductor (MOS) structures are described. Special attention is paid to ammonia-sensitive MOS devices with thin (*ca.* 3 nm) iridium or platinum gates. It is shown how these devices can be used in combination with immobilized enzymes to develop bioprobes or biosensing systems. The temperature dependence of the gas sensitivity of MOS structures with catalytic metal gates is considered. It is demonstrated that at low temperatures (30–40 °C) iridium gates have a faster response to ammonia than platinum gates, and that Ir-MOS structures thus are better suited for the development of biosensors. It is also shown that at high temperatures (190–200 °C) platinum gates can be used to detect unsaturated hydrocarbons such as ethylene. Gas evolution from ripening fruits was monitored with such a sensor.

Some biosensing applications of ammonia sensitive Ir-gate MOS devices are described; for example, the determination of urea and creatinine. The devices are used both to measure a pulse of ammonia in a flowthrough system and to measure *in situ* steady-state responses as a bioprobe. The special features of gas sensors used for biosensing purposes are summarized.

1. Introduction

There are several types of semiconductor device which may be used as biosensors in combination with a suitable biochemical reaction. The ion-sensitive field effect transistor, discussed by Covington (this symposium) can be used, for example, to measure pH and ion concentrations in solution. There are, however, also other possibilities for biosensing, namely by using gas-sensitive semiconductor devices. We shall describe one class of semiconductor gas sensors and their applications as biosensors in a general sense. The devices are based on the use of a catalytic metal as a gate of a field-effect structure, where the hydrogen-sensitive Pd-gate metal-oxide semiconductor (MOS) structure is the prototypical device (Lundström *et al.* 1975; Lundström 1981; Lundström & Svensson 1985). It has, however, been demonstrated that, by a modification of the metal gate, very sensitive sensors for ammonia may be obtained (Winquist *et al.* 1983; Spetz *et al.* 1983).

The development of sensitive and specific gas sensors is rather interesting in view of the general biosensor concept illustrated in figure 1. In many biochemical reactions, gaseous species are either produced or consumed. This fact has, of course, already been utilized in the so-called enzyme electrodes where, for example, ammonia molecules produced by immobilized enzymes are detected as ammonium ions, giving a pH change in a solution behind a gas-permeable membrane. It may, however, be advantageous to measure the gaseous species directly. This is the approach we take, which has been shown to be useful for the determination of substances like urea, creatinine and several amino acids by the use of immobilized enzymes and ammonia-sensitive MOS structures, with thin iridium gates (Winquist *et al.* 1984, 1985, 1986).

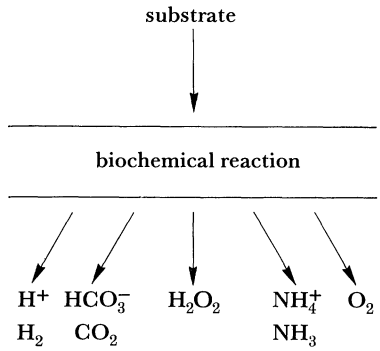

FIGURE 1. Schematic illustration of biosensors based on the combination of a monitor for a gaseous or ionic species produced from the substrate to be determined in a biochemical reaction. Only a few examples have been shown. For oxygen, consumption is also possible. The biochemical reaction is often enzymic, for example:

$$\text{urea} \xrightarrow{\text{urease}} \begin{cases} 2\,NH_4^+ + HCO_3^- & \text{(in solution)} \\ 2\,NH_3 + CO_2 & \text{(in gas phase).} \end{cases}$$

A hydrogen-sensitive Pd-gate MOS device has been used to monitor enzymic reactions and hydrogen produced by microbes (Winquist *et al.* 1982; Danielsson *et al.* 1983). It has also been used to detect anaerobic conditions in fermentation (Cleland *et al.* 1984) and to measure hydrogen in the breath of people to diagnose lactose malabsorption (Berg *et al.* 1985).

Furthermore it has been employed for the study of antibiotic resistance of microorganisms through their hydrogen production rate under anaerobic conditions (Hörnsten *et al.* 1985).

There are several other examples where the monitoring of a gaseous species may be of interest for biosensing purposes. In this paper, we describe gas sensors based on catalytic metal gates and some of their applications as biosensors. We are aware of other semiconductor-based gas sensors, such as those based on semiconducting metal oxides (ZnO, SnO_2, etc.) and organic semiconductors (e.g. phthalocyanines). Instead of making a survey of different types of gas sensor, we have, however, chosen to concentrate on one type of device. This means that we have not written a review paper, but a summary of the work at our own laboratory. We will describe the basic principles of hydrogen- and ammonia-sensitive metal-oxide semiconductor structures and give some examples of their use as biosensors. We also point out how the operating temperature of the gas sensors can be used to promote the detection of other molecules of biological interest, and thus demonstrate the detection of ethylene (or unsaturated hydrocarbons) emanating from ripening fruits.

2. GAS-SENSITIVE METAL-OXIDE SEMICONDUCTOR STRUCTURES

(*a*) *Principles*

Figure 2 shows schematic illustrations of the two types of device that we have investigated so far. The structure to the left has a thick (200–300 nm) palladium gate. Palladium is a catalytic metal, which dissociates hydrogen molecules on its surface. Some of the produced hydrogen atoms diffuse through the palladium layer and adsorb at the Pd–SiO_2 interface, where they are polarized and form a dipole layer. This dipole layer causes a voltage drop across the Pd–SiO_2 interface, which adds to the externally applied gate voltage, shifting the capacitance–voltage

curve of an MOS capacitor, or the I_d–V_g curve of an MOS field-effect transistor, along the voltage axis as illustrated in figure 2*c*, *d*. In figure 2*a*, it is indicated that, in the presence of oxygen (e.g. in air), water is continuously produced on the palladium surface. Normally this type of device is operated at an elevated temperature to speed up the chemical reactions on the palladium surface. When hydrogen is taken away from the ambient, the palladium surface, bulk and interface are emptied of hydrogen atoms because of the chemical reactions on the palladium surface. The association of two hydrogen atoms to form a hydrogen molecule is rather unlikely compared with water formation. This also means that the hydrogen sensitivity will be very large in an inert atmosphere. Sensitivities like one part per thousand million (1 p.p.b.) by volume in an inert atmosphere and one part per million (1 p.p.m.) by volume of hydrogen in air are achievable. It should be mentioned that the sensitivity, i.e. the voltage shift, ΔV, shows a Langmuir-like behaviour in the square root of the hydrogen pressure, p_{H_2}:

$$\Delta V = \Delta V_{\max} \frac{(k p_{H_2}^{\frac{1}{2}})}{(1 + k p_{H_2}^{\frac{1}{2}})}, \tag{1}$$

where k is a constant at a given oxygen pressure and operating temperature. $\Delta V_{\max}$, i.e. the maximum observable voltage shift, is around 0.5 V. The mechanisms behind the hydrogen sensitivity of the Pd-MOS devices have been fairly well elucidated and they are further described in a recent review (Lundström & Svensson 1985).

The structure shown in figure 2*b* has a very thin layer (typically 3–10 nm) of a catalytic metal (Ir, Pt, Pd) as the active gate. To contact the thin layer, a thick contact is used (not shown). This contact can, however, be made of any type of metal, for example aluminium. In the presence of ammonia, the electrical characteristics of the part of the structure belonging

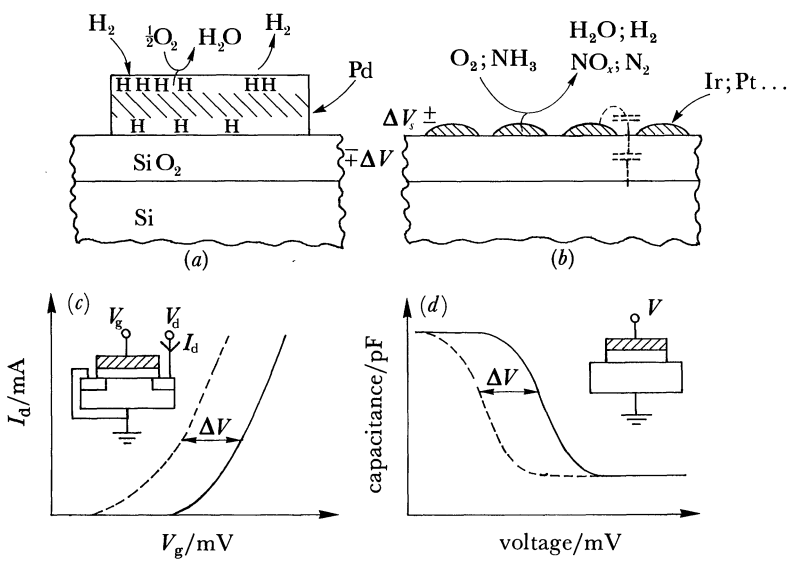

FIGURE 2. Schematic illustration of the MOS-gas sensors with catalytic metal gates. (*a*) Hydrogen-sensitive palladium gate; (*b*) ammonia-sensitive thin platinum (or iridium) gate; (*c*) gas-induced shift of the I_d–V_g curve of a gas-sensitive MOS field effect transistor. This shift is equivalent to a change in the so-called threshold voltage. (*d*) Gas-induced shift of the capacitance–voltage curve of a gas-sensitive MOS capacitor, equivalent to a change in the so-called flatband voltage. Tentative explanations of the gas-sensitivity are given in (*a*) and (*b*). The hydrogen sensitivity (*a*) is rather well understood, whereas the ammonia sensitivity (*b*) still needs further investigation. (See text for further details.)

to the thin catalytic metal shift along the voltage axis; this result is similar to the hydrogen-induced shift of the Pd-MOS structure. The detection mechanism is, however, different, because ammonia-sensitivity requires a porous catalytic gate. A thick Pt or Pd gate has, for example, a very small sensitivity for ammonia. The thin catalytic films are also sensitive to hydrogen (figure 3*a*) but since the thickness dependence of the ammonia and hydrogen sensitivities, respectively, is quite different, we conclude that the detection mechanisms for the two molecules are different. The device aspects of the ammonia sensor such as the dependence of the ammonia sensitivity on the Pt-layer thickness, at operation at 150 °C, are treated in a recent publication (Spetz *et al.* 1987). The details behind the ammonia-induced voltage shift are not completely understood at present. Ammonia molecules apparently take part in chemical reactions on the thin catalytic metal-oxide surface. One suggestion is that a surface potential change of the metal, due to dipoles formed on the metal islands, is capacitively coupled through the pores in the metal to the semiconductor surface (Lundström *et al.* 1987). It should be mentioned that ammonia molecules adsorbed on platinum surfaces can cause a very large decrease in the work function of the metal, up to 3 eV (Fisher 1981). The ammonia sensitivity shows also a Langmuir-like behaviour (figure 3*b*) although the relation between the ammonia pressure and

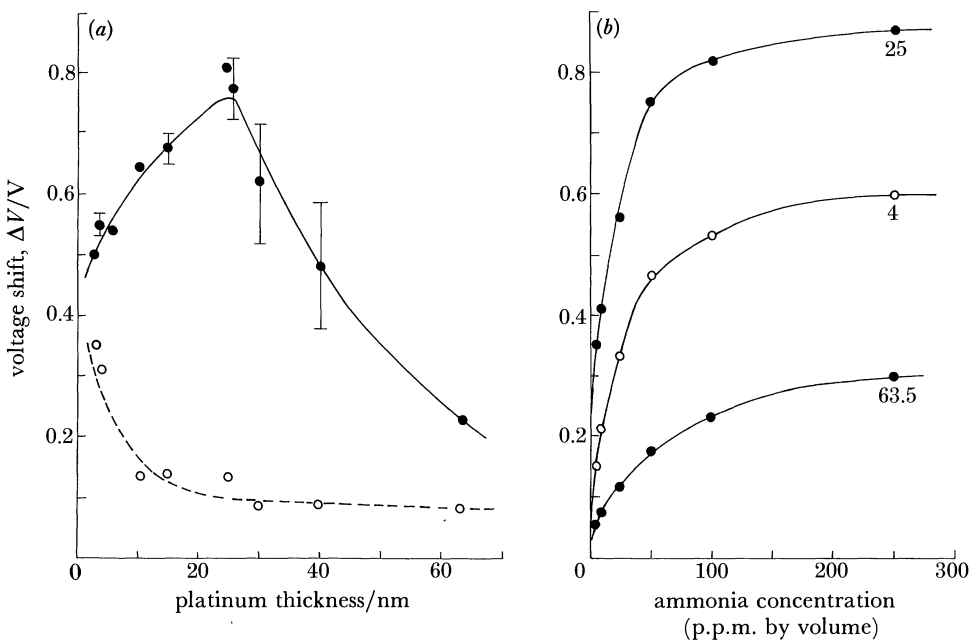

FIGURE 3. (*a*) Experimentally observed voltage shift plotted against the thickness of the platinum gate during exposure to 100 p.p.m. (by volume) NH_3 (filled circles) and 100 p.p.m. H_2 (open circles) in air. Device temperature 150 °C. (*b*) Relation of voltage shift to ammonia concentration in air for platinum films of different thicknesses. Device temperature 150 °C. The structures used to obtain the data had a thick, evaporated aluminium contact; the area ratio between the thin and thick contact was 3:1.

voltage shift does not appear to follow a simple formula like that of hydrogen (equation (1)). This is not surprising in view of the rather complex chemical reactions taking place between NH_3 and O_2 on the catalytic metal (Gland & Korchak 1978). It is observed that for Pt gates operating at elevated temperatures, it would be possible to detect about 1 ppm (by volume) of NH_3 in air.

(*b*) *Temperature dependence*

We have so far discussed the behaviour of the palladium-gate and thin-platinum-gate MOS structures at a high temperature (150 °C). It turns out that the temperature of the sensor is an interesting parameter, which can be used to alter the sensitivity of the structures towards different gases. The sensitivity of thick Pd-gate MOS structures to ethanol is, for example, observed to increase above 150 °C (Ackelid *et al.* 1986, 1987). Similar observations have been made for thin platinum gates with ethanol, ethylene or other unsaturated hydrocarbons (Winquist & Lundström 1987; Ackelid *et al.* 1987). A summary of observed high-temperature responses of thin platinum gates is given in figure 4*a*. It is thus observed that the sensitivity to gases other than ammonia can be enhanced at a higher operating temperature. Ethylene is an interesting molecule, because it is involved in the ripening of fruits and vegetables.

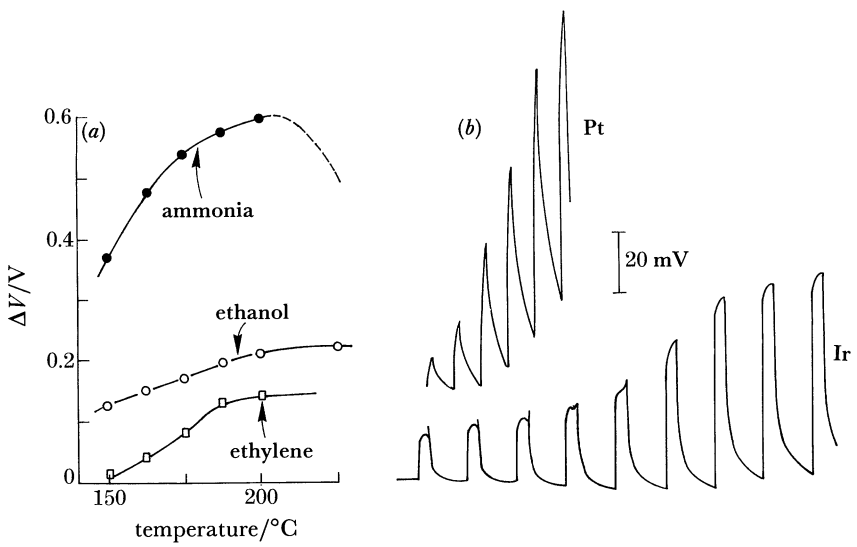

FIGURE 4. (*a*) High-temperature response of thin (6 nm) Pt-gate MOS structures to 30 s pulses of three different gases. The gas concentration was 100 p.p.m. (by volume) in air. The curves were recorded for three different test structures at different occasions. The dashed part of the response to ammonia is a preliminary observation, which has to be elucidated further. The voltage shift is the change in flatband voltage during the gas pulse. (Data partly from Ackelid *et al.* (1987).) (*b*) Response of thin Pt- and Ir-MOS structures to ammonia in air. Both gates were about 3 nm thick and operated at 40 °C. The ammonia pulses were 1 min long with 3 min between the pulses. Different timescales were used for Pt and Ir respectively. The ammonia concentrations were (from left to right), for Pt: 3, 11, 50, 100, 250, 500 p.p.m.; for Ir: 3, 7, 11, 23, 50, 100, 250, 350, 500 p.p.m.

It is often possible for the sensor to be kept at a high temperature. However, sometimes (for example, in the development of bioprobes for *in situ* or *in vivo* measurements) it will be difficult to keep a sensor at a temperature significantly higher than that of its surroundings. It is thus of considerable interest that thin platinum or iridium films retain a substantial ammonia sensitivity at about room temperature (20 °C) (figure 4*b*); iridium has a more distinct pulse response than platinum at low temperatures, although the voltage shifts are somewhat smaller. It is thus more practical to use iridium-gated devices for ammonia sensing at low temperatures.

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3. Biosensing with gas sensors

(*a*) *General principles*

Gas sensors can be used for biosensing in several ways, some of them illustrated in figure 5. They can be put directly above a reaction vessel (figure 5*a*) or in the exhaust of it (figure 5*b*). Furthermore, it is possible to use the sensor behind a gas-permeable membrane; for example, in a flowthrough system or as in a probe (figure 5*c*, *d*). Another feature of a gas sensor

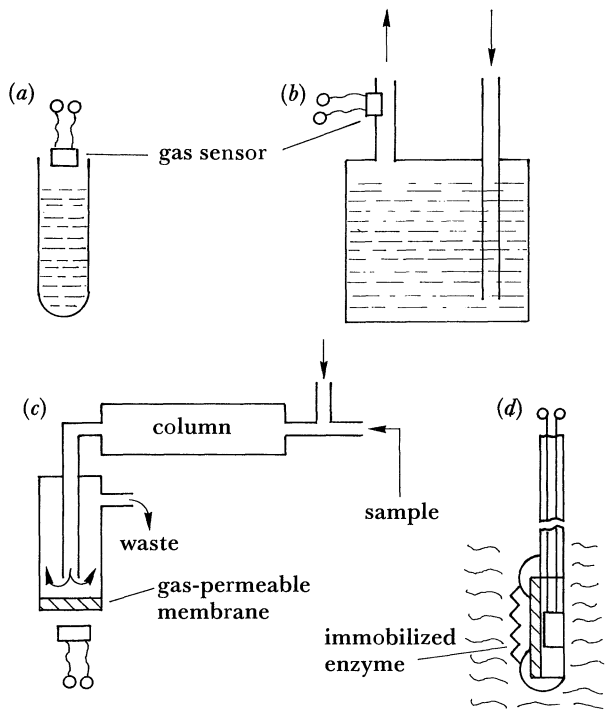

Figure 5. Schematic illustrations of some different ways of using gas sensors for biosensing purposes. Parts (*c*) and (*d*) are further described in the text.

is that the sample can often be diluted in the gas phase to a concentration corresponding to that of the maximum differential sensitivity of the sensor. In addition, the sample can often be employed in pulses over the sensor; this treatment minimizes the influence of drift in the baseline of the sensor on the measured response. The pulse response may also be linear in the concentration of the species to be measured even if the steady-state response is nonlinear. The response of the semiconductor gas sensors treated in §2 can be approximated by

$$\mathrm{d}(\Delta V)/\mathrm{d}t = c_1 P(\Delta V_{\max} - \Delta V)^{\alpha} - c_2 \Delta V^{\beta}, \tag{2}$$

where c_1 and c_2 are rate constants, α and β are constants depending on the reaction order of the rate-limiting steps of the chemical reactions on the metal surface (in the Pd-MOS hydrogen sensor, $\alpha = \beta = 2$) and P is the partial pressure (concentration) of the species to be measured. If the test pulse is short enough, then ΔV is very much smaller than $\Delta V_{\max}$ and

$$\Delta V(t_{\mathrm{p}}) \approx c_1 P \Delta V_{\max}^{\alpha} t_{\mathrm{p}}, \tag{3}$$

where t_{p} is the pulse length; it is assumed that the concentration P is constant during the pulse.

In the following sections we give some examples of the applications of the gas-sensitive MOS structures. Although the hydrogen sensor has been used for biosensing in several cases, as mentioned in the Introduction, we shall describe the application of the ammonia-sensitive device in combination with immobilized enzymes and the use of thin metal gates at high temperatures to detect ethylene and other unsaturated hydrocarbons from ripening fruits or vegetables.

(*b*) *The* NH_3*-sensor and immobilized enzymes in a flowthrough system*

A flowthrough system (shown schematically in figure 5*c*) has been used to investigate the usefulness of the NH_3-sensor, in combination with enzymes, for substrates that produce ammonia. Two such possibilities of clinical interest are urea and urease, and creatinine and creatinine iminohydrolase. Several other combinations have, however, also been investigated. Table 1 gives a summary of some of the results obtained by using enzymes immobilized in a column as indicated in figure 5*c*. The sample was injected as a short pulse into the column. By having a large excess of enzyme activity in the column (and a suitable flow rate) all the substrate was converted into ammonia during the passage through the column. When the ammonia pulse passed over the gas-permeable membrane, ammonia molecules diffused through the membrane according to the equilibrium

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O, \tag{4}$$

and were detected by an iridium-gate NH_3-sensor, operated at 40 °C. The response (table 1) was the voltage shift during one pulse as defined in figure 6. If suitable substrate concentrations

TABLE 1. SOME ENZYME–SUBSTRATE PAIRS STUDIED WITH A FLOWTHROUGH SYSTEM

(Substrate (10 μM) was injected into the flow (flow rate 0.125 ml min^{-1}, pH = 8.5–9) for 30 s. The sensor was an Ir-gate MOS device kept at 40 °C.)

substrate	enzyme	pulse response, ΔV/mV
urea	urease	16
creatinine	creatinine iminohydrolase	8
adenosine	adenosine deaminase	8
asparagine	asparaginase	6
glutamine	glutaminase	9
glutamate	glutamate dehydrogenase	8
alanine	alanine dehydrogenase	9
adenosine monophosphate (AMP)	AMP-deaminase	10

were chosen, equation (3) was observed to be followed. The fact that all substrates (except urea) gave about the same voltage shift (at a given concentration) is due to the excess of enzyme activity in the column as discussed above. Urea gives twice as large a response, because it is the only substrate that is converted into two ammonia molecules (or ammonium ions).

The flowthrough system has so far been used to develop assays for urea (Winquist *et al.* 1984) and for creatinine (Winquist *et al.* 1986). Creatinine will be further discussed below, but before doing so we shall point out an observation related to the pH-dependence of the urease–ammonia sensor combination. Figure 7*a* shows the experimentally observed pH-response of the flow-through system for the detection of urea (broken line). The solid line is the expected pH-dependence, calculated from the known pH-dependence of the enzymic reaction (which has

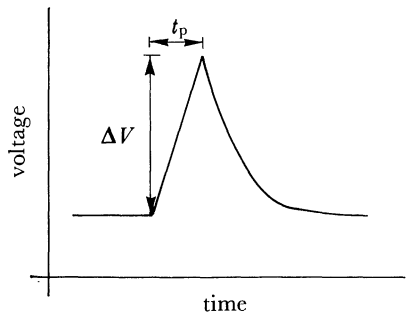

FIGURE 6. Definition of the response during an ammonia pulse in the flowthrough system.

a maximum at about pH 7.2 and the pK value of the reaction in equation (4) (p$K \approx 9.3$). In the experiments and in the calculations, there was no excess of enzyme in the column. This would, of course, have altered the pH-dependence, giving a constant response over a certain pH-range. We observe that the experimentally observed pH-dependence is broader than the calculated one and that the optimum is displaced towards a somewhat lower pH. Another observation, which explains the displacement of the pH-value of the maximum sensitivity, is shown in figure 7*b*. This solid line is the theoretically expected ammonia gas (NH_3) concentration at a given total ammonia concentration as calculated from (4) with p$K_a \approx 9.3$. The broken line is the experimentally observed pH-dependence of the response of the NH_3-sensor behind the gas-permeable membrane to a given total ammonia ($NH_3 + NH_4^+$) concentration in solution on the other side of the membrane. The pK_a value of this combination appears to be about 8.4, probably because of kinetic effects.

The flowthrough system has also been used to determine creatinine in plasma, whole blood and urine (Winquist *et al.* 1986). Because the levels of creatinine in blood (and plasma) are rather small, it is necessary to remove endogenous ammonia in the sample before the determination of creatinine. The flowthrough system was therefore provided with an extra enzyme column in front of the one containing creatinine iminohydrolase. The extra column

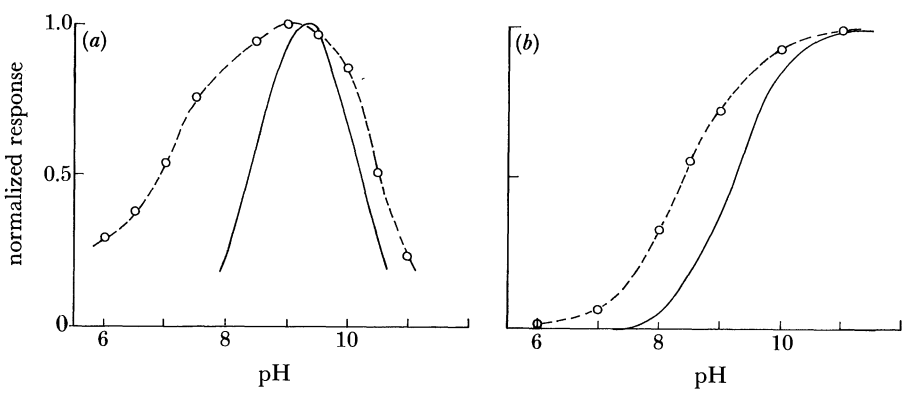

FIGURE 7. (*a*) Normalized response to urea of the flowthrough system with immobilized urease against the pH of the buffer flowing through the system. Solid line: theoretical calculation; circles and dashed line: experimental results. The column did not contain any excess of enzyme activity and the urea concentration was rather large (50 µM, 30 s, flow rate 0.125 ml min^{-1}). Total conversion of the substrate was thus not obtained. Sensor: (3 nm) Ir-MOS, 40 °C. (*b*) Normalized response of the flowthrough system to ammonia versus the pH of the buffer. Solid line: theoretically expected response from equation (4) with p$K_a \approx 9.3$. Circles and broken line: experimental results (ammonia concentration 50 µM, pulse length 30 s, flow rate 0.125 ml min^{-1}). Sensor: (3 nm) Ir-MOS, 40 °C.

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contained immobilized glutamate dehydrogenase, which catalyses the amination of α-ketoglutarate in the presence of NADH. In this way, by using a buffer containing NADH and α-ketoglutarate, endogenous ammonia in the sample will be consumed (Tanganelli *et al.* 1982; Tabata *et al.* 1983). Figure 8 shows the pulse response of the flowthrough system (as defined in figure 6) for creatinine. It is observed that it is a straight line, i.e. it obeys equation (3). Furthermore, the pulse responses are rather reproducible, as seen in the insert of figure 8, although the baseline is changing.

The insert also shows the response to a 25-fold dilution of the plasma sample. It was found that the results from the flowthrough system correlated well with a considerably more complicated and time-consuming spectrophotometric method for determination of creatinine. It is interesting to note that the gas-sensor method can be used also in turbid samples, such as whole blood.

Possible interferences are of course of large interest for the development of a practical monitor for creatinine (or other substrates). Table 2 shows some of the observed results when a plasma

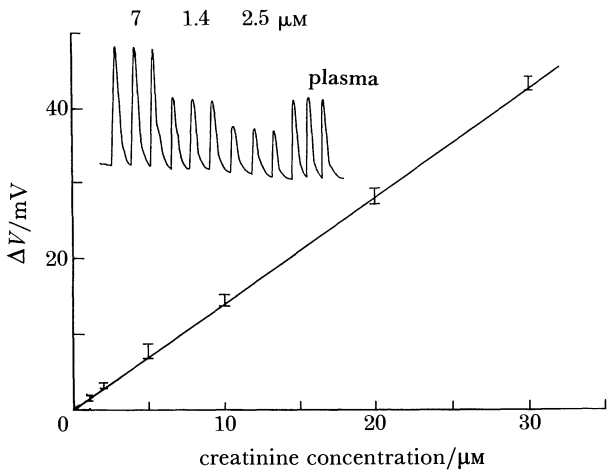

FIGURE 8. Calibration graph of the flowthrough system for creatinine (see text for details). ΔV during one sample pulse (40 s) is defined in figure 6. The bars connect the maximum and minimum values for five measurements at each concentration. The straight line follows equation (3) with concentration in micromoles equal to 0.71 ΔV/mV. The inset shows a strip-chart recording of the response to creatinine of different concentrations (one 85 μl sample was injected every 4 min). The response to a 25-fold diluted plasma sample is also shown. The pulse height for the plasma sample is about 5.6 mV. (Some data from Winquist *et al.* (1986)).

TABLE 2. TEST OF POSSIBLE INTERFERENCE IN THE DETERMINATION OF CREATININE IN PLASMA

(Interferents were added to 25-fold diluted plasma, and 85 μl of this mixture were injected into the flowthrough system (Ir-MOS, 35 °C). (See text for discussion of the creatinine determination.))

interferent	concentration / mg l^{-1}	response / mV
none	—	5.65
acetylsalicylic acid	50	5.5
ascorbic acid	20	5.6
asparagine	10	5.7
ethanol	100	5.6
urea	15	5.7
methylamine	0.15	6.2
ethylamine	0.2	6.05

sample was intentionally contaminated with a possible interferent. We note that in the table there are only two substances, the amines, which give a considerable change in the measured response. The reason for this is that amines by themselves give a signal on the NH_3-sensor (a relative sensitivity, compared with NH_3, of 0.1 and 0.05 respectively) (Winquist *et al.* 1985) but they are not consumed in the column with glutamate dehydrogenase.

(*c*) *Bioprobe for urea*

The possibility shown in figure 5*d* has been tested with urea immobilized directly on the gas-permeable membrane, which was mounted just above the surface of the NH_3-sensor, and put into a probe (figure 5*d*). Figure 9 shows the response of this probe when it was put into different concentrations of urea and ammonia. Because each urea molecule gives rise to two ammonia molecules, the response is larger for urea at a given molar concentration. If urea is going to be determined in an endogenous background of ammonia, a reference probe with, for example, heat-denatured enzyme (i.e. insensitive to urea) should also be used.

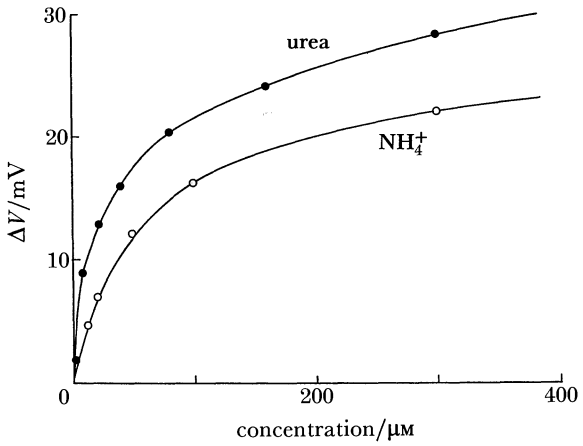

FIGURE 9. Response of a bioprobe, according to figure 5*d*, to urea and ammonia. The sensor was a (3 nm) Ir-gate MOS structure, operated at 40 °C. The probe was used in aqueous solution, buffered at pH 8.5. The response is the steady-state response of the probe (data from Winquist *et al.* (1985)).

(*d*) *Gas evolution from fruits and vegetables*

In this example, we describe the use of a thin-film Pt-MOS structure operated at 195 °C, where it is sensitive not only to hydrogen and ammonia but also to a number of unsaturated hydrocarbons. The sensitivity to ethylene is in the order of 1 p.p.m. (by volume); this result implies that these sensors could be useful in plant physiology to follow ethylene, or more general (unsaturated) hydrocarbon, production.

It has long been known that ethylene plays a key role as a hormone and regulator in the process of cell growth and ripening of fruits. Only recently, however, have the biochemical pathways leading to ethylene production and its regulation in growing cells been elucidated (Yang & Hoffman 1984). The rate of ethylene production during ripening of fruits may go up to 10 μl kg^{-1} h^{-1}. Analytical procedures for ethylene thus demand specific and very sensitive techniques (of the order of a few parts per million by volume), such as mass spectroscopy or gas chromatography.

In a preliminary experiment, the production of unsaturated hydrocarbons from tomatoes

was followed. Air was continuously pumped through a glass jar, containing tomatoes, to a three-way valve, which exposed a thin Pt-MOS field-effect transistor to the airstream for 5 min in every 20 min. The signal from the sensor was recorded for five days (figure 10*a*). During the first few days, no significant response was obtained, showing that no detectable gas was evolved. After four days, a response from the sensor was seen, which could implicate ethylene or, more generally, unsaturated hydrocarbon production.

In a second experiment, with the same experimental conditions, the tomatoes were subjected to external ethylene exposure after one day. The response of the sensor is shown in figure 10*b*. One day after the injection, a response from the sensor was observed. The slope of the response–time curve (figure 10*b*) is steeper than that of the previous experiment (without ethylene injection). These two experiments thus indicate that it is possible to follow ethylene (hydrocarbon) production from tomatoes and thus also the ripening process. It is, however, premature to draw wider conclusions about the applicability of these sensors during practical conditions. More investigations have to be performed, for example, to elucidate the selectivity properties and long-term stability.

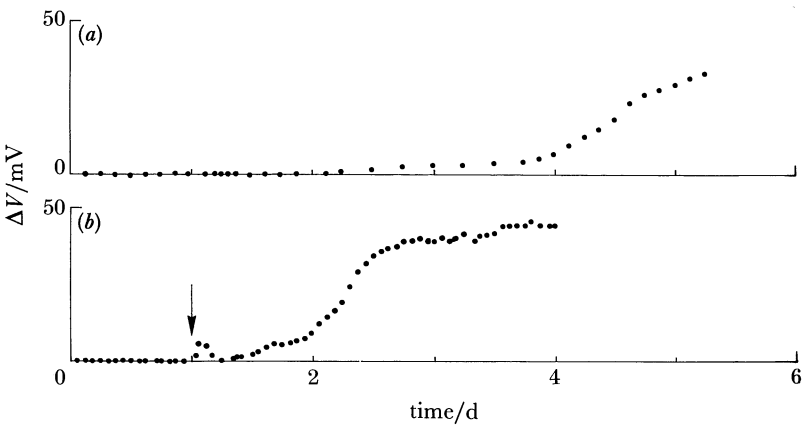

FIGURE 10. Gas evolution from ageing tomatoes. A thin (6 nm) Pt-MOS device was used at 195 °C to monitor the evolution of ethylene and/or other (unsaturated) hydrocarbons from tomatoes. (*a*) Spontaneous evolution; (*b*) triggered evolution through ethylene injection (at the arrow).

4. DISCUSSION

There are very few biosensors described in the literature where the concentration of a gaseous species is measured directly. There are, however, several cases where such an approach may be advantageous. Gas chromatography has, for example, been used to measure gas concentrations in breath for medical diagnosis (Christman & Hamilton 1982). In general a gas sensor can be applied, if necessary, far away from the sample to be tested. A gas sensor is naturally electrically insulated from liquid samples. In a bioprobe application, the sensor is separated from the biological surroundings by a gas-permeable membrane, which inflicts the problem of biocompatibility and clogging on the membrane only. Furthermore, the buffering capacity of the biological system does not influence the measured signal.

We have considered the use of one class of gas-sensitive semiconductor devices for biosensing purposes. The ammonia-sensitive thin catalytic metal-gate MOS structure appears to be very

useful in several applications. Because of the large sensitivity of this device, it is possible, in a flowthrough system, to use strongly diluted samples; this approach minimizes problems of clogging on the gas-permeable membrane.

We have also shown how the temperature of the device can be used to promote the detection of other gaseous species of biochemical interest. Like most other chemical sensors, the catalytic metal-gate MOS structures are not totally selective towards one particular substance; the specificity has to come from a biochemical reaction. The use of several sensors with different patterns of selectivity is, however, quite feasible, because each sensor or sensor array is easily manufactured.

In our exploratory work, we normally use MOS-capacitors heated by an external heater. Practical devices are, however, often based on MOS field-effect transistors, as in the ethylene detector, with a catalytic-metal gate, a heating resistor and a temperature-sensing diode on the same chip.

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Discussion

W. J. Albery, F.R.S. (*Department of Chemistry, Imperial College of Science and Technology, South Kensington, London, U.K.*). I was interested in the results that Professor Lundström showed for the ammonia sensor, where ΔV was plotted against the concentration of ammonia at pH 12.2 and at pH 7.7. The gradients of the two plots differ by a factor of about 2; this difference was attributed to the fact that at pH 12.2 all the ammonia will be present as NH_3, whereas at pH 7.7 it will be protonated. The pK_a of NH_4^+ is 9.5 and therefore at pH 7.7 only 1.6% of the ammonia species will be present as NH_3. The much larger signal seen at pH 7.7 may arise from the displacement of the NH_4^+–NH_3 equilibrium, in the presence of a buffer HB–B, by the removal of NH_3 into the gas phase:

$$NH_4^+ + B \rightleftharpoons HB + NH_3.$$

If this is the case then the response of the system will vary with the kinetics of the equilibrium and hence with the concentration of buffer species. Has the response been studied as a function of buffer concentration?

I. Lundström. This remark is very interesting and points to a phenomenon we have not investigated in a systematic way. The possible effects of a displacement of the $NH_4^+ + NH_3$ equilibrium are demonstrated in figure 7. This is a large difference between the theoretical sensitivity and the measured one at low pH (6–9), which appears to be too large to be explained by the displacement of the NH_4^+–NH_3 equilibrium only. One possible explanation is that the pressure drop across the membrane may 'push' gas molecules through the membrane. Another possibility is the hydrophobicity of the membrane, which leads to adsorption of ammonia molecules in the membrane. The concentration of gaseous ammonia at the membrane, and hence its transport through the membrane, may thus be increased in this way.

J. D. R. Thomas (*Applied Chemistry Department, UWIST, Cardiff, U.K.*). This paper is of great interest to all concerned with monitoring ammonia in biomedical monitoring, especially since the sensors can be employed at ambient temperature. However, Professor Lundström mentions that the sensor has to be kept at 35 °C, that is, above room temperature, to avoid water condensation. This will have the effect of reducing the ammonia level in the sensing volume lying between the gas-permeable membrane and the sensor surface. Can he comment on the extent of the reduced sensitivity arising from this effect?

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I. Lundström. A temperature of 35 °C means only a small change in the absolute temperature (from 298 to 308 K). The changes in both gas concentration above the sensing surface and the sensitivity of the sensor will be very small.

The experiments described in the paper are based on a flow-injection technique, that is, solution at room temperature is continuously flowing on one side of the gas-permeable membrane. This will thus create a temperature gradient in the sensing volume between the sensor and the gas-permeable membrane, marking detailed considerations on temperature effects quite difficult. One of the main advantages of flow-injection techniques is, however, that each sample is treated equally and is compared to a standard sample; this procedure ensures reliable measurements independent of (in this case) a possible loss of sensitivity owing to the increased sensor temperature.

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