行政院國家科學委員會補助專題研究計畫成果報告

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計畫類別:個別型計畫

計畫編號:NSC89-2215-E-009-025

執行期限:88年8月1日-89年10月31日

計畫主持人:趙書琦

本成果報告包括以下應繳交之附件：
　　□赴國外出差或研習，心得報告一份
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　　□國際合作研究計畫國外研究報告書一份

執行單位:國立交通大學電子物理系

中華民國:　　　89年10月26日

WO_3-IrO_2 二極體陣列感測元件之抗干擾特性研究
Interference attenuation in WO_3-IrO_2 diode array microsensors

一、中文摘要

以 WO_3-IrO_2 二極體形成之陣列元件，經 Ta_2O_5 薄膜表面修飾，以及內含葡萄糖氧化酶的聚合物覆蓋後，可於室溫下，依葡萄糖濃度梯度，產生具有空間分佈性的類似二極體般整流特性。實驗中發現，在 Ta_2O_5 的阻擋下，待測環境中的氧化還原物質，例如氧，透過電子傳輸對元件電性進行的干擾，可以被有效的消除。表面修飾後的元件，並依然保有原來的可逆性、再現性，和陣列中二極體正向電流為葡萄糖濃度的函數特性。實驗證明，以這種元件為基礎可以發展並建構耐用的二極體陣列型生物感測器，使用於例如食材的內外葡萄糖濃度感測。獲得的生物分子空間分佈資訊，可實用於食材的新鮮度量測，並且，不受待測環境中含氧量變化的影響。本元件除了其獨具的整流機制所賦予的耐久性特質外，現在，抗干擾效能的增強，更使其邁入了實用的境界，這是在應用傳統二極體建立的微感測器裡無法做到的。
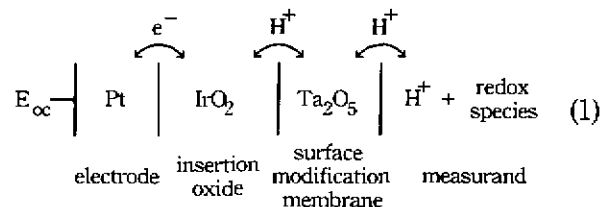
關鍵詞：表面修飾、氧化鉭、酵素二極體陣列、氧化銥、三氧化鎢、新鮮度、微感測器

Abstract

Arrays formed by the WO_3-IrO_2 diode that are surface modified by a Ta_2O_5 membrane and covered by a glucose oxidase containing polymer can exhibit spatially resolved, glucose-dependent, diodelike current rectification at room temperature. Experiments show that the Ta_2O_5 can block electron transport from the redox species, such as oxygen, in the measuring environment. The blocked flow of electrons eliminates redox interference on the device IV characteristics. The Ta_2O_5-modified diode arrays is still capable of generating the reversible and reproducible currents in the forward direction as a function of glucose concentration. Experiments here also provide evidence for the successful use of the Ta_2O_5 surface modification scheme to build more practical biosensors. For example, the modified arrays have been used, in air, to measure the bulk-to-surface glucose concentration gradient in food material. The measurement, without any interference from the changing oxygen content in the atmosphere, is an indication of its freshness. The arrays are thus practical for use as microsensors for the resolution of such food freshness. Their ability to attenuate redox interference, and also their durability as a result of the unique rectification mechanism, are therefore key to their practicality, that is not attainable in previous microsensors built from conventional diodes.

Keywords : Surface, Modification, Ta_2O_5, Enzyme, Diode, Array, IrO_2, WO_3, Freshness, Microsensor
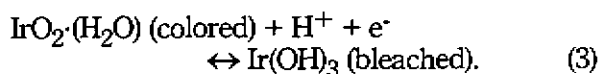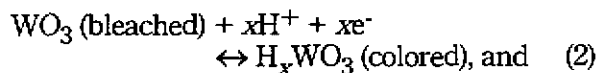
二、緣由與目的

A surface modification scheme employing Ta_2O_5 membrane coated over the WO_3-IrO_2 array to eliminate redox species interference is investigated this year. It is unfortunate that WO_3 and IrO_2 also conduct electronically. Microsensor arrays built with these exceptionally stable insertion oxides will respond strongly to environmental redox species and suffer from interference errors in measurements. The conception of a Ta_2O_5 membrane integrated over the surface of WO_3-IrO_2 is based on the notion that Ta_2O_5 is an ionic conductor with electronic insulation. Use an IrO_2 electrode having its surface modified by Ta_2O_5 as an example, the transport of carriers across the interfaces is:

$$E_{\infty} - \boxed{Pt} \quad \boxed{IrO_2} \quad \boxed{Ta_2O_5} \quad \boxed{H^+} + \begin{array}{c}\text{redox}\\\text{species}\end{array} \quad (1)$$

電極 — Pt electrode; IrO_2 insertion oxide; Ta_2O_5 surface modification membrane; measurand

Clearly, the membrane can mediate the transport of released protons from enzyme

catalyzed reaction of glucose and block the transport of electrons from redox species. Insertion of protons by IrO_2 is not hindered whereas redox interference is eliminated. The conduction of both electrons and protons by insertion oxides preserves the continuity of current[1] without incidence in (1).

The present investigation is an expansion of the previous diode array usable as a microsensor for the spatial resolution of glucose concentration gradient in food materials. It is constructed of single diodes that are operable in liquids at room temperature, Fig. 3. The previous diode device is based on pH-sensitive WO_3 and IrO_2, which interact with H^+ in the reversible redox reactions,[2,3]

$$WO_3 \text{ (bleached)} + xH^+ + xe^-$$
$$\leftrightarrow H_xWO_3 \text{ (colored), and} \quad (2)$$

$$IrO_2 \cdot (H_2O) \text{ (colored)} + H^+ + e^-$$
$$\leftrightarrow Ir(OH)_3 \text{ (bleached).} \quad (3)$$

Both WO_3 and IrO_2 reductions to the conducting H_xWO_3 and insulating $Ir(OH)_3$ occur at more positive electrochemical potentials in acidic media over a range of pH values between ~2-12[4,5] (reactions (2) and (3)). These redox transformations arise due to the insertion of ionic species into the oxides, which can produce large conductance changes.[5,6] The present work is inspired by the earlier discoveries in this laboratory that a bicarbonate (HCO_3^-)-doped, polyvinyl alcohol (PVA) solid polymer matrix interfaced with WO_3 or IrO_2, can respond to CO_2 in terms of resistance or potential across closely spaced microelectrodes at 1 atm and room temperature.[7,8] Despite the advantages of such relatively simple chemical-sensitive resistors and potentiometers, practical use is less attractive due to the lack of a built-in current "turn-on" capacity commonly found in diode and transistor-based microsensors.[9] However, WO_3 and IrO_2 are known to be complementary cathodic and anodic electrochromic materials,[10] and have been used to demonstrate optical attenuation. By connecting WO_3 and IrO_2 in series, as shown in Fig. 3, both oxides become conducting under positive bias in the forward direction and insulating under negative bias in the reverse direction. However, the device function cannot be fully explained by treating the oxides simply as variable series resistors. Since H_xWO_3/WO_3 is cathodically electroactive whereas $Ir(OH)_3/IrO_2$ is

anodically electroactive, the transport of charge across the WO_3/IrO_2 interface is allowed only in the forward direction and forbidden in the opposite direction. The current growth in the forward direction can occur readily via the thermodynamically favored reduction of IrO_2 by the reduced H_xWO_3. In fact, the attenuation of current in the reverse direction is more an indication that the oxidation of $Ir(OH)_3$ by the oxidized WO_3 is thermodynamically not feasible. In our view, this type of rectification governed by thermodynamic free energies is the major advantage for constructing such diodes based on the contact of WO_3 and IrO_2. The devices will be durable since they are made of robust materials. Unlike previous microsensors based on conventional or organic diodes,[9,11] their electrical functions in gases and liquids are not susceptible to the environmental variabilities arising from interfacial or material instability. Previous exploratory experiments in this area have led to our results[12] that the electrical contacts of solid WO_3 and IrO_2 films, sputtered on adjacent Pt electrodes and covered by the polymer blend $PVA \cdot KHCO_3$, can be used to generate diodelike current-voltage outputs that respond to CO_2 gas. We have later reported similar devices that are operable in aqueous solutions. When devices based on the contact of sputtered WO_3 and IrO_2 are covered by a glucose oxidase containing polymer, they can exhibit reversible and reproducible glucose-dependent, diodelike current rectification in liquids at room temperature. We now report a new device, a Ta_2O_5 surface modified diode array based on the serially connected contacts of sputtered WO_3 and IrO_2, Fig. 3.

三、實驗方法

The Pt-pad electrodes in Fig. 3 are fabricated using similar procedures as described earlier.[7] The electrodes are typically 600 μm wide and 1,400 μm long and are separated by a distance of 400 μm. To avoid annealing the IrO_2 due to heating by subsequent WO_3 deposition, sputtering of the WO_3 target (99.99%, Pure Tech) at radiofrequency is carried out first under 20% O_2 in Ar at a total pressure of 90 mTorr, in the same apparatus as used before.[8] A stainless-steel sheet with laser-opened holes or a Si wafer with micromachined V-grooves is used as the deposition mask. The mask and devices on the wafer are pressed onto a heated substrate platen at 676 K.[4] The WO_3 films

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exhibit broad cyclic voltammograms in aqueous 1.0 M $HClO_4$ over the potential range of 0.3 to -0.3 V vs a saturated calomel electrode, as reported previously.[4] Characterization of the films on indium-tin oxide glass shows electrochromism in the visible region and a wide range of absorption change under potential cycling in aqueous 1.0 M H_2SO_4. The change is ~15-85% in transmittance at 650 nm, near the wavelength of maximum difference in the absorption band. The process for derivatizing the Pt electrodes with the amorphous IrO_2 film, next to the WO_3 films (Fig. 3), by the reactive sputtering method and the characterizations have been described earlier.[8]

The deposition of the Ta_2O_5 membranes (~300 Å thick) is carried out by sputtering of a Ta_2O_5 target (99.99%, Pure Tech) under 45% O_2 in Ar at a total pressure of 100 mTorr, in the same apparatus as used before. The integrity of the sputtered Ta_2O_5 membrane as a barrier to electron transport from solution redox species is tested on a flat Pt electrode. Potential cycling of the Pt/Ta_2O_5 electrode in aqueous 10 mM $K_3Fe(CN)_6$ in 0.10 M KCl electrolyte has yielded no discernible cyclic voltammetric waves that are characteristic of the redox species even at the highest current sensitivity of the potentiostat (BAS CV-50W, Bioanalytical Systems). The prepared WO_3, IrO_2 and Ta_2O_5 films are robust and adhere strongly to the Pt surface. No difficulty such as peeling has been encountered throughout the course of our experiment. After deposition of the oxide films, electrical contact of individual Pt electrodes is made using Ag epoxy, which is later encapsulated using insulating epoxy. Optical microscopy reveals that uniform bleaching and coloring can occur in each of the oxide films on Pt. As shown in Fig. 3, each film has only one underlying Pt electrode. This forces the potential drop to be confined to the WO_3/IrO_2 interfaces, leaving the individual films on Pt approximately at equipotential and of the same color.

In the next step, the whole device active area is covered by the insolublized polyvinyl alcohol (PVA, average molecular weight = 1.33 × 10^5 g/mole, Polysciences) with immobilized glucose oxidase (GOD, Type VII from *Aspergillus niger*, Sigma) (Fig. 3). This newly developed enzyme layer, PVA-GOD, is used for creating a glucose-modulated pH environment specifically for the diode. The procedure for the insolublization of PVA and the immobilization of GOD is performed separately in two sequential steps. The first

step involves the photoinitiated crosslinking of PVA. The reason for the choice of PVA over the other polymers is due to its solubility in polar solvents. The second step involves the preparation of immobilized enzyme derivatives through the use of the bifunctional reagent glutaraldehyde in the presence of the protein albumin.[13] Since intramolecular crosslinking is favored by low enzyme and reagent concentrations, it is common practice to increase the overall protein concentration with the use of a second "carrier" or "sacrificial" protein. Since control of the pH, ionic strength, temperature, and reaction time are also factors that affect the degree of intramolecular crosslinking and the mechanical strength of the insoluble polymer, determination of the optimal conditions for the retention of enzyme activity is a trial-and-error process. Before insolublization, GOD (1,200 units or 10 mg) and 10 mg of Bovine Serum Albumin (BSA, RIA grade, Fraction V, Sigma) is dissolved in 0.2 ml of a photopolymer solution (15 mg 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone, BA, Aldrich, 20 mg PVA and 0.2 ml H_2O). No other supporting electrolyte has been added since the enzyme layer functions only as a source or sink for H^+, and not as an electrolyte to carry ionic current. The photopolymer solution (100 µl) is placed with a microsyringe over the exposed active area of the diode array as shown in Fig. 3. After exposure to UV irradiation (365 nm, 100 W, 90 s), the treated diode array is immersed in aqueous 25% glutaraldehyde solution for 5 min at room temperature to form the insoluble glucose oxidase enzyme layer. After washing with deionized H_2O, the diode array is immersed in 0.1 M glycine for 15 min to terminate the cross-linking reaction by glutaraldehyde. The insolublized enzyme layers form light-yellow films that are highly adhesive to WO_3 and IrO_2. No peeling of the films has been encountered throughout the electrical experiments. Enzyme layers insolublized with the same procedure separately on glass slides have been immersed in H_2O at 90 °C for at least 1 week without any visible sign of disintegration.

The potential of the $Pt/IrO_2/Ta_2O_5$ electrode in (1) as a function of pH has been measured by use of a Keithley 617 electrometer. A 10 mM Britton-Robinson buffer[14] solution, initially at pH ~2.0, is titrated with KOH in steps to generate the pH buffer solutions, reaching a final pH value of ~12, Figs. 1 and 2. The current-voltage

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sweeps are generated using a Keithley 236 source-measure unit (Fig. 4). The diode array packaged into integral flow cells are used, as earlier,[15] to allow exposure to glucose solutions with different concentrations, Fig. 4. Low concentration aqueous phosphate (10 mM, pH 7.0) buffer solution is used as the carrier solution for glucose to facilitate pH change in PVA·GOD. The conditions of glucose or the interfering redox solution concentrations carried in the buffer solution are generated by ratioing the flowrate of microprocessor-controlled infusion pumps (Sage 341B) as shown in Fig. 4. Bovine meat samples are first incubated at 37°C for 24-48 hours in air to induce depletion of glucose by micro-organisms at the surface. A vertical incision is made to half the sample, which is then carefully laid onto the microsensor with the incised surface pressed against the array, Fig. 8.

四、結果與討論

Experiments show that the surface modification by Ta_2O_5 membrane has not affected the electrochemistry of the IrO_2, Fig. 1. Potential developed on a Ta_2O_5 modified IrO_2 electrode toward H^+ still exhibits Nernstian behavior as before (E^o is ~0.726 V vs Ag/AgCl, and ΔE^o is -59.7 mV per ΔpH). When O_2 is purged through the solution every other hours as a source of redox interference, Fig. 2, the Ta_2O_5 modified IrO_2 electrode under test still shows long-term potential stability toward H^+ without any change or drift. This shows that Ta_2O_5 membrane can block electron transfer from solution redox species in (1) as expected.

The glucose-dependent current-voltage characteristic of a single Ta_2O_5 modified diode on the array based on WO_3 and IrO_2 are shown in Fig. 5. The glucose creates a pH-regulated environment for the diodes (Fig. 3), through its pH-lowering effect, as previously reported.[16] It equilibrates in the glucose oxidase-blended polymer PVA·GOD according to the reaction

$$Glucose + 1/2O_2 \xrightarrow{\quad GOD \quad} Gluconate^- + H^+. \quad (4)$$
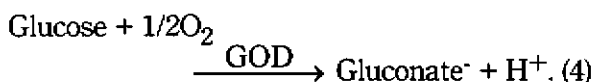
As results of the glucose-dependent experiment in Fig. 5 show, diodes based on WO_3 and IrO_2 undergo a current decrease in the forward direction when the glucose concentration is increased within the range of

0.2-10 mM in phosphate buffer solutions. This result is consistent with the WO_3 and IrO_2 redox processes (reactions (2) and (3)). The electrochemical potentials in both reactions become more positive in more acidic environments. This shift can be considered to result from the pH-dependent changes in the potential drop across the Helmholtz layer at the surface of both oxides, as stated earlier.[7,8] On the potential scale, the shifting of redox potentials towards the positive region renders reductions more favorable than oxidations under fixed driving force. When the bias voltage is unchanged, the current passing through a less fully oxidized IrO_2 in series with a more fully reduced H_xWO_3 in the forward direction still shows a loss due to the current-limiting effect exerted by the higher resistance (Fig. 5). The fact that the experimentally determined pH sensitivity for our IrO_2 (~58 mV/ΔpH) in aqueous solutions,[8] is higher than that for our WO_3 (~52 to 54 mV/ΔpH), is relevant and should be noted. This means that up to ~5 mV/ΔpH positive displacement of IrO_2 potential in excess of that of WO_3 can be expected in the more acidic diode environments created by glucose, that should further contribute to the current loss in the forward direction. The current loss in Fig. 5 in the forward direction is also confirmed by the observed progressive coloration in H_xWO_3 and discoloration in IrO_2 as the pH is lowered in PVA·GOD by glucose solution under a fixed positive bias.

The Ta_2O_5 modified diodes on our new array are markedly durable and reproducible. Repeated voltage sweeps under each glucose concentration in Fig. 5 give almost identical current-voltage signals. Neither the rectification behavior nor the current amplitudes show signs of degradation. Under a fixed 1.6 V bias, switching the solution between 0.2 to 10 mM glucose in phosphate buffer solution turns the diodes on the new array to the "on" and "off" states with ~-5 to -25 μA and 0 μA current change in the forward direction, respectively (Fig. 6). The current switchings are free of interference from injected O_2 solutions, reversible and reproducible and can be carried out for all diodes on the array without significant deterioration for >48 h.

The sensitivity or dynamic range of the Ta_2O_5 modified diodes on the array for glucose concentration is found to be dependent on the pH value of the carrier buffer solution used. Glucose is capable of inducing a larger pH drop in PVA·GOD when

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the buffer solution is more basic. As shown in Fig. 7, the Ta_2O_5 modified diodes on the array have a range valid for glucose concentrations between ~0.2-10 mM. Separate experiments show that the diodes can actually respond to glucose from 50 mM down to the 0.01 mM concentration range with still acceptable signal-to-noise ratio. It has also been determined that the glucose-induced current change in the forward direction is proportional to the logarithm of the glucose concentration to which PVA·GOD is exposed, as shown in the inset in Fig. 7.

The Ta_2O_5 modified diode array capable of resolving the spatial distribution of glucose concentration in food material in uncontrolled air atmosphere with changing oxygen content is demonstrated next. A bovine tissue incubated for bacterial growth (controlled at 37 °C in air for 24 hours) at the surface is placed over the array, Fig. 8. The diodes on the array are biased individually and generate the spatially resolved rectifying I-V characteristics, Fig. 9. The detected forward currents at 1.6 V plotted in the inside in Fig. 9 indicate bulk-to-surface blood glucose distribution due to bacterial consumption of glucose at the tissue surface. Since glucose is progressively depleted at the surface as time goes by, such result can be used for building a quantitative database on sensing the freshness[17] in food materials. The new diode array therefore has a potential for practical use as a food freshness microsensor in air.

This work reveals that oxide-based microsensors are durable and that, in principle, many oxides with widely varying properties can be used to fabricate sensing devices with special electrical characteristics, including even the capacity for interference attenuation. Work is already under way, in this laboratory, to incorporate this diode in other microsensors. Other details of the device can be found in Ref. 18.[18]

五、計畫成果自評

　　本研究內容與原計畫相符程度約 95%，達成預期目標包括創新元件之發現、其理論之推導和模式建立、實驗原型、系統之建立、及人才培育等。本研究成果之學術、應用價值高，可發表於國外期刊和申請專利，本研究主要發現包括以獨具的整流機制，建構感測用二極體元件，並以表面修飾法阻擋干擾源，賦予元件關鍵的耐久性和抗干擾特質。以這種元件為基礎，可以發展其他的二極體陣列微感測器，使用於各種生物液體中，並獲得

生物分子的空間分佈資訊，向感測資訊影像化邁進。這是應用傳統二極體建立的微感測器無法做到的。

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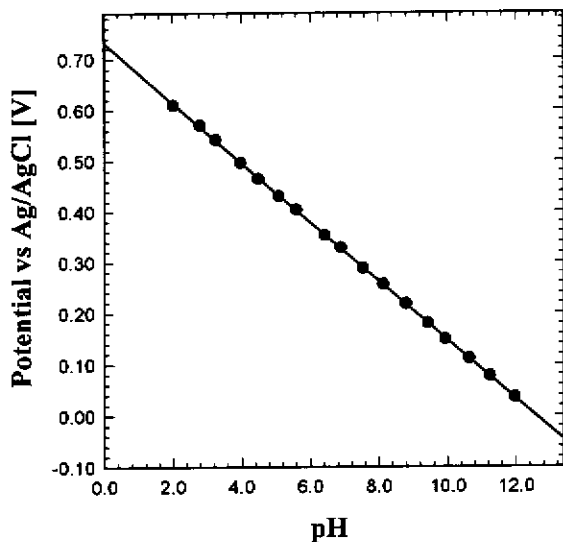18) C. C. Hsu and C. C. Lin: M. S. Thesis, Dept. Electrophys., NCTU, 1999.

七、圖表

Fig. 1. Nernstian potential developed on a Ta_2O_5 modified IrO_2 electrode toward H^+ (E^o is ~0.726 V vs Ag/AgCl, ΔE^o is -59.7 mV per ΔpH), showing that the surface modification has not affected the electrochemistry of IrO_2.

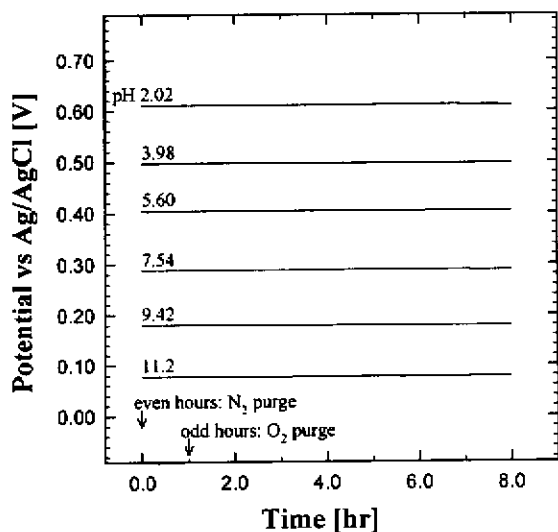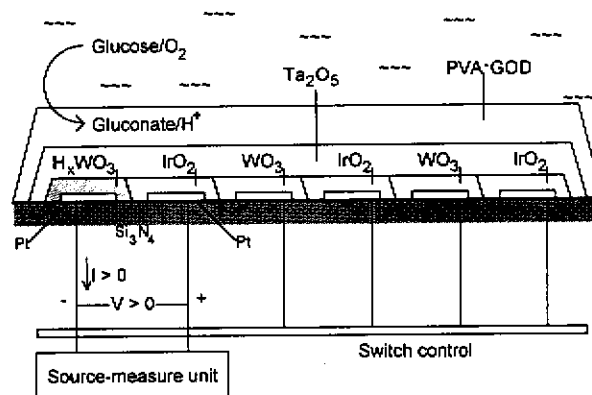Fig. 3. Scheme showing the Ta_2O_5 surface modified diode array and one forward-biased diode based on the contact of WO_3 and IrO_2 on Si under glucose modulation in H_2O.
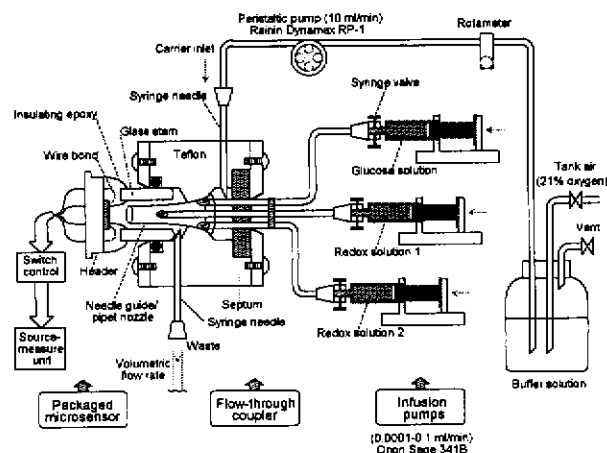

Fig. 2. Long-term potential stability test of a Ta_2O_5 modified IrO_2 electrode toward H^+. As a source of redox interference, O_2 is purged through the solution every other hours to show that the potential of IrO_2 is not affected.

Fig. 4. Flow-injection apparatus for the generation of interfering redox solution concentrations and the diode array packaging.
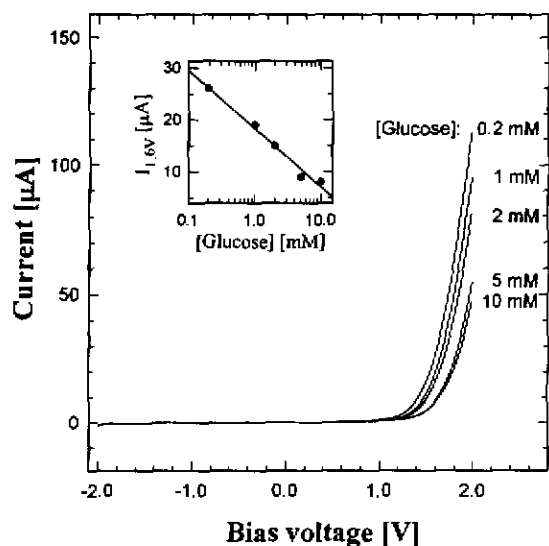
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Fig. 5. Typical single diode current-voltage characteristics on a Ta_2O_5 surface modified, 5-diode array under the modulation of glucose concentrations in phosphate buffer solution at 298 K.
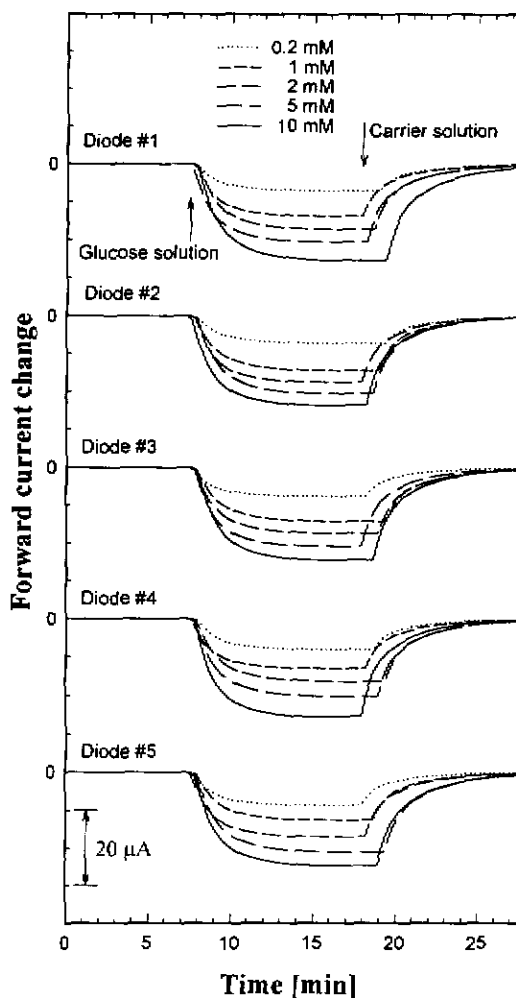
Fig. 6. Typical interference-free electrical response of a Ta_2O_5 surface modified, 5-diode array. The glucose concentration-induced switching behaviors of each diode are produced under a forward bias of 1.6 V at 298 K. The carrier flows are 10 mM aqueous phosphate buffer solution adjusted to pH 7.0. The source of redox interference is a burst of 0.2 ml O_2-saturated H_2O, injected every minute into the carrier flow.
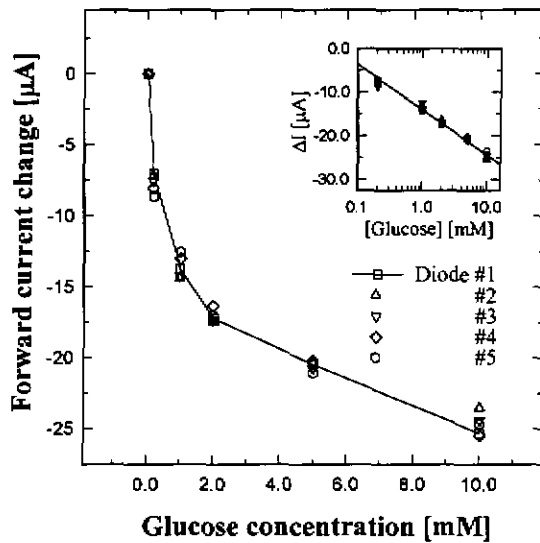
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Fig. 7. Typical current changes in the forward direction (1.6 V bias) of a Ta_2O_5 surface modified, 5-diode array induced by glucose concentrations in phosphate buffer solution at pH 7.0 and 298 K.
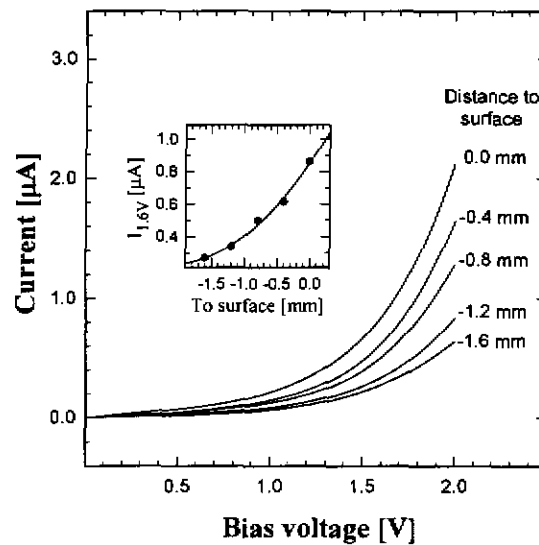

Fig. 9. Typical current in the forward direction of a Ta_2O_5 surface modified, 5-diode array induced by the glucose concentration gradient in an incubated meat sample at 298 K in uncontrolled air atmosphere. (Diodes are labeled by their distance to the incubation surface, Fig. 8).
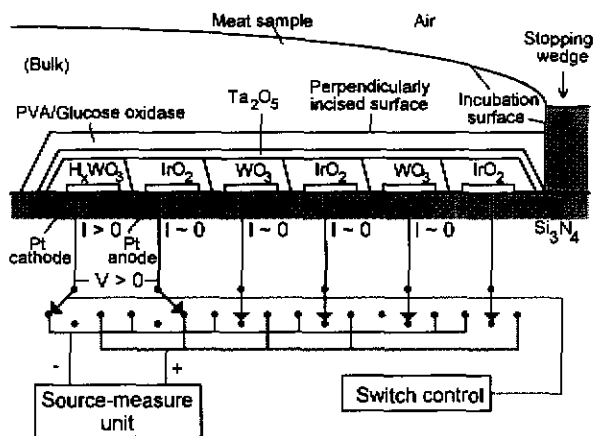

Fig. 8. The Ta_2O_5 surface modified diode array and one forward-biased diode based on the contact of WO_3 and IrO_2 on Si and the incubated meat sample measurement scheme.

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