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Dynamic switching rate in enzymatic WO$_3$ - IrO$_2$ diode array microsensors for glucose

一、中文摘要

以 WO$_3$ - IrO$_2$ 之類材料組成的食物新鮮度感測器，會因為二極體陣列所使用之 Pt 電極間距較大，而形成很大的 RC 時間延遲，造成元件的感測切換反應速率遲緩。本計畫係以一種溝渠影蔭鍍膜技術，製作出具有次微米間距之 Pt 電極陣列，大幅降低元件的 RC 時間延遲，有效的提昇了元件的反應速率，使其邁入更實用化的境界。

關鍵詞：溝渠影蔭鍍膜、反應速率、二極體、微感測器、新鮮、WO$_3$、IrO$_2$

Abstract

In food freshness microsensors built from WO$_3$ - IrO$_2$, slow electrical signals can arise due to large inter-electrode gaps in the Pt array. Particularly, the RC-time delay associated with these large gaps will severely limit the device’s switching rate. Through a newly developed Pt patterning technique termed “trench-shadow deposition,” the inter-electrode gap has been reduced to ~0.4 µm. These submicron gaps can diminish the RC-time delay, achieving enhancement of the device’s switching rate to that of a few seconds.

Keywords : Trench-shadow Deposition, Switching Rate, Diode, Microsensor, Freshness, WO$_3$, IrO$_2$

二、緣由與目的

This investigation calls for the enhancement of the dynamic switching rate in the present device through reducing the inter-electrode gap in the Pt array. Due to their exceptional stability, insertion oxides (such as WO$_3$, IrO$_2$ and Ni(OH)$_2$) have attracted wide research interests. Unfortunately, in the case of food freshness microsensors built from insertion oxides, past incorporation of large gaps between Pt electrodes (~200-400 µm) introduced large resistance and capacitance components in the device. This RC-time delay has significantly limited the device's switching rate:

![Diagram 1](image1)

To alleviate the problem, we propose to replace the previously used Pt array with a new design, (2), featuring submicron gaps (~0.4 µm). As shown in (2), we also placed WO$_3$ and IrO$_2$ in indirect contact via a bridging Pt electrode. This measure will avoid difficulties in the alignment of the WO$_3$ and IrO$_2$ films within a ~0.4 µm-wide gap.

The present investigation is an expansion on the previous microsensor diode that is usable in resolving 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,

\[
\begin{align*}
\text{WO}_3 \text{(bleached)} + x\text{H}^+ + xe^- & \leftrightarrow H_x\text{WO}_3 \text{(colored), and} \\
\text{IrO}_2\cdot(H_2O) \text{(colored)} + H^+ + e^- & \leftrightarrow \text{Ir(OH)}_3 \text{(bleached)}.
\end{align*}
\]

Both WO$_3$ and IrO$_2$ reductions to the conducting $H_x$WO$_3$ and insulating Ir(OH)$_3$ occur at more positive electrochemical
potentials in acidic media over a range of pH values between ~2-12.3,4) (reactions (3) and (4)). These redox transformations arise due to the insertion of ionic species into the oxides, which can produce large conductance changes.4,5) 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.6,11) 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" function commonly found in diode and transistor-based microsensors.7) However, WO$_3$ and IrO$_2$ are known to be complementary cathodic and anodic electrochromic materials.8) 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$_2$WO$_3$/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$_2$WO$_3$. 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 infeasible.

For this reason, rectification in -WO$_3$ - IrO$_2$ -, (1), should not be affected as we opt for -WO$_3$ - Pt - IrO$_2$ -, (2), since it is governed by thermodynamic free energies. As reported previously,5) our experiments show exactly that. The WO$_3$ and IrO$_2$ films, when contacted via a third Pt electrode in between can still be used to generate the same rectification as before. The results also show that geometrical areas involved in the direct overlap of WO$_3$ and IrO$_2$ affect the magnitude of the current, but the overall rectification is not changed.

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,7,10) 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 results11) that the electrical contacts of solid WO$_3$ and IrO$_2$ films, sputtered on adjacent Pt electrodes and covered by the polymer blend PVA-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. A surface modification scheme employing the bi-layer Ta$_2$O$_5$/Al$_2$O$_3$ membrane coated over the WO$_3$ - IrO$_2$ array to eliminate redox species interference was investigated last year. We now report a new device, a submicron-gap microsensor diode array based on the serially connected contacts of sputtered WO$_3$ and IrO$_2$ via Pt, Figs. 3, 5 and 9.

三、実験方法

The Pt-pad electrodes in Fig. 3 can no longer be fabricated by use of the lift-off procedure described earlier.6) "Lift-off" requires a relatively thick photoresist. From our past experiments, resolution worsening due to thick photoresist limits the inter-electrode separation to > 4 µm. Since Pt is a robust material that forms essentially ohmic contacts with WO$_3$ and IrO$_2$,9) it is deemed irreplaceable. The patterning problem involving Pt is solved by employing a technique we have termed “trench-shadow deposition.” First, trenches are cut into Si$_3$N$_4$ using reactive-ion etching. The Pt deposition is then carried out at an oblique angle, utilizing the well-formed steps generated in the trenches as shadow masks, Fig. 4. This technique has been used to yield Pt arrays separated by submicron gaps ~0.4 µm wide, as illustrated in Fig. 5. The dimensions of Pt electrode themselves are typically 200-300 µm wide and 3,000 µm long.

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.\textsuperscript{9} 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.\textsuperscript{3} The deposited WO$_3$ films 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.\textsuperscript{3} 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$_2$SO$_4$. The change is $\pm$15-85% in transmittance at 650 nm, near the wavelength of maximum difference in the absorption band. The process for derivatizing the Pt electrodes with the amorphous IrO$_2$ film, next to the WO$_3$ films (Fig. 3), by the reactive sputtering method and the characterizations have been described earlier.\textsuperscript{9}

The deposition of Ta$_2$O$_5$ and Al$_2$O$_3$ membranes (~200-300 Å thick) is carried out by sputtering of a Ta$_2$O$_5$ or Al$_2$O$_3$ 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$_2$O$_5$ or Al$_2$O$_3$ membrane as a barrier to electron transport from solution redox species is tested on a flat Pt electrode. Potential cycling of the Pt/Ta$_2$O$_5$ or Pt/Al$_2$O$_3$ electrode in aqueous 10 mM K$_3$Fe(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$_2$ Ta$_2$O$_5$ and Al$_2$O$_3$ 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 Au epoxy, which is later encapsulated using insulating epoxy. Optical microscopy reveals that uniform bleaching and coloring can occur in each of the insertion oxide films on Pt. As shown in Fig. 3, each film has most of its lower surface in contact with the underlying Pt electrode. This forces the potential drop to be confined only to the WO$_3$ and IrO$_2$ within the two submicron gaps across the Pt/WO$_3$/Pt/IrO$_2$/Pt 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 with covalently immobilized glucose oxidase (GOD, Type VII from \textit{Aspergillus niger}, Sigma) (Fig. 3). This enzyme layer is used for creating a glucose-modulated pH environment specifically for the diode. The procedure for the covalent immobilization of GOD is performed separately in two sequential steps:\textsuperscript{12,13}

\begin{align*}
\text{Al}_2\text{O}_3 & \quad \text{Cl} \quad \text{N} \quad \text{Cl} \quad \text{Cl} \\
\text{H}_2\text{N} & \quad \text{GOD} \\
\text{Al}_2\text{O}_3 & \quad \text{N} \quad \text{Cl} \quad \text{HN} \\
& \quad \text{GOD}
\end{align*}

The first step involves the activation of the exposed Al$_2$O$_3$ surface (Fig. 3) by cyanuric chloride. The reason for the choice is due to the simplicity of the cyanuric chloride reaction over the other reagents. The second step involves the preparation of immobilized enzyme derivatives via covalent coupling in the presence of glucose.\textsuperscript{12,13} In order to protect the active site, it is common practice to carry out this reaction in the presence of the substrate. The reaction also needs to be performed under mild conditions. Low temperature, low ionic strength and pH in the physiological range are important factors in preventing the enzyme from denaturing. The determination of the optimal conditions for the maximum retention of enzyme activity is a trial-and-error process. Before activation, the exposed Al$_2$O$_3$ surface is pretreated in aqueous 0.1 M KOH for 60 s to grow hydroxyl groups. After washing with deionized H$_2$O and dried, the surface is reacted with 0.05 M cyanuric chloride in benzene for 2 h at 50°C. Before covalent coupling, GOD (1,200 units or 10 mg) is dissolved in 0.2 ml aqueous 0.1 M phosphate solution (pH 8.0) saturated with glucose. 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 enzyme solution (100 µl) is placed with a microsyringe over the exposed active area of
the diode array (Fig. 3) and chilled at 4°C for 12 h to form the non-leaching glucose oxidase enzyme layer. This covalently immobilized enzyme layer forms light-yellow films that are highly adhesive to Al₂O₃. No peeling of the films has been encountered throughout the electrical experiments. Enzyme layers immobilized with the same procedure separately on glass slides have been immersed in H₂O at 37°C for at least 4 months without any visible sign of disintegration.

The switching current of the submicron-gap microsensor diode, reaching equilibrium as a function of time in Fig. 1, has been measured by use of a Keithley 236 source-measure unit, Fig. 3. The glucose solution flows are generated by switching between the streams of two digital peristaltic pumps (Rainin Dynamax RP-1). Aqueous 0.01 M phosphate buffer (pH 7.0) is used as the carrier solution in both streams but only one contains 0.2 mM glucose. The glucose injection experiment in Fig. 2 is performed on a flow-injection apparatus. This FIA includes a combination of one syringe pump and one peristaltic pump.¹⁴) The diode arrays packaged into integral flow cells are used, as earlier,¹⁴) to allow exposure to glucose solutions with different concentrations. The conditions of glucose concentrations carried in the buffer solutions are generated by proportioning the flowrate of microprocessor-controlled infusion pumps (Sage 341B). Low concentration aqueous phosphate (0.01 M, pH 7.0) buffer solution is always used as the carrier for glucose to facilitate pH change in immobilized GOD.

Bovine meat samples are first incubated at 37°C for 24-48 h in air to induce depletion of glucose by microorganisms 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. 9.

四、結果與討論

Experiments show that reducing the inter-electrode gap to ~0.4 μm has largely enhanced the dynamic switching rate of the device. In Fig. 1, when a current switching experiment for the submicron-gap microsensor diode is performed, the device under test shows a fast response toward glucose concentration. The equilibration time is ~5 s, which is faster than before by a factor of ~10². This result has also enabled us to run glucose flow-injection experiments for the first time on the microsensors built from WO₃ and IrO₂, Fig. 2. The fast current transients induced by glucose injections have a pulse rise-time on the order of ~1 s. Finally, we have improved the switching rate of our device to fully comply with the specification set by the sensor industry.

Experiments also show that neither reducing the inter-electrode gap to ~0.4 μm nor adding the bridging Pt electrode, Fig. 3, has affected the rectification of WO₃ - IrO₂. Fig. 6 shows the glucose-dependent current-voltage characteristics of 1 single submicron-gap diode within an array of 5 diodes based on WO₃ and IrO₂. The glucose creates a pH-regulated environment for the diodes (Fig. 3), through its pH-lowering effect, as previously reported.¹⁵) Glucose equilibration catalyzed by the immobilized glucose oxidase proceeds according to the reaction

\[
\text{Glucose} + \frac{1}{2}\text{O}_2 \xrightarrow{\text{GOD}} \text{Glucuronate}^- + \text{H}^+. \tag{6}
\]

As results of the glucose-dependent experiment in Fig. 6 show, diodes based on WO₃ and IrO₂ 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 WO₃ and IrO₂ redox processes (reactions (3) and (4)).

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.⁶,⁹) 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 IrO₂ in series with a more fully reduced H₂WO₃ in the forward direction still shows a loss due to the current-limiting effect exerted by the higher resistance (Fig. 6). The fact that the experimentally determined pH sensitivity for our IrO₂ (~58 mV/ΔpH) in aqueous solutions,⁹) is higher than that for our WO₃ (~52 to 54 mV/ΔpH), is relevant and should be noted. This means that up to ~5 mV/ΔpH positive displacement of IrO₂ potential in excess of that of WO₃ 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. 6 in the forward direction is also confirmed by the observed progressive coloration in HWO3 and discoloration in IrO2 as the pH is lowered in the immobilized GOD layer by glucose solution under a fixed positive bias.

The new submicron-gap diodes are markedly durable and reproducible. Repeated voltage sweeps under each glucose concentration in Fig. 6 give almost identical current-voltage signals. Neither the rectification behavior nor the current amplitudes show signs of degradation. Figure 5 shows the layout and the diode numbering scheme of the new array. Under a fixed 1.6 V bias, switching the solution between 0.2 to 10 mM glucose in phosphate buffer turns the new diodes in the 5-diode array to the "on" and "off" states (Fig. 7). They exhibit ~8 to 18 mA and 0 mA current change in the forward direction, respectively (Fig. 8). The current switchings are 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 submicron-gap 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 immobilized GOD layer when the buffer solution is more basic. As shown in Fig. 8, the submicron-gap diodes have a valid range for glucose concentrations between ~0.2-10 mM. Separate experiments show that the diodes can actually respond to glucose from 60 to 0.01 mM with 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 immobilized GOD is exposed, as shown in the inset in Fig. 8.

Next, experiments are performed to demonstrate that the new diode array is capable of resolving the 1-dimensional spatial distribution of glucose concentration in food material. A bovine tissue incubated for bacterial growth at the surface (controlled at 37°C in air for 24 h) is placed over the array, Fig. 9. The diodes on the array, each biased individually, show spatially resolved rectifying I-V characteristics, Fig. 10. The detected forward currents at 1.6 V bias in Fig. 10 produce a 1-dimensional, 5-point plot of the blood glucose distribution that has been generated by the surface-to-bulk bacterial consumption near the tissue surface. Since glucose is progressively depleted from the surface as a function of time, this result can be used to build a quantitative database on the sensing of freshness15 in food materials. In particular, the new linear diode array has a potential for practical use as a microsensor for acquiring the "freshness plots" of foods in several seconds.

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. In addition, functions can be readily built-in to enhance the switching rate. Work is already under way, in this laboratory, to incorporate this diode function in some other microsensors.

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，所用實驗原型和系統之建立，以及人才培育等。本研究成果具有學術、應用價值，可發表於國際期刊和申請專利。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，並以溝渠影蔭鍍膜技術，製作關鍵的次微米間距電極陣列，賦予元件快速反應的特質。以這種元件為基礎，可以發展出其他二極體的陣列感測器，能用於各種生物液體中，獲得生物分子的空間分佈資訊，並向感測資訊快速化邁進。這是應用傳統二極體建立感測器無法做到的。

六、參考文獻

7) J. W. Gardner: Microsensors, Principles and Applications (John Wiley, Chichester,
17) 荒井林

Fig. 1. A current switching experiment for the submicron-gap microsensor diode, showing fast on-off response (equilibration time ~5 s) induced by glucose solution flows (0.2 mM). The forward bias was 1.6 V and the flow conditions were produced by switching between the streams of two peristaltic pumps.^[14]^[14]

Fig. 2. A flow-injection experiment for the submicron-gap microsensor diode, showing fast current transients (pulse rise-time ~1 s) induced by repeated glucose injections (0.3 ml, 0.2 mM).

Fig. 3. Scheme showing the new submicron-gap microsensor diodes under test, with one forward-biased enzymatic diode based on the contact of WO$_3$ and IrO$_2$ on Si in the array under glucose modulation in H$_2$O.
Fig. 4. The “trench-shadowing” method for the deposition of Pt generating electrode arrays with sub-micron gaps.

Fig. 5. Typical layout and numbering scheme of Pt electrodes and the diode array based on the contact of WO₃ and IrO₂ on Si.

Fig. 6. Typical single diode current-voltage characteristics in a 5-diode submicron-gap microsensor array (Fig. 3) under the modulation of glucose concentrations in phosphate buffer at 298 K.
Fig. 7. Typical electrical response of a 5-diode, submicron-gap microsensor 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.

Fig. 8. Typical current changes in the forward direction (1.6 V bias) of a 5-diode, submicron-gap microsensor array induced by glucose concentrations in phosphate buffer at pH 7.0 and 298 K.

Fig. 9. The sub-micron gap enzymatic diode array, one forward-biased diode based on the contact of WO$_3$ and IrO$_2$ on Si, and the incubated meat sample measurement scheme.
Fig. 10. Typical spatially resolved current in the forward direction (1.6 V bias) of a 5-diode, submicron-gap microsensor 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. 9).