Doping dependence of the adsorption of proteins on proton-doped WO$_3$ film

V Asimakopoulos$^1$, C Mastichiadis$^2$, S Kakabakos$^2$ and D Davazoglou$^1$

NCSR “Demokritos”, $^1$Institute of Microelectronics, $^2$Institute of Radioisotopes and Radiodiagnostic Products, POB 60228, 153 10 Agia Paraskevi, Attiki Greece.

E-mail: bassimak@freemail.gr

Abstract. Configurations consisting of electrochromic (EC) strips of the kind SnO$_2$:F/WO$_3$ were fabricated on glass substrates with dimensions 5X5 cm$^2$. For the fabrication, SnO$_2$:F strips were formed first with standard lithography and etching. A tungsten film was subsequently deposited on the patterned substrate by low pressure chemical vapor deposition from W(CO)$_6$ vapors, which was removed from the uncovered (by SnO$_2$:F) parts of the glass substrate and oxidized to WO$_3$ in O$_2$ at 550 °C. These EC strips were colored at various degrees in an aqueous solution of NaCl using potentials varying from 0 to 5 V. After coloration strips were coated with a protein solution with pH varying between 6 and 9, then with a fluorescent antibody, washed and dried. The adsorption of proteins on EC strips was studied by fluorescent microscopy. It was found that rabbit gamma globulins were adsorbed on uncolored strips and as soon as the coloration voltage increased, adsorption decreased. Adsorption was also slightly dependent on pH and increases as the pH decreases.

1. Introduction

Biosensors represent a powerful tool in analytical science. Multi-analyte approach is an advance in the biosensor technology that makes them more suitable for proteomics and genomics compared to conventional, classical analytical techniques [1]. Selective patterning of biomolecules is a crucial step towards the development of multi-analyte biosensors [2]. Various strategies such as photolithographic or liquid dispensing techniques have been employed to achieve this goal [2, 3]. The recent trend in the field of biosensing device fabrication is the development of integrated optoelectronic microsystems, where the sensing process is based on waveguiding of the light emitted either by an appropriate source or label [4].

In this report, we describe a device for biosensing that is based on electrochromic “sandwiches” of the kind fluorine-doped SnO$_2$ films/tungsten oxide films fabricated on glass substrates using standard lithographic-etching techniques [5]. Electrochromic “sandwiches” of this kind are colored when in contact with an electrolyte and a positive voltage is applied to the last. Then, the positive ions present in the electrolyte are inserted in the WO$_3$ film and, simultaneously, electrons are injected from the SnO$_2$ into the WO$_3$ film (SnO$_2$ when doped with F is a transparent and conductive material). The above injection of charges causes the change of color of the WO$_3$ film, which originally is transparent and is colored blue. The intensity of coloration depends on the nature of the electrolyte and the magnitude of the applied voltage, which, for the case of aqueous electrolytes is of the order of 1-2 V. When the voltage is reversed, the positive ions return in the electrolyte, the electrons in the SnO$_2$ and
the WO₃ film returns to its initial transparent state. From the above description it is seen that WO₃ films may be doped with positive ions at various concentrations, reversibly and easily and doping is accompanied by coloration. This reversible doping is probably related to the surface properties of the material. Hence, in this work it was studied whether selective patterning of biomolecules could be accomplished on WO₃ films colored at various degrees, i.e., doped at various concentrations. If biomolecules respond selectively to the doping state of the WO₃ film, then a spatial separation of the various biomolecules present in a solution could be achieved driven by voltages of the order of 1-2 V and the corresponding concentrations could be measured using a measuring technique. Moreover, since the phenomena described so far are reversible, more than one measurement could be made in every case to confirm the obtained results.

2. Experimental
For the fabrication of devices, SnO₂:F covered glass substrates with dimensions 5X5 cm² were patterned with AZ 5214™ photoresist using a photomask to form strips with dimensions of 5 mm and separated by 5 mm spaces, and etched. W films were then deposited on the patterned substrates in a horizontal, radiatively heated CVD reactor at low pressure (0.1 Torr) at 400 °C, from decomposition of W (CO)₆ vapors [6]. These films were patterned using the same photomask as for the SnO₂ films and etched with an aqueous solution containing tetra-methyl ammonium hydroxide (TMAH). After patterning the W films were oxidized to WO₃ in a horizontal furnace at temperatures varying between 550 and 650 °C and at various times dependent on film thickness. The edge of the SnO₂ strips was left uncovered by the WO₃ films so a different voltage could be applied to the strips with a flat connector and a ribbon cable. The coloration of strips was made in an aqueous NaCl solution with voltages varying between 0 (uncolored) and 5 V with various increments.

After coloration all strips were coated with a 10 µg/mL rabbit gamma globulins (RgG) solution prepared either in 50 mM carbonate buffer, pH 9.2, or in 50 mM phosphate buffer, pH 6.0, for 1h at room temperature (RT). Then, the strips were washed with distilled water and 50 mM phosphate buffer, pH 7.5, containing 0.9% NaCl and blocked with 0.1 M NaHCO₃ solution containing 1% (w/v) Bovine Serum Albumin (BSA) for 1h at RT. Then were incubated for 2 h at RT with a solution of 20 µg/mL fluorescein-labeled anti-rabbit IgG in 0.15 M Tris-HCl, pH 8.25, containing 0.5% (w/v) BSA, 0.05% (w/v) bovine gamma globulins and 0.2% (w/v) thimerosal. Finally, the strips were washed with the same washing buffer containing 0.05% (v/v) Tween-20 and the fluorescence intensity was measured using an epifluorescence microscope, accommodated with an excitation filter at 485 nm and an emission filter at 532 nm. The bioreaction between adsorbed biotinylated-BSA and the conjugate of streptavidin with R-Phycoerythrin was also tested as a model.

3. Results and Discussion
Fluorescent microscopy measurements have shown that for samples on which BSA was deposited there was no difference in signal between EC strips, colored or uncolored and glass substrate. For samples on which RgG was deposited similar measurements have revealed differences between EC strips and glass. Fluorescent microscopy photos taken on colored and bleached strip at various potentials and on the glass substrate next to them are shown in figure 1. It can be observed that for the colored (doped) strip the fluorescence intensity is almost equal on the strip and on the glass substrate indicating that RgG are present at equal concentrations on the strip and on the substrate. On the contrary, the fluorescence intensity measured on the bleached strip is much higher than that on the substrate, which means that RgG is preferentially stabilized on the bleached strip. Additionally, the photoluminescence intensity seems to be dependent on the coloration voltage and drops with it while it increases with that of the bleaching voltage. Similar observations are made when comparing neutral (not colored, virgin) strips and colored ones. The corresponding results are shown in figure 2 where the photoluminescence intensity of an EC strip (number 2 in figure) colored at 3.5 V is compared with that measured on two virgin strips (number 3 and 4) and a bleached one (number 5) at
Figure 1. Photos depicting the adsorption of RgG (assayed with fluorescein-labeled antirabbit IgG antibody) on colored and bleached stripes using fluorescence microscopy. On the left photos, a colored stripe at high voltage (upper) and one at lower voltage (lower) are shown. On the right photos a bleached strip at high voltage (upper) and one bleached at lower voltage (lower) are shown. For the colored strips the photoluminescence intensity is almost equal on the strip and on the glass substrate (left photos). The photoluminescence intensity on the bleached strips is much higher than on the glass (right photos).

Figure 2. Photoluminescence density measured at two different pHs on a colored (doped) (2), two uncoloured (3, 4) and a colored and bleached (5) strip. The error bars were obtained from many measurements taken at various locations on each photograph.

a voltage of 3.5 V. RgG solutions prepared at pH 6 and 9.2 were used. It can be observed that in all cases proteins are preferentially adsorbed on the virgin and the bleached strips. A dependence of the photoluminescence on the pH can also be observed; a lower pH seems to favor adsorption on glass and EC strips.

Hydrophilicity measurements taken with the method of contact angle on undoped and colored EC strips have shown that it does not depend on doping. Therefore, the observed phenomena have a pure electronic nature. At equilibrium a Fermi level is established in the virgin (or bleached) WO₃, which is
a semiconductor, and in the protein solution. When the two come in contact the two Fermi levels are aligned and during this alignment process the proteins stick on the surface of the WO₃. This means that before contact a difference existed between the Fermi levels in the virgin WO₃ and the solution. When the WO₃ is doped with H⁺, the Fermi level moves towards higher values (the conduction band). Now, during contact proteins are not moving toward the doped WO₃ surface meaning that before contact the two Fermi levels are almost aligned. A similar phenomenon is observed when pH in the solution changes: When it decreases, which means that the Fermi level in the solution increases, proteins move towards the EC strip because the difference between the two Fermi levels increases.

The adsorption of RgG on bleached EC strips is seen in figure 3 where the photoluminescence measured on virgin and bleached strips is compared. It can be observed that in spite of the fact that there are always charges remaining into the WO₃ film after bleaching [7] the photoluminescence is comparable to that on virgin strips and in sometimes even higher than it. This could be explained assuming that the Fermi level at the surface is slightly different than that in the bulk of the WO₃ strip.

![Figure 3](image-url)

**Figure 3.** Photoluminescence density measured on three bleached and three virgin strips on which RgG was deposited.

4. Conclusions

Electrochromic (EC) strips of the kind SnO₂: F/WO₃ were fabricated on glass substrates which were colored at various degrees and bleached. It was shown that RgG were preferentially adsorbed on virgin and bleached strips than on colored ones and that this is an electrostatic effect. Adsorption depends on the difference between Fermi levels in the WO₃ films and the protein solution, which may be influenced by the doping (coloration) of the strips and the pH of the solution.

5. References

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