# Urea-EnFET biosensor based on pH-EGFET using FTO and ITO support films

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Urea sensors are applied in fields as food industry, pharmaceutical, environmental protection, fertilizers and especially urease clinical analysis and also in renal therapy[1]. Pathologies such as renal insufficiency, hyperpyrexia, leukemia, diarrheal diseases, diabetes mellitus and hyperthyroidism may be diagnosed by causing a variation in urea concentration in blood, serum or urine [2]. The normal level of urea in serum is 15–45 mg/dl (2.5–7.5 mM). At levels above 180 mg/dl (30 mM) renal insufficiency is critical and hemodialysis is required [3]. In this study the urea-enzymatic field effect transistors (EnFETs) was investigated based on extended-gate field effect transistors (EGFETs) using commercial FTO (fluorine doped tin oxide) and ITO (indium doped tin oxide). EGFETs were separated into two parts: the sensitive film and the MOSFET. To fabricate the EnFET, urease was immobilized directly onto the surface of the sensing films, which were connected by a wire into a commercial CD4007UB MOSFET. In order to fully retain biological activity, proteins should be properly immobilized onto surfaces without affecting their three dimension conformation and biological function. Immobilization techniques are mainly based on three mechanisms: physical adsorption, covalent bonding and bioaffinity immobilization. The covalent bonding method was choosen and Glutaraldehyde (GA), which is a dual function group, was used to cross-link amine bonds on urease and the supports surface as the protocol described by Pijanowska & Torbicz [4]. Urea hydrolysis reaction by urease is as follow:

$$CO(NH_2)_2 + 3H_2O \longrightarrow CO_2 + 2NH_4^+ + 2OH^-$$

The reaction products cause an increase in pH on surface's micro-region. However, the measurements are performed in a phosphate buffer solution (PBS), then a gradient between the sensing gate area and the sample solution allow the pH-based EGFET to indirectly determine the urea concentrations. Therefore the sensors' sensing properties are dependent on the performance of the enzymatic layer and also on the sensing film of the EGFETs. To evaluate the pH sensing properties of the films, commercial pH buffer solutions from pH 2 to pH 12 were used for calibration curves. The sensitivities obtained were 54.10 mV/pH for FTO and 53.56 mv/pH for ITO. The enzymatic layer property depends of the catalytic enzyme activity. For immobilized enzymes the ideal environment ambient is quite different than that for free enzymes. It would be determined by each sensitive membrane. An optimal measurement environment is pH 6 and 5 mM phosphate buffer solution [6-8]. Using this environmental conditions, in range of $10^{-2}$ to 300 mM (-0.5 to -5 $pC_{urea}$), the urea sensitivity for FTO was 8.92 $\mu A/pC_{urea}$ (Fig. 1) and 85.33 $\mu A/pC_{urea}$, for ITO (Fig. 2).

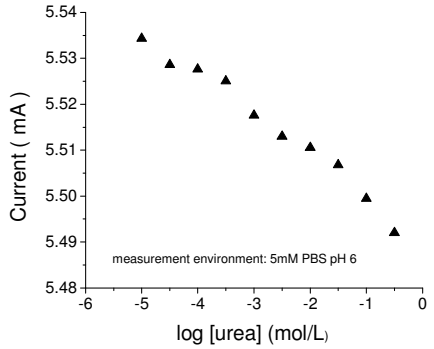


*Fig. 1 Calibration of urea biosensor with FTO.*

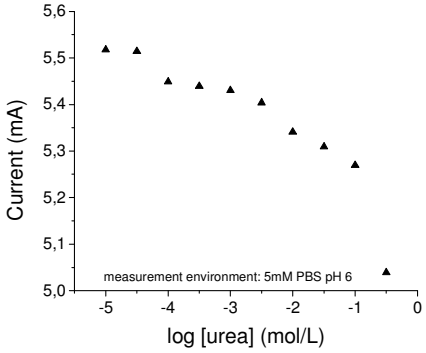


*Fig. 2 Calibration of urea biosensor with ITO.*

In conclusion we will present the sensing properties of FTO and ITO for urea detection and discuss the influence of environment conditions such as different pHs, buffers capacity and ionic strength. This work was supported by FAPESP, CNPq and CAPES.

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