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Application of original assemblies of polyelectrolytes, urease and electrodeposited polyaniline as sensitive films of potentiometric urea biosensors

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ABSTRACT

Original assemblies were prepared for use as sensitive films of potentiometric enzyme urea sensors, and compared to identify the more efficient structure with respect to stability. These films included electrodeposited polyaniline, used as transducer, urease, used as catalyst, and biocompatible polyelectrolytes, used as a matrix to preserve the integrity of the enzyme in the sensitive film. Two kinds of assemblies were done: the first one consisted in the adsorption of urease onto a polyaniline film followed by the adsorption of a chitosan-carboxymethylpullulan multilayer film, while the second one consisted in the adsorption of a urease-chitosan multilayer film onto an electrodeposited polyaniline film. The morphological features and growth of these assemblies were characterized by scanning electron microscopy and quartz crystal microbalance, respectively. This allowed us to demonstrate that the assemblies are successfully formed onto the electrodes of the sensors. The potentiometric responses of both assemblies were then measured as a function of urea concentration using a home-made portable potentiostat. The electrochemical response of resulting sensors was fast and sensitive for both types of assemblies, but the stability in time was much better for the films obtained from alternative adsorption of urease and chitosan onto a layer of urease adsorbed over electrodeposited polyaniline.

1. Introduction

Enzyme-based sensors have attracted much attention in recent years due to their potential applications in clinical diagnostics [1], pharmaceutical [2], environmental analysis [3], as well as food industry [4]. Enzyme sensors are particularly interesting since enzymes interact with specific substrates leading to a very selective response, whereas other sensors generally suffer from a lack of selectivity in presence of interferents. Amongst all analytes that could be detected by enzyme sensors, urea has received considerable attention. Indeed, the detection of urea is of interest: i) in clinical diagnosis since it is the main reliable index of kidney diseases [5], ii) in environmental analysis since it is a nitrogenous fertilizer that causes environmental stress [6], and since urea levels can be used to track adulteration in dairy milk or assess the nutritional program of lactating dairy cows [4].

To develop efficient urea sensors, it is important to optimize three points that are generally considered as crucial in the elaboration of an enzyme-based sensor: the detection technique, the sensitive material and the immobilization of the enzyme. Thus, many various detection techniques have already been successfully used to obtain optical [7,8], piezoelectric [9,10], conductimetric [11,12], impedimetric [13], amperometric [14–16] or potentiometric urea sensors [4,17–19]. Amongst these detection modes, potentiometry is one of the most attractive due to its easy fabrication, effectiveness and simplicity. However, to obtain an efficient potentiometric enzyme sensor, it is necessary to use a sensitive material that efficiently converts the presence of urea into a variation of electrochemical potential. In this aim, materials sensitive to pH changes are generally used since the hydrolysis of urea, in presence of urease, leads to the apparition of alkaline products (Eq. (1)) that increase the pH, and

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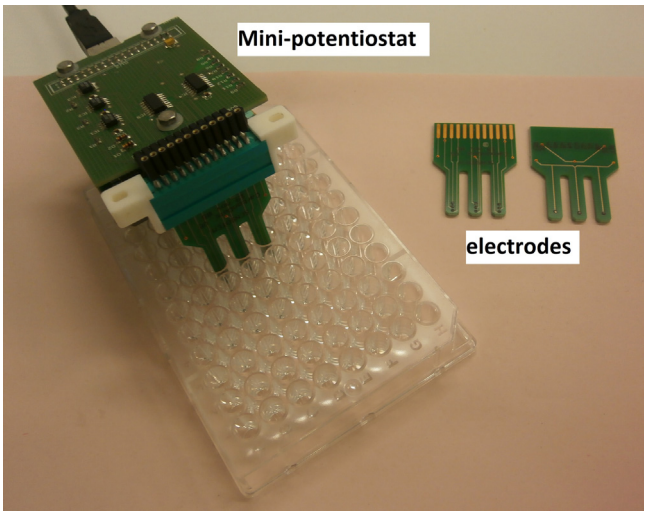

Fig. 1. Pictures of the home-made portable potentiostat and of its electrodes.

subsequently modify the potential measured by the transducer according to Nernst equation.

$$H_2N - CO - NH_2 + 3H_2O \overset{\text{urease}}{\rightarrow} 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

Thus, some oxide metals have been used as pH sensitive materials and incorporated into urea sensors [20,21]. However, the vast majority of potentiometric urea sensors are based on electrodeposited conducting polymer films such as polypyrrole [22–24], polyaniline [25] or polyparaphenylenediamine [26]. Indeed, these polymer materials have several advantages for this application including tunable intrinsic conductivity, presence of pH-sensitive functional groups in their backbone such as amino groups that can be easily protonated and deprotonated, easiness of (electro) deposition and possibility to precisely control their thickness. The last crucial step in the development of an enzyme-based sensor is the effective immobilization of an enzyme on the electrode surface. When electrodeposited polymers are used, immobilization of the enzyme is generally performed by physical adsorption [18], covalent binding between the polymer and the analyte through the carbodiimide coupling reaction [27] or entrapment in the film during electropolymerization [18]. Each of these immobilization methods possesses its advantages and drawbacks. Physical adsorption leads to sensors with high sensitivity and poor lifetime due to enzyme leakage. Covalent binding improves the lifetime of the sensor but generally results in a decrease of its sensitivity. Finally, entrapment could result in a loss of the enzyme activity during the electropolymerization. Taking into account these limitations, some attempts have been recently done to perform an efficient immobilization of urease using original immobilization strategies. A possibility to optimize enzyme immobilization is the use of organic cyclic molecules such as calixarenes or cyclodextrines which form a complex with urease or the use of polyelectrolyte complexes and polyelectrolyte multilayer films to entrap urease at the vicinity of the transducer. The first strategy was successfully used by Hamilton et al. who developed a highly sensitive urea sensor incorporating urease into a polypyrrole film in one simple electropolymerization step, using a sulfonated-β-cyclodextrin dopant [28]. This led to the formation of an inclusion complex between urease and a sulfonated-β-cyclodextrin host in an aqueous solution, and subsequently to an efficient immobilization of the enzyme. The second strategy was used by Osaka, Komaba et al. [29,30] to prepare polypyrrole with a polyion complex as a composite film. The polyion enzyme complex was cast over polypyrrole by the successive deposition of polyacrylate or polystyrenesulfonate solutions, of an urease solution, and of a polylysine solution. The addition of these polyelectrolytes resulted in urea sensors with rapid, sensitive and stable response signal. In more recent works, our groups have also developed urea sensors based on conducting polymers and polyelectrolytes films [31,32]. The active layer of these sensors was prepared by the sequential deposition of a polyaniline film, a polyelectrolyte multilayer film composed of chitosan (CHI) and carboxymethylpullulan (CMP) used as anchoring layer, and of urease grafted over the anchoring layer. The combination of electrodeposited polyaniline, acting as transducer, and polyelectrolyte multilayer film, acting as protective layer of the enzyme, resulted in sensitive and stable sensors.

Following these works, the study presented here aims at developing new urea sensors based on original assemblies combining three components: a conducting electrodeposited polymer, an enzyme and a polyelectrolyte multilayer film (PEM). More specifically, the composition and the location of these components in the assembly were varied to determine their respective impact on the sensor response. Indeed, a first assembly consisted in urease deposited onto a PANI film and covered by a

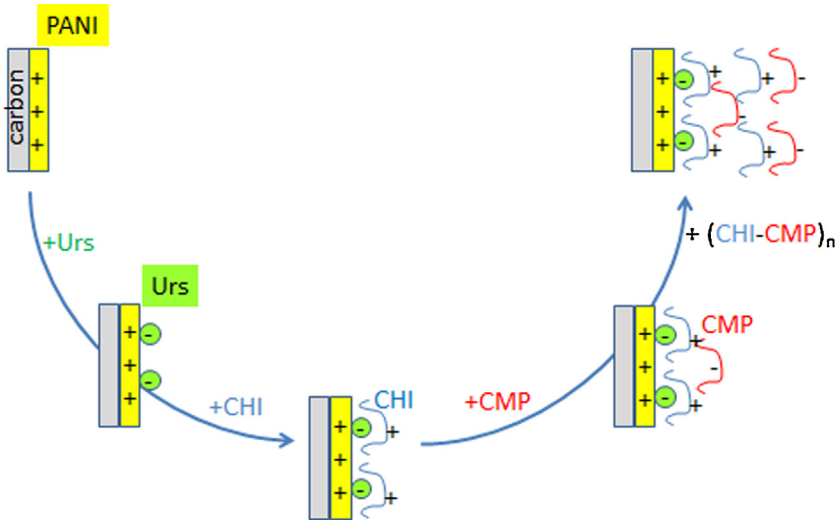

Fig. 2. Schematic description of the preparation of PANI-Urs-$(CHI\text{-}CMP)_n$ assemblies.