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Potentiometric detection of chemical species by spin-assisted assembly of vanadium pentoxide nanorods

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ABSTRACT

Vanadium pentoxide nanorods (V_2O_5-nanorods) and poly(allylamine hydrochloride) (PAH) were assembled onto gold-coated substrates via spin-assisted assembly technique and used as a chemically sensitive electrodes. PAH/V_2O_5-nanorods detected H^+ ions (pH) with sensitivity between 52–61 mV/pH (close to Nernstian theoretical value). As a proof-of-concept, a urea biosensor has been developed, upon immobilization of urease enzyme on PAH/V_2O_5-nanorods electrodes. The biosensor could detect urea in a 0.05–5 mM dynamic range. The spin-assisted assembly technique enables the combination of different materials in a simple way and offers advantages for the construction of functional electrodes.

1. Introduction

Metal-oxides obtained in one dimensional (1D) nanostructures, including nanowires, nanotubes and nanorods, display enhanced physical and chemical properties in comparison to their similar bulk structures [1]. Particularly, vanadium pentoxide (V_2O_5) has emerged as a promising 1D nanostructure for ion sensing, specially H^+ ions (pH-sensing), because of the V_2O_5 property of either donating or accepting protons [2]. In fact, the monitoring of the pH value is very important from a biochemical point of view. Some biochemical processes are related to the pH value or result in the release or consumption of protons, as it occurs in many enzymatic reactions [3]. We compared the pH-sensing properties of different 1D $V_2O_5{\cdot}nH_2O$ nanostructures synthesized in a one-step hydrothermal route [2]. All nanostructures showed pH sensitivity close to the theoretical value expected by Nernst equation (59.15 mV pH^{-1}), which indicates the pH sensitivity was not dependent on the morphology and structure of 1D $V_2O_5{\cdot}nH_2O$ [2].

Unlike most metal oxides, 1D $V_2O_5{\cdot}nH_2O$ nanostructures can be dispersed in water, which enables their manipulation in the form of thin nanostructured films or composites, unusually desirable for applications in cost-effective sensors. The spin-assisted assembly technique represents an effective methodology for the combination of various materials and formation of nanocomposites onto different types and sizes of substrates [4–6]. Moreover, the combination of alternating layers of materials enables the functionalization of substrates with appropriate functional groups in the last layer, as in the case of covalent immobilization of biomolecules [7].

This study explored the feasibility of the spin-assisted assembly technique for the construction of pH-sensitive electrodes by the combination of V_2O_5 nanorods (denoted as V_2O_5-nanorods) and poly(allylamine hydrochloride) (PAH) on gold-coated substrates. As a proof-of-concept, PAH/V_2O_5-nanorod electrodes were functionalized with urease enzyme and applied as a potentiometric urea biosensor. PAH is a cationic polyelectrolyte commonly used in self-assembly. It bears amino groups suitable for the binding of biomolecules while V_2O_5-nanorods act as a pH-sensitive material. The proposed system is easy to construct and can detect pH and urea with high sensitivity in a 0.05–5 mM dynamic range.

2. Materials and methods

1D V_2O_5 nanorods were synthesized by a hydrothermal method at 200 °C for 24 h. The synthesis and a complete characterization of V_2O_5-nanorods is described in details elsewhere [8,9]. We denoted the as-synthesized samples as V_2O_5, without

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H_2O molecules, because in this synthesis conditions, V_2O_5-nanorods is composed mainly of adsorbed H_2O molecules [8,9]. Poly(allylamine hydrochloride) average Mw ~ 17,500 were purchased from Sigma–Aldrich.

As previously introduced by Cho and Chiarelli, the assembly technique used to build up PAH/V_2O_5-nanorods was based on the alternating deposition of PAH and V_2O_5-nanorods using a spin coater [4,5]. Fifty microliters of PAH (0.5 mg mL^{-1}) and V_2O_5-nanorods (1 mg mL^{-1}) aqueous solutions were alternately deposited onto gold-covered substrates with approximately 23 mm^2 of active area (Fig. S1). The solutions were prepared using ultra-pure water (Milli-Q source, 18.3 MΩ cm) without pH adjustment and with no addition of salt or additive compounds. In these conditions, the pH of both solutions was ca. 5. The deposited layers were allowed to dry for 1 min at 3000 rpm. This procedure was repeated for a desired number of layers, that varied from 1 to 5 layers of PAH and V_2O_5 nanorods. The assembly of the PAH/V_2O_5-nanorods was monitored via UV–vis absorbance (Hitachi U-2001 spectrophotometer) after the materials had been deposited onto quartz slides.

Urease (EC 3.5.1.5, 109 U/mg) from Jack beans, serum bovine albumin (BSA), glutaraldehyde (GA) and urea were purchased from Sigma–Aldrich and used without purification. The enzyme immobilization method involved the cross linkage of urease on three bilayers of PAH/V_2O_5-nanorods. The last layer was formed by PAH and contained exposed amine groups. Ten microliters of a mixture containing urease (50 μL, 40 mg mL^{-1}), BSA (50 μL, 20 mg mL^{-1}) and GA (18 μL, 2.5% in phosphate buffer (pH 7.4) were dropped on the PAH/V_2O_5-nanorods [7].

We have constructed an easy and cheap potentiometer readout circuit based on an instrumentation amplifier operating as a unity gain buffer. The system can measure the open circuit potential between a work electrode and a reference electrode [2,7]. PAH/V_2O_5-nanorods were connected to the input pin of the amplifier and an Ag/AgCl electrode was utilized as a reference electrode. The schematic diagram of the sensor/biosensor is shown in Fig. S2 and the complete circuit configuration can be seen in details in our previous papers [2,7]. All pH or urea sensing measurements were performed at 25 °C.

Scanning electron microscopy (SEM) images were obtained in the Inspect F50 equipment (Fei, The Netherlands).

3. Results and discussion

3.1. Characterization of the PAH/V_2O_5-nanorods assembly

Fig. 1a shows a comparison of the normalized absorbance spectra of an aqueous solution containing only V_2O_5-nanorods and the as-prepared PAH/V_2O_5-nanorods formed by five layers of each material. The two bands observed around 407 nm and 257 nm for the V_2O_5-nanorods solution are associated with charge transfer transitions of an electron from the π orbital of the oxygen atom to the d level of vanadium for the vanadium electronic configuration in the oxidized state [10,11]. The PAH/V_2O_5-nanorods spectrum shows a small blue shift, which may be related to environmental effects due to the formation of a nanocomposite, i.e., interaction of V_2O_5-nanorods with PAH [12]. Fig. 1b shows the absorbance spectra of each deposition cycle of the PAH/V_2O_5-nanorods. The linear dependence (inset of Fig. 1b) of the absorbance indicates that a same amount of V_2O_5-nanorods is deposited at each deposition cycle. The regular deposition of the film may be related to electrostatic interactions between NH_3^+ terminal groups from PAH and OH^- from V_2O_5-nanorods. However, a contribution of H-bonding for the formation of the PAH/V_2O_5-nanorod layers, as reported by

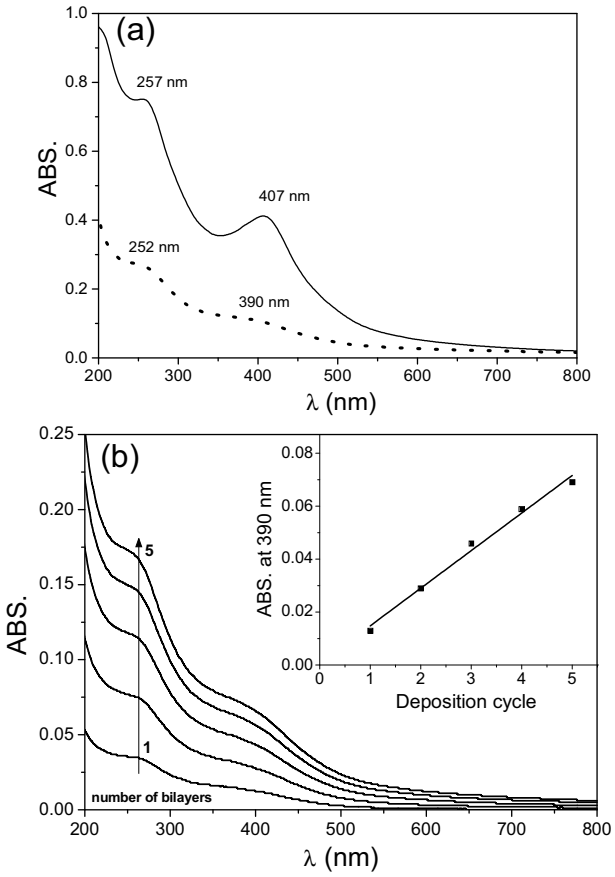

Fig. 1. (a) Absorbance spectra of an aqueous solution of V2O5 nanorods (0.01 mg.mL-1) (solid line) and a PAH/V2O5-nanorods assembly (dotted line). (b) Relationship between film absorbance at 390 nm and number of deposition cycles of PAH/V2O5-nanorod bilayers.

Ferreira et. al., in the multilayered films of V_2O_5 and a conducting polymer that also has terminal amino groups is expected [12].

Fig. S3 shows the scanning electron microscopy (SEM) images of the samples containing 3 and 5 bilayers of PAH/V_2O_5. Due to morphologic characteristics of V_2O_5 sample, i.e., nanorods, it is not expected a preferential orientation of the nanoparticles after deposition onto the PAH film. As it can be seen on Fig. S3, V_2O_5 nanorods are well dispersed onto the PAH surface. The V_2O_5 nanorods presented a diameter around 55 nm and a length varying from 1 to 5 μm. From electron microscope images, we estimated that the thickness of each deposited bilayer was around 150 nm.

3.2. PAH/V_2O_5-nanorods as a pH sensor

The pH-sensitivity of PAH/V_2O_5-nanorods electrode was analyzed through the immersion of the nanocomposite grown in Au into different buffer solutions (from pH 2 to 12) and the time-dependent output potential of the system was measured along time. The typical dynamic pH-response of PAH/V_2O_5-nanorods, shown in Fig. 2, is similar to that observed for V_2O_5-nanorods deposited directly onto Au-coated substrates and addressed in our previous paper [2]. It is important to note that there is a drift in output voltage. As shown in Fig. 2, the drift was: 38 mV; −16 mV, −21 mV, −25 mV, −29 mV; −24 mV; and 17 mV for pH values of: 2; 4; 6; 7; 8; 10 and 12, respectively. Drift voltage is a very common behavior and is inherent for pH sensitive electrodes composed of metal oxides, such as V_2O_5-nanorods, because metal oxides participate in redox reactions with the ions in the electrolyte [13]. The

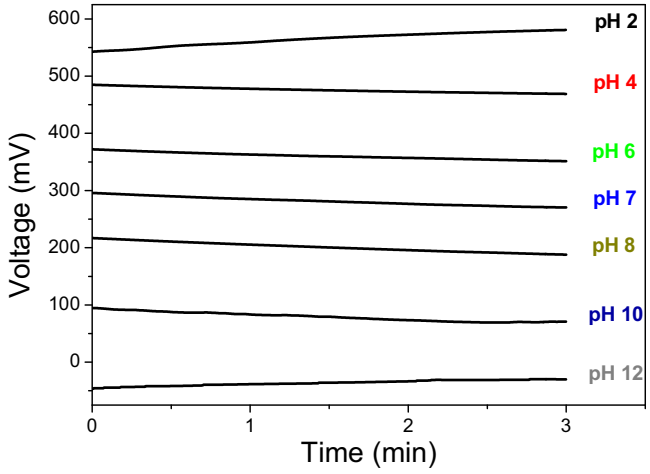

Fig. 2. Dynamic response of PAH/V_2O_5-nanorods (3 bilayers) toward pH variations.

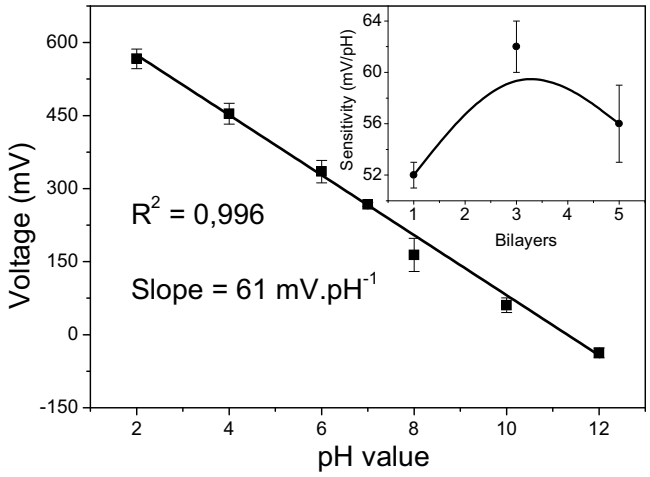

Fig. 3. pH-sensitivity of PAH/V_2O_5-nanorods (3 bilayers). Inset: sensitivity as a function of number of PAH/V_2O_5-nanorod cycles deposited.

drift voltage is higher in the first minutes tending to stabilize over time.

Fig. 3 shows the pH-sensitivity for an electrode formed by three bilayers of PAH/V_2O_5-nanorods and sensitivity as a function of the number of deposited cycles is shown in the inset of Fig. 3. The pH-sensitivity was estimated by reading the voltage value at 3 min in pH dynamic measurements (Fig. 2). The pH-sensitivity is higher for films composed of three bilayers and close to both the value found for the free V_2O_5 nanorods and the theoretical value predicted by Nernst equation (ca. 59 m mV/pH at 25 °C) [2]. An Au electrode without modifications shows pH-sensitivity of ca. 30 mV/pH under similar conditions [14,15]. The results suggest the pH-sensitivity of the nanocomposite is governed by V_2O_5-nanorods in the last layer. However, multilayered nanocomposites are expected to be permeable to the H^+ diffusion and the V_2O_5 inner layers probably contribute to the pH sensitivity [7,16].

From Fig. S4, it is clear that the pH-sensitivity is the same for voltages observed at the beginning or at the end of each measurements (after 3 min), indicating that the system works well even with the drift voltage. In addition, the results are in good agreement with Fig. 3, which the error bars corresponding to three different PAH/V_2O_5-nanorods electrodes. In addition, we performed five successive measurements in a 5-day interval for PAH/V_2O_5-nanorods electrode (3 bilayers). The results are shown in Fig. S5. As it can be seen, the pH sensibility did not vary significantly, being within the experimental errors.

Our results are in agreement with findings of previous reports on V_2O_5 as a pH-sensing material and even better in terms of the film sensitivity and stability [17,18] through pH variations. V_2O_5 synthesized via sol–gel was used as a pH-sensitive membrane in extended gate field-effect transistor (EGFET) apparatus [17]. This system showed 58 mV/pH sensitivity. However, under strongly alkaline conditions (i.e. pH 12), it is not stable [17]. Mixed oxides containing V_2O_5 and WO_3 were also utilized as a pH-sensitive material in a same SEGFET sensor and displayed a super-Nernstian behaviour (68 mv/pH of sensitivity) [19]. On the other hand, V_2O_5/hexadecylamine membranes showed supra-Nernstian pH-sensitivity of 38 mV/pH [18].

3.3. PAH/V_2O_5-nanorods as a urea biosensor

The high pH-sensitivity and stability showed by PAH/V_2O_5-nanorod electrodes suggest their application in biosensors, since H^+ or OH^- ions are generated in various reactions catalyzed by enzymes. As a proof-of-concept, we have functionalized a 3-bilayer PAH/V_2O_5-nanorods film with urease enzyme for urea detection. In the biosensor configuration, the last layer was formed by PAH, which contains NH_3^+ groups. These groups are activated by glutaraldehyde, a common cross-linking agent used in protein immobilization [20]. According to the literature, glutaraldehyde reacts with various functional groups of proteins, such as amine, thiol, phenol, and imidazole [20]. In the present case, we believe that glutaraldehyde reacts with amine groups of lysine residues presents in urease enzyme, as reported by Crespilho et al. in a similar strategy for enzyme immobilization [21]. Glutaraldehyde reacts with free amino groups present in the urease forming a protein network between the enzyme molecules and the support, which also contains NH_3^+ functional groups [21].

Urease is an oxidoreductase enzyme that catalyzes the hydrolysis of urea into ammonia according to the following reaction [22]:

$$CO(NH_2)_2 + 3H_2O \overset{urease}{\rightarrow} CO_2 + 2NH_4^+ + 2OH^-$$

this reaction is expected to be monitored by a pH sensor due to local changes in pH induced by the OH^- ions produced in the urea catalytic transformation. The versatility of the spin-assisted assembly technique enables the surface activation with NH_3^+ groups for the immobilization of enzymes (by a simple choice of material of the last layer, in this case, the PAH polymer). Metal oxides, as V_2O_5, require a previous surface modification with silane agents for the immobilization of biomolecules [23]. Therefore, our methodology is an alternative approach for an easy enzyme immobilization in metal oxides, as it avoids the use of non-volatile organic solvents commonly employed in silanization processes.In order to reduce the drift voltage influence, biosensor measurements were carried out after the system had reached a stable baseline. The time required to achieve a stable baseline differed slightly from electrode to electrode. Fig. S6 shows a typical curve of output voltage stabilization for PAH/V_2O_5-nanorods/urease electrodes.

Fig. 4 shows a typical response of PAH/V_2O_5-nanorods acting as a biosensor when successive aliquots of urea (mM) are added into the buffer solution (pH 7.5, 10 mM). A decrease in the H^+ concentration occurs near PAH/V_2O_5-nanorods/urease electrode due to acid-base equilibrium with the products of the enzymatic reaction. Specifically, hydroxyl OH^- induces a decrease in the biosensor response. Considering the response time as time needed for reaction saturation, after urea injection, it is not larger than 80 s. The calibration curve for the PAH/V_2O_5-nanorods biosensor is shown in Fig. 5, where the error bars correspond to three different PAH/V_2O_5-

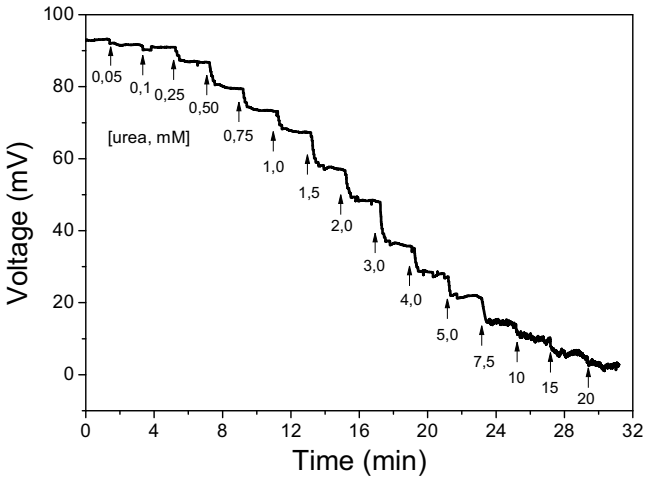

Fig. 4. Typical response of PAH/V_2O_5-nanorods as a biosensor against different additions of urea to the solution measured. Measurement conditions: 10 mM phosphate buffer, pH 7.5.

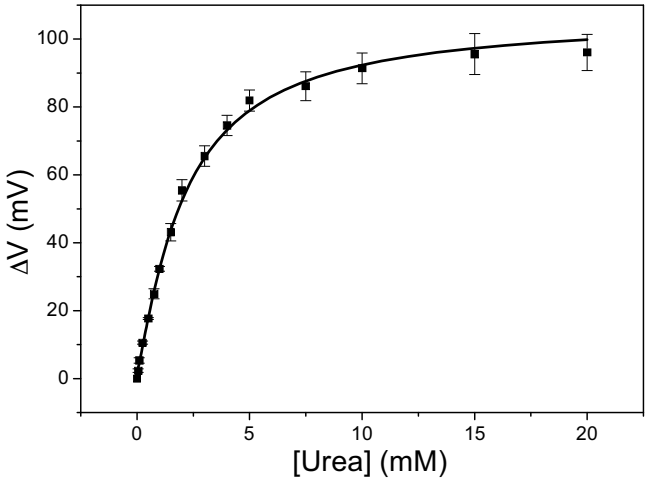

Fig. 5. Calibration curve of PAH/V_2O_5-nanorods as a urea biosensor. Measurement conditions: 10 mM phosphate buffer, pH 7.5.

nanorods/urease electrodes. The proposed biosensor was evaluated in a wide range of urea concentrations (from 0.05 up to 20 mM). The normal urea concentration in human blood ranges from 2.5 to 7.5 mM. A human serum sample, for example, needs to be diluted before use in our system. Analyzing the calibration curve, the proposed biosensor is able to measure urea concentrations of 0.05 mm (the lowest detected concentration) to 5 mM, where the calibration curve tends to saturation. From 5 mM urea concentration, Fig. 5 shows that the signal is no longer distinguishable considering the experimental error. Therefore, in practical applications, a previous sample treatment would be necessary for adaptation with our biosensor. Its good biosensing performance can be directly related to the immobilization process (an oriented position of the enzyme without loss of its activity) along the high pH-sensitivity of PAH/V_2O_5-nanorods.

Although PAH/V_2O_5-nanorods acting as a biosensor had been proposed here as proof-of-principle, some comparisons deserve to be made with others potentiometric biosensors for the detection of urea. We highlight the operational range and the response time of recent potentiometric biosensors for the detection of urea. Marchenko et al. developed a potentiometric urea biosensor based on two identical pH-sensitive field-effect transistors using recombinant urease entrapped into PVA/SbQ photopolymer, while bovine serum albumin entrapped in PVA/SbQ photopolymer on the second transistor was used for reference. The latter system acted in a 0.5–40 mM urea concentration range and presented a response time between 1–2 min [24]. Extracellular crude urease from Arthrobacter creatinolyticus was immobilized on poly(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) membrane by Ramesh et al. The system exhibited a large operational range from 1 to 100 mM with a response time of 2 min [25]. Nguyen et al. showed that an indium tin oxide electrode modified with carbon nanotube, urease and a mixture of poly-L-lysine hydrobromide and poly (sodium 4 styrenesulfonate) can detect urea in a range of 0.01–3 mM with response time of ca. 60–90 s [26]. Potentiometric urea biosensor using urease immobilized on polypyrrole detected urea in a 0.5–10 mM concentration range with response time of 1 min, as reported in Ref. [27].

4. Conclusions

The feasibility of the spin-assisted assembly of V_2O_5-nanorods in conjunction with PAH has been demonstrated for the construction of potentiometric chemical sensors. PAH/V_2O_5-nanorods showed great potential as an ion sensor and pH-sensitivity between 52 and 61 mV/pH, depending on the number of PAH/V_2O_5-nanorods layers. Urease enzyme was immobilized on PAH/V_2O_5-nanorod electrodes and the system was applied as a urea biosensor, presenting a wide range of detection from 0.05 to 20 mM, comprising normal concentrations in human blood. The spin-assisted assembly technique enables the combination of different materials to form active electrodes of potential applications to other sensors and biosensor.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2016.02.010.

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Valmor R. Mastelaro is currently the associate professor at the Institute of Physics of Sao Carlos, University of Sao Paulo, Brazil. He received his doctorate in Science from University Paris XI (France) in 1992. His research interests mostly deals with structural characterization of inorganic materials by X-ray diffraction spectroscopy (XAS) and electrical properties of perovskite oxide based nanostructure and ceramic materials. He is the author of over 170 papers in international peer-reviewed journals.

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