Effect of the polymerization bath on structure and electrochemical properties of polyaniline-poly(styrene sulfonate) hydrogels

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ABSTRACT

Conducting polymer hydrogels combine the mechanical and swelling properties of hydrogels with the specific electron transport and redox properties of conducting polymers. Such hydrogels are typically obtained by oxidative polymerization of the monomer at high concentration in the presence of a polyanion, which acts as a electrostatic cross-linking agent. In this contribution, we study polyaniline-poly(styrene sulfonate) (PANI/PSS) hydrogels synthesized in 1 M HCl (PANI/PSS/pH 0) or in phosphate solution at pH = 5 (PANI/PSS/pH 5). The composition of the polymerization bath influences the electrochemical properties of the hydrogel. Cyclic voltammetry responses of PANI/PSS/pH 0 studied at pH = 6 show a single redox pair with large potential distance (0.59 V) between the anodic and the cathodic peak. The distance between peaks for PANI/PSS/pH 5 is much smaller (0.15 V). Cyclic voltammetry results are correlated with vibrational spectra of hydrogels. Both PANI/PSS/pH 0 and PANI/PSS/pH 5 were tested as supports for horseradish peroxidase (HRP). The amperometric responses to H_2O_2 were immediate in the case of the enzyme immobilized in PANI-PSS/pH 5, while the enzyme immobilized in PANI/PSS/pH 0 exhibited a much slower response. The differences are explained by various ion-exchange properties of the two hydrogels and presence of phenazine oligomers in PANI/PSS/pH 5. The sensitivities of the enzyme immobilized in the two hydrogels were similar at 0.184 $\mu A\ cm^{-2}\ \mu M^{-1}$ and 0.148 $\mu A\ cm^{-2}\ \mu M^{-1}$ for PANI/PSS/pH 0 and PANI/PSS/pH 5, respectively. The limit of detection for HRP in PANI/PSS/pH 5 was equal to 76 nM. The limit of detection value was worse (0.9 μM) due to larger current noise value.

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1. Introduction

Intrinsically conducting polymers together with polyanions form three-dimensional hydrogel structures stabilized primarily by electrostatic interactions between cationic charge carriers and the polyanions [1–4]. Such hydrogels are obtained by the oxidation of monomers in the presence of an anionic polyelectrolyte. The hydrogel formation depends on the concentrations of the monomer, oxidant and polyanion. Reagent concentrations that are too low yield colloidal solutions of complexes of polyaniline (PANI) and polyanion [1]. Typically, PANI, polypyrrole or poly(3,4-ethylenedioxythiophene) (PEDOT) is the intrinsically conducting polymer, and polystyrene sulfonate (PSS), poly-(1-acrylamido-2-methylpropanesulfonate) (PAMPS) [5] or anion of poly(-acrylic) acid (PAA) [6] is the polyanion. The polyaniline hydrogel may also be obtained by the reaction of phytic acid (PA), which acts as a counterion [4].

Compared to standard conducting polymer films, hydrogels containing conductive polymers are characterized by higher electrical capacitance, making these types of hydrogels interesting materials for energy storage [7–10]. The application of PANI containing hydrogels as drug carriers with electrically driven release has also been investigated [11].

Conducting polymer hydrogels readily swell in water, making them attractive for sensor construction due to the facile diffusion of analyte molecules. Similar materials based on non-conductive cross-linked polymer networks are widely used for biomolecule immobilization [12,13]. Intrinsically conducting polymers may also serve as redox mediators for enzymatic reactions, which is an advantage compared with non-conductive polymer gel scaffolds. Examples of conducting polymer hydrogels as enzyme supports without any additional redox mediators include the use of PANI/PSS as a support for urease [14] and PANI/PA as a glucose oxidase support [4,15]. PEDOT/PSS/poly-4-vinylpyridine hydrogel was used for horseradish peroxidase (HRP) [2], although in this system, osmium ions were used as redox mediators for the enzyme. The considerable advantages of conductive polymer hydrogels include the very simple synthesis and low cost of these materials.

Among the conductive polymers, PANI stands out due to its facile synthesis, long-term environmental stability, high electronic and protonic conductivity and reversible redox behavior [16,17]. PANI can

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serve also as a redox mediator for HRP [18,19] and glucose oxidase [20, 21]. HRP is one of the most studied peroxidases (EC1.11.1.7) [22,23] containing heme at the active site. HRP is widely used for hydrogen peroxide sensing and is a model compound for other peroxidases [24–29]. The resting state contains a heme-iron at the Fe^{3+} oxidation state and a neutral porphyrin ring (P^0). HRP is active in a quite broad pH range, with an optimum pH value between 6 and 8. Presence of proton donors, like NH_4^+ increases rate of the catalytic reaction [26]. The good proton conductivity of PANI might be an advantage for the immobilization of HRP. PANI shows also an ability of direct electron transfer between the enzyme and the polymer. Several amperometric biosensors based on HRP immobilized in bare PANI or PANI composite materials have been constructed [18,30–34] Typical detection limits of PANI-HRP biosensors fall in the micromolar range with a millimolar linear response range. The conductivity of PANI depends on the proton concentration. Because protons are consumed during H_2O_2 reduction, conductometric sensors employing HRP and PANI have been studied [24,35]. The detection limits for these conductometric sensors reach 0.7 nanomole, but their linear response ranges are limited to 1 μmol [24]. Another limitation of PANI conductometric sensors is the strong dependence of the conductivity of PANI on the pH of the solution. Therefore changes of pH not related to the enzymatic reaction – like CO_2 concentration, affect the sensor response, what can hinder potential applications.

The oxidation and reduction of conducting polymers is accompanied by the exchange of ions with the supporting electrolyte, which is necessary to compensate charge changes induced by the redox reaction. The conducting polymers doped with polyanions exchange cations with the supporting electrolyte due hampered movement of the large polyanion, but mixed anion-cation charge compensation have been also proposed [36,37]. The cation or anion exchange properties of conducting polymers has been investigated in order to construct ion sensors [38–40]. The presence of polyanion influences properties of the resulting polymer. It has been reported that PANI polymerized in presence of PSS is conductive in a wider pH range [34,41].

In this contribution we investigate the PANI/PSS hydrogels synthesized in 1 M HCl or 0.1 M KH_2PO_4 + NaOH solution at pH = 5 to verify the influence of the polymerization environment on the molecular structure and electrochemical properties of the hydrogel. The role of phosphate ions is discussed. The hydrogels are studied as possible supports for HRP. Up to our knowledge HRP immobilized in a PANI/PSS hydrogel was not studied. Spectroscopic and electrochemical methods are used to investigate the structure and properties of PANI/PSS hydrogels synthesized in 1 M HCl or 0.1 M KH_2PO_4 + NaOH solution at pH = 5.

2. Experimental

2.1. Chemicals and reagents

Aniline, C_6H_7N (>98%); poly(sodium 4-styrenesulphonate), PSSNa (average Mw ~ 70,000, powder); ammonium persulfate, $H_8N_2O_8S_2$ (ACS reagent, ≥98%); N-hydroxysuccinimide (NHS), $C_4H_5NO_3$ (98%); N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), $C_8H_{17}N_3$·HCl (purum, ≥98%); horseradish peroxidase, HRP (type VI-A lyophilized powder; E.C.1.11.1.7, 250–330 units per mg); 5% Nafion-117 solution were purchased from Sigma-Aldrich (International). KH_2PO_4; NaOH were purchased from POCH (Poland). Hydrogen peroxide (H_2O_2, 30%, v/v aqueous solution) was supplied by Chempur (Poland), and the solutions were freshly prepared prior to use. The phosphate buffer solution with a pH of 6 was prepared using a concentrated phosphate buffer (pH 6) supplied by POCH (Poland). A1 M HCl solution was prepared using 35–38% HCl from POCH (Poland). All the chemicals were of analytical grade and used without further purification. All the aqueous solutions were prepared using ultrapure water from a Millipore water purification system ($R \geq 18$ MΩ).

2.2. Hydrogel preparation

Hydrogels were synthesized using the procedure described by Dai and Jia [1] with modifications. The concentrations of reagents we used to obtain the mechanically stable hydrogels were equal to 0.5 M for aniline, PSSNa and ammonium persulfate. The reaction was carried out in an environment of 1 M HCl (PANI/PSS/pH 0) or in the 0.1 M KH_2PO_4 solution brought to pH = 5 by adding 0.1 M NaOH (PANI/PSS/pH 5). The pH was measured by the pH meter (ELMETRON, CPO-401). Received hydrogels were deposited on a gold electrode, left for drying in air for 5 min and subsequently rinsed with distilled water. 4 μl of the 5% Nafion 117 solution was cast on the electrode and left for 10 min in air. After that the electrode was transferred into the electrochemical cell. The infrared and Raman spectra were studied without the Nafion addition.

2.3. Swelling degree

The freshly prepared samples of hydrogels were weighed on the analytical balance. Subsequently the samples were placed in the laboratory drying oven at 70 °C for two hours to remove water and weighed again. The samples were placed again into the oven for 0.5 h and weighed until the stable mass was reached. The swelling degree was calculated as: $S = \frac{m_w - m_d}{m_w}$, where m_w is the mass of the wet sample and m_d is the mass of the dried sample. Five samples of each hydrogel were studied. The swelling degree values are average of the five measurements. The error was calculated as the standard deviation.

2.4. HRP immobilization

The HRP enzyme was immobilized via covalent bonding using a carbodiimide reaction. The covalent immobilization of HRP was achieved by the formation of amide bonds between the amine groups of PANI and the carboxylic groups of HRP using EDC as the coupling agent and NHS as the activator. The EDC/NHS combination has been widely used for the immobilization of biomolecules in PANI matrices [42,43]. For the binding reaction, 8 μl of a mixture containing 40 mM EDC and 10 mM NHS was poured onto the PANI/PSS/Au electrode (3.14 mm^2 area) and incubated for 1 h at 4 °C in a humidified atmosphere solution to allow the reagent to diffuse throughout the entire hydrogel volume. Next, 20 μl of the HRP solution (20 mg/ml) in phosphate buffer at pH = 6 was poured onto the electrode. The electrode was left in a humidified atmosphere at 4 °C for 24 h. The electrode was rinsed with Milli-Q water. Subsequently, 4 μl of the 5% Nafion 117 solution was poured on the electrode. The electrode was left in air for 10 min in air and then transferred into the electrochemical cell.

2.5. Electrochemical experiments

Electrochemical measurements were conducted in a three-electrode cell equipped with a Pt counter electrode and a Ag/AgCl reference electrode. The potential values given in the text were measured with respect to the Ag/AgCl electrode. The Au disc electrode, which was 2 mm in diameter, was used as the working electrode. Cyclic voltammograms were recorded using a PGSTAT 204 potentiostat (Metrohm-Autolab, The Netherlands), which was operated by the NOVA 1.10 software. The sweep rate (v) was set to 20 mV/s in the cyclic voltammetry experiments.

2.6. Resonance Raman spectra

Raman spectra were recorded with a LabRAM HR spectrometer (Horiba Jobin Yvon). A LION semiconductor laser (Sacher Lasertechnik) operating at 632.8 nm was used as the excitation source. The selected wavelength fell within the absorption band of the fully oxidized PANI