



Evaluation of enzymatic formation of polyaniline nanoparticles



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ARTICLE INFO

Article history:

Received 10 January 2017

Received in revised form

22 February 2017

Accepted 14 March 2017

Available online 18 March 2017

Keywords:

Glucose oxidase

Polyaniline nanoparticles

Spectrometry

Polymerization

Nanocomposite

ABSTRACT

Polyaniline (PANI) nanoparticles were synthesized by enzymatic polymerization of aniline monomers using glucose oxidase (GOx). The influence of aniline and GOx concentrations as well as the duration of the polymerization on the formation of PANI nanoparticles was investigated. The UV/Vis spectra of polymerization solution showed absorption peaks at 325 and 434 nm, which are characteristic for PANI emeraldine base. The increase of absorption at 434 nm was exploited for the monitoring of PANI formation. The most optimal duration for the formation of PANI nanoparticles was in the frame of 100–115 h. Extension of the polymerization time does not increase the efficiency of here proposed polymerization procedure. The highest rate of PANI nanoparticle formation was observed in solution containing 0.50 mol L⁻¹ of aniline and 0.75 mg mL⁻¹ of GOx. Hydrodynamic diameter of synthesized PANI nanoparticles was evaluated using dynamic light scattering technique. Hydrodynamic radius of formed GOx/PANI-nanoparticles increased from 15.8 nm up to 142 nm by prolongation of polymerization process. Synthesized PANI nanoparticles were characterized by cyclic voltammetry and two oxidation peaks at +0.51 and +0.68 V were observed in cyclic voltammograms. These peaks are attributed to emeraldine form of PANI.

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1. Introduction

Polyaniline (PANI) is one of mostly studied organic conducting polymers that have been developed over the past 30 years [1–3]. PANI is not toxic and relatively stable. Even in aggressive chemical environments polyaniline is characterized by high thermal stability, low manufacturing cost and loss of conductivity only at relatively high temperature [1,4–6]. The electrochemically conductive (10³ S cm⁻¹) polyaniline could be synthesized by oxidative polymerization [1,7]. Mostly PANI is synthesized by electrochemical [8–10] or chemical [11–14] oxidation of aniline monomers, and it is characterized by pronounced electron donor properties [4]. Formed PANI could be doped by counterions. Usually aniline polymerization reaction is stoichiometric in the number of electrons accepted from aniline monomer [1] and during the process the dopant anion mostly is incorporated into the structure of formed PANI [7]. Electrochemical polymerization is faster at lower pH values [2]. Chemical polymerization/oligomerization of aniline is typically

initiated by relatively strong chemical oxidants such as H₂O₂, (NH₄)₂S₂O₈, which are able to oxidize dissolved aniline what is leading to the formation of cation radicals of aniline [1,12,15,16]. These cation radicals further react with other monomers or oligomers, ensuring formation prolonged polymeric chains [1]. During such synthesis formed PANI mostly is conductive and is characterized by poor solubility in water [4,17]. But in order to avoid this disadvantage a new strategies, which allow to synthesize water soluble PANI, were developed recently [18–22]. The enzymatic polymerization of PANI can be carried out in aqueous solutions [16,18,19]. Protonated and non-protonated secondary amino groups, which are present in aniline monomer, have different reactivity [23], therefore efficiency of enzymatic polymerization of aniline is strongly depended on the pH of polymerization bulk solution [16,18,22,24]. The conducting polyaniline is produced at lower pH (4.0–4.5). More branched and not conducting form of polyaniline is produced at pH 6.0 or at higher values of pH [18]. Depending on the pH applied during synthesis formed PANI could be doped by negatively or positively charged dopants including enzymes, which are used for initiation of polymerization [10]. Additional advantages of enzymatic polymerization are: (i) possible

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control of the reaction kinetics, (ii) simple, one-step procedure based process [18]. The formation of polyaniline-based films [8,9,11,25], colloidal nanoparticles [19], nanocomposites materials [10,15,26,27] and water-soluble PANI-based materials [18,20,24,28] were reported. The polymerization conditions and composition of initial bulk solution are important factors, which affect the size and morphology of formed nanoparticles and other PANI-based nanostructures [1,7,27].

Chains of polyaniline have ordered repeating structure consisting of phenyl rings and secondary amino groups, which are forming a zigzag lying in one plane, and π -electron clouds overlap with other PANI chains above and below this plane [4]. Depending of the ratio amine/imine nitrogen atoms synthesized electroactive polyaniline might be in various oxidation states. PANI exists in three well-defined stable forms: (i) fully reduced (all nitrogen atoms are in amine form) leucoemeraldine form, (ii) 50% oxidized (amine/imine forms ratio is ~ 0.5) emeraldine form and (iii) fully oxidized (all nitrogen atoms are imine form) pernigraniline form [1,3]. Depending on oxidation state and pH each of these forms can exist in the form of its base or in the form of its protonated (doped) salt, which have different colours (pale yellow-green-blue-violet), stabilities and electric-conductivities [6]. Leucoemeraldine and electronically conducting emeraldine both can be synthesized by standard chemical or electrochemical oxidation [1,4]. Upon further oxidation of leucoemeraldine and emeraldine a fully oxidized pernigraniline can be formed [1]. Leucoemeraldine, pernigraniline or emeraldine forms may be easily turn one to the other one by the redox processes [29]. The emeraldine form is characterized by high conductivity of 10^0 – 10^1 S cm^{-1} [4]. The formation of aggregates between growing chains of polyaniline could lead to incorporation of some ions within formed aggregates [4].

Polyaniline has attracted a great deal of research interest because electrical, electrochemical and optical properties together with its chemical tunability and easy derivatization together with great promise for applications in rechargeable batteries, light-emitting diodes, electrochromic display devices, a corrosion inhibitors [1] and detecting material for sensors [8,11,30,31]. Due to high stability and unique complex of properties, polyaniline could contribute to the development of portable, sensitive biosensors. PANI-based various micro-fabricated electrochemical biosensors, such as enzymatic-, DNA or immuno-sensors offer many advantages and new possibilities for the determination of biologically important compounds [7,17,32–34]. Polyaniline nanoparticles are used as an electro-catalysts and immobilization matrixes for biomolecules [1,8,11,30], because it has good environmental stability and biocompatibility [9]. It was demonstrated that in the presence of polyaniline mediatorless redox coupling between the electrode and biomolecular components could be achieved [10,11]. Glucose biosensor based on composite consisting of gold nanoparticles and conductive polyaniline nanofibers was developed, where polyaniline film was exploited not only as excellent matrix for enzyme immobilization, but as perm-selective membrane, which was blocking diffusion of interfering species [15]. In the construction of electrochemical biosensors [30] polyaniline on the electrode leads to a smoother, slightly ordered conductive nanostructured film with good mechanical properties [25,30].

In this research GOx/polyaniline nanoparticles were synthesized by polymerization/oligomerization of aniline monomers using hydrogen peroxide, which was formed during glucose oxidase (GOx) catalyzed enzymatic reaction. During this process GOx was encapsulated within formed PANI nanoparticles. One of the aims of presented research was to select the optimal conditions for enzymatic formation of PANI nanoparticles.

2. Materials and methods

2.1. Materials

Glucose oxidase (EC 1.1.3.4, type VII, from *Aspergillus niger*, 208 units mg^{-1} protein) and D-(+)-glucose were purchased from Fluka (Buchs, Switzerland) and Carl Roth GmbH&Co (Karlsruhe, Germany), respectively. Before investigations glucose solution was allowed to stay overnight for the formation of equilibrium between α and β optical isomers. All other chemicals used in the present study were either analytically pure or of highest quality. All solutions were prepared using deionized water purified with water purification system Millipore S.A. (Molsheim, France). The solution of sodium acetate (SA) buffer ($0.05 \text{ mol L}^{-1} \text{ CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$) with 0.1 mol L^{-1} KCl was prepared by mixing of sodium acetate trihydrate and potassium chloride, which were obtained from Reanal (Budapest, Hungary) and Lachema (Neratovice, Czech Republic). Ethanol ($\text{C}_2\text{H}_5\text{OH}$) was purchased from Vilniaus degtine (Lithuania). Alumina powder (Al_2O_3 , grain diameter $0.3 \mu\text{m}$, Type N) was purchased from Electron Microscopy Sciences (Hatfield, USA). Aniline and hydrochloric acid (HCl) were purchased from Merck KGaA (Darmstadt, Germany). Aniline was filtered before measurements through 5 cm column filled by Al_2O_3 powder to remove coloured components. All solutions were stored between measurements at $+4 \text{ }^\circ\text{C}$.

2.2. The separation procedure of polyaniline nanoparticles

Polyaniline nanoparticles were prepared at room temperature in darkness in the solution of 0.05 mol L^{-1} SA buffer, pH 6.0, 0.05 mol L^{-1} of glucose, 0.50 mol L^{-1} of aniline and 0.75 mg mL^{-1} of GOx. PANI nanoparticles after 21 h lasting synthesis at $20 \pm 2 \text{ }^\circ\text{C}$ were separated from the synthesis solution by centrifugation (5 min , $16.1 \times 10^3 \text{ g}$). PANI nanoparticles were washed with (i) $\text{C}_2\text{H}_5\text{OH}$; (ii) 0.05 mol L^{-1} SA buffer, pH 6.0; (iii) 0.001 mol L^{-1} HCl or (iiii) 1.0 mol L^{-1} HCl solution. Washing procedure was repeated 3 times and PANI nanoparticles were collected by centrifugation. Then separated and washed PANI nanoparticles were resuspended in SA buffer and used for further investigations.

2.3. The optimization of PANI nanoparticle formation

The efficiency of PANI nanoparticles formation depends on the initial aniline and GOx concentrations in polymerization solution. The selection of optimal aniline concentration was performed by changing the aniline concentration from 0.10 to 0.90 mol L^{-1} in the polymerization solution consisting of 0.05 mol L^{-1} SA buffer, pH 6.0, 0.05 mol L^{-1} of glucose and 0.50 mg mL^{-1} of GOx during 21 h period. The optimization of GOx concentration was performed by changing GOx concentration from 0.125 to 0.75 mg mL^{-1} in the polymerization solution with 0.50 mol L^{-1} of aniline. The optimal duration of polymerization was studied changing the polymerization time from 1 to 331 h in 0.05 mol L^{-1} SA buffer, pH 6.0, 0.05 mol L^{-1} of glucose, 0.50 mol L^{-1} of aniline and 0.75 mg mL^{-1} of GOx. The separation and washing of PANI nanoparticles were carried out in the same way as it was described in the previous section. All experiments were performed at $20 \pm 2 \text{ }^\circ\text{C}$. The influence of aniline and GOx concentrations, as well as polymerization duration was evaluated by UV/Vis spectroscopy.

2.4. UV/Vis spectroscopy based monitoring of polyaniline particle formation

The absorbance of solutions was investigated by UV/Vis spectrometer Lambda 25 (Shelton, USA) in 300 – 700 nm wavelength

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