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Synthesis of Conducting Polyelectrolyte Complexes of Polyaniline and Poly(2-acrylamido-3-methyl-1-propanesulfonic acid) Catalyzed by pH-Stable Palm Tree Peroxidase

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Comparison of the stability of five plant peroxidases (horseradish, royal palm tree leaf, soybean, and cationic and anionic peanut peroxidases) was carried out under acidic conditions favorable for synthesis of polyelectrolyte complexes of polyaniline (PANI). It demonstrates that palm tree peroxidase has the highest stability. Using this peroxidase as a catalyst, the enzymatic synthesis of polyelectrolyte complexes of PANI and poly(2-acrylamido-3-methyl-1-propanesulfonic acid) (PAMPS) was developed. The template polymerization of aniline was carried out in aqueous buffer at pH 2.8. Varying the concentrations of aniline, PAMPS, and hydrogen peroxide as reagents, favorable conditions for production of PANI were determined. UV−vis−NIR absorption and EPR demonstrated that PAMPS and PANI formed the electroactive complex similar to PANI doped traditionally using low molecular weight sulfonic acids. The effect of pH on conformational variability of the complex was evaluated by UV−vis spectroscopy. Atomic force microscopy showed that a size of the particles of the PANI−PAMPS complexes varied between 10 and 25 nm, depending on a concentration of PAMPS in the complex. The dc conductivity of the complexes depends also on the content of PAMPS, the higher conductivity being for the complexes containing the lower content of the polymeric template.

Introduction

In recent years, a great interest has arisen in the production of π-conjugated polymers which can be used as organic conductors for a new generation. Polyaniline (PANI) is one of the most extensively investigated conducting polymers because of its high environmental stability and promising electronic properties. The potential use has a wide range of applications including lightweight batteries, light-emitting diodes, optical display, anticorrosive protection, biosensors, and so forth.[1−6]

There are two forms of emeraldine polyaniline: as a base, which is not a conductor; and as a salt, capable to conduct current (Scheme 1). The salt form is usually produced from emeraldine base via protonation of its imine sites with sufficiently strong acids such as organic sulfonic and phosphoric acids.[3] This process is named "doping".

Doped PANI cannot be processed because of its poor solubility in common solvents.[7,8] However, PANI can produce polyelectrolyte complexes after its interaction with soluble polymers carrying negatively charged groups to form stable dispersion of nanoparticles in aqueous media and, hence, processability of such complexes is higher than that of PANI itself.[9,10] Furthermore, in such complexes, PANI is in the doped and usually chiral state because of interaction of imine groups of PANI with polymeric anions.[3,9,11−14]

The polyelectrolyte complexes of PANI are synthesized either chemically or enzymatically. The chemical polymerization of monomer aniline is carried out under strongly acidic conditions (usually 1 M HCl or H_2SO_4) using ammonium persulfate as oxidant. The mechanism of this reaction was described earlier.[15,16] The reaction is exothermal,

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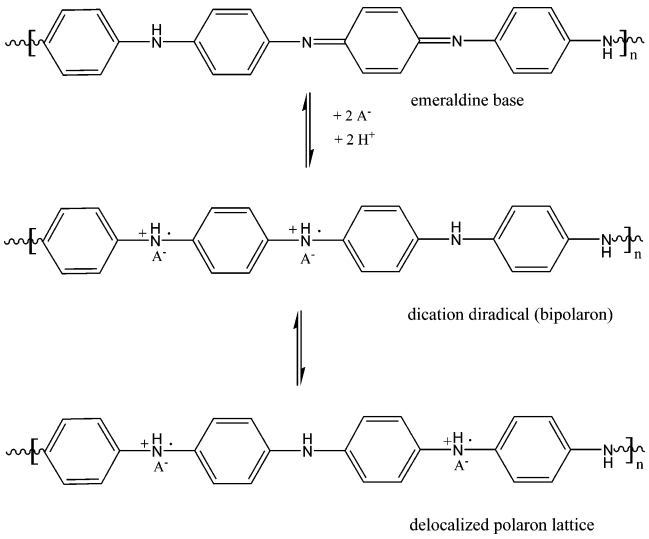
Scheme 1. Chemical Structure of Polyaniline



and, therefore, controlling the kinetics of the reaction is rather difficult.[17] Moreover, ammonium persulfate is a strong oxidant, and its use in high concentration can result in a degradation of polymeric chains of the complex. The removal of the byproduct (ammonium sulfate) from the synthesized polyaniline is difficult; therefore, PANI preparations produced chemically usually contain the contaminating salt.[17,18]

Conversely, the enzymatic reaction can be performed under environmentally friendly conditions under a kinetically controlled regime. The synthesis of the PANI complexes catalyzed by horseradish peroxidase (HRP) was reported previously.[19,20] HRP-catalyzed polymerization of monomer aniline was carried out in the presence of hydrogen peroxide as reducing substrate. The byproduct of this reaction is water; therefore, contrary to the chemical reaction, pure polyaniline is formed in the enzymatic polymerization.

Unfortunately, HRP showed a low stability at pH below 4.5,[19,21,22] that is, at the same pH interval where the polyelectrolyte complex between PANI and negatively charged polymers can be formed. This results in a consumption of large amounts of the enzyme in polymerization. To develop this process further, alternative peroxidases capable of effectively polymerizing aniline under acidic conditions were used.

This paper describes a comparison of the stability of five plant peroxidases under conditions favorable for synthesis of polyelectrolyte complexes of PANI. It demonstrates that under acidic conditions the peroxidase isolated from royal palm tree leaves (RPTP) showed the highest stability. Consequently, using RPTP we have developed a successful "green" synthesis of PANI in the presence of poly(2-acrylamido-3-methyl-1-propanesulfonic acid) (PAMPS) and studied some properties of the obtained complexes of PANI.

Materials and Methods

Materials. Aniline, Na_2HPO_4, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and citric acid were purchased from Sigma Chemical Co. Commercial aniline was purified by distillation before use. Poly(2-acrylamido-3-methyl-1-propanesulfonic acid) (MW ca. 1.000.000) was produced by Aldrich Chemical Co. Inc. Soybean peroxidase (RZ 1.5) and HRP (RZ 3.0) were purchased from Enzymol International Inc. and Biozyme, respectively.

The anionic isoenzyme of peanut peroxidase and RPTP were purified to homogeneity as described.[23,24] The cationic isoenzyme of peanut peroxidase was purified by column chromatography.[25]

pH stability of the peroxidases was studied in 20 mM universal buffer (CH_3COOH, H_3PO_4, H_3BO_3−NaOH).[26] The peroxidases ($[E] = 4 \times 10^{-8}$ M) were incubated at 25 °C for 140 min at different pH values. Then, 10-μL aliquots of the enzyme solution were taken, and their activity was measured. The activity was usually determined spectrophotometrically as follows: 10 μL of enzyme solution was added to 2 mL of 10 mM citrate−phosphate buffer, pH 3.0, containing 60 μM ABTS and 0.3 mM H_2O_2 as substrates, and the absorbance change at 414 nm was measured at 25 °C.[26]

The syntheses of PAMPS−PANI complexes were carried out usually in 0.1 M citrate−phosphate buffer, pH 2.8, at ambient temperature. The pH value of the reaction medium was adjusted using the concentrated phosphoric acid. The concentration of aniline and PAMPS in the reaction varied from 25 to 100 mM and 5 to 50 mM, respectively. The H_2O_2 concentration was 0.1−5 mM. The enzymatic reaction was initiated by adding RPTP (6×10^{-8} M). In the presence of RPTP inactivated by its treatment in boiling water for 1 h, the aniline polymerization did not proceed. Finally, the obtained complexes were purified by dialysis against distilled water for elimination of low molecular weight compounds. The aniline polymerization was evaluated using the UV−vis spectroscopy.

UV−Vis−NIR Spectroscopy. The UV−vis−NIR spectra of PANI samples were recorded on a Shimadzu UV-2401 PC spectrophotometer. In each measurement, distilled water was used as a control. The films were formed by casting of the PANI complexes on thin glass slides and then drying at ambient temperature for 1 day.

EPR Studies. EPR spectra of the PANI samples were recorded on a "Varian E-3" spectrometer. The spectra of samples of dispersed PAMPS−PANI complexes (150-μL aliquots in quartz cuvette) were recorded using microwave power of 4 mW and modulation amplitude of 0.5 G at temperature of 293 K. The spectra of the complex films were recorded at quartz sample tube using a power of 6.3 mW and a modulation amplitude of 1 G at 293 K. The films were formed by casting of the PANI complexes on thin glass slides and then drying at ambient temperature for 1 day. A *g*-value was measured by a conventional method using standard containing signal of Mn(II) diluted by MgO and crystal 2,2′-diphenyl-1-pycrylhydrazine.

Atomic Force Microscopy (AFM). The AFM images were recorded on a Solver P-47-MDT microscope (NT-MDT, Russia). Noncontact "Golden" Silicon cantilevers NSG 11 (NT-MDT) with less than 10-nm curvature radius of tip, 22° cone angle, 5.5 N/m force constant, and 150 kHz resonant frequency were used. Typical amplitude was 20−50 Å. The preparation of a sample was carried out as follows: a 20-μL drop of the studied solution was placed on a fresh surface

of high oriented pyrolytic grafite (HOPG) and incubated for 5 min. Then, the HOPG surface was washed by 20-μL drops of MilliQ ultrapure water (conductivity 18 MΩ) three times. Finally, the sample was dried with a silicagel at ambient temperature for 1 h.

Conductivity. For electric conductivity measurements, several parallel Au electrodes were produced by a thermal evaporation in a vacuum device VUP-4 at the residual pressure of 10^{-3} Pa on a surface of PANI films formed by casting of the PANI complexes on thin glass slides and then drying at ambient temperature for 1 day. The distance between the electrodes was 1 mm, the electrode width was 8 mm. Current−voltage characteristics were recorded between two neighboring electrodes (two-point probe method) using a digital voltmeter-electrometer V7-57/2 (Belvar, Belarus). An applied voltage varied in a range 1−100 V. dc conductivity of the PANI films was calculated in Ohmic range of VAC at room temperature.

Results and Discussion

Typically, HRP-catalyzed synthesis of polyelectrolyte complexes of PANI and polymers carrying negatively charged groups such as DNA, sulfonated polystyrene, poly(vinylphosphonic acid), and so forth is carried out in the pH range 4.0−4.3.[19,20] However, these conditions are not optimal for the formation of complexes between PANI and polymeric anions. Since the pK_a value of amine group of aniline is 4.6,[19] the favorite conditions for the formation of such polyelectrolyte complexes are at pH below 3.6. However, at these pH values, HRP is inactivated over a very short time period.[19,21,22] Therefore, availability of novel peroxidases stable and active under acidic conditions might allow developing more technological process of the enzymatic polymerization of aniline.

Comparison of pH stability of five plant peroxidases, namely, HRP, RPTP, soybean peroxidase, and cationic and anionic peanut peroxidases, was carried out in the pH range 2−7. Figure 1 showed that at ambient temperature all studied peroxidases were stable under neutral conditions. However, when these enzymes were incubated under acidic conditions (pH 2−3), they were inactivated quickly. Only RPTP retained practically its initial activity. Previously, the extremely high stability was reported for the peroxidase purified from other species of palm trees, namely, African oil palm tree.[27−29] Reasons for this stability are not clear yet, however, the high pH stability of RPTP induced us to use this enzyme as the catalyst in the synthesis of PANI under acidic conditions.

RPTP-catalyzed synthesis of PANI was carried out in the presence of PAMPS (Scheme 2), containing negatively charged sulfonated groups. Since pK_a values of amine group of aniline and sulfonic group of PAMPS are 4.6 and 0.7, respectively, the polymerization of aniline was studied at pH 2.8, because under these conditions a maximal amount of charged molecules capable of producing ionic complexes is present in the reaction medium. Because of the formation of ionic bonds between the components of the polyelectrolyte complexes, PANI is transferred in doped form. This conclusion was made on the basis of UV−vis spectra recorded for the complexes. As seen in Figure 2, in the course of the enzymatic polymerization of aniline, a strong polaron absorption band at around 750 nm is observed indicating the formation of conducting polyaniline. After the completion of the polymerization of aniline, a bathochromic shift of the band from 750 to 800 nm is observed because of the reorganization of the 3-D structure of the polyelectrolyte complex.

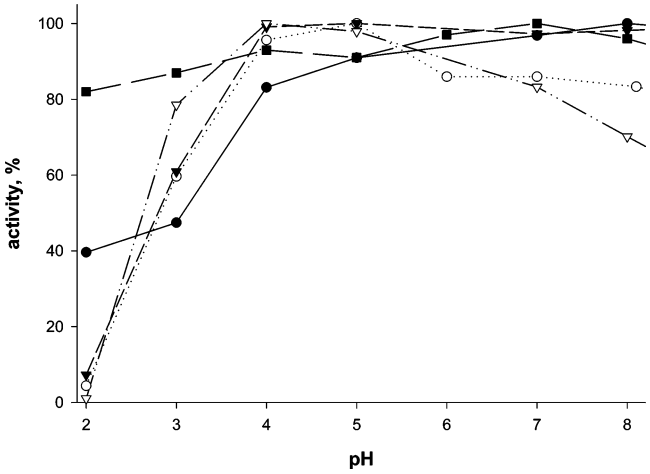


Figure 1. pH stability of cationic isoenzyme c of horseradish peroxidase (circles), anionic palm tree peroxidase (closed squares), anionic soybean peroxidase (closed circles), and cationic (triangles) and anionic (closed triangles) peanut peroxidases. The experimental conditions: the activity was measured toward ABTS after the sample incubation ([*E*] = 4×10^{-8} M) in 20 mM universal buffer with different pH values at 25 °C for 140 min. The maximum value of the activity was taken as 100%.

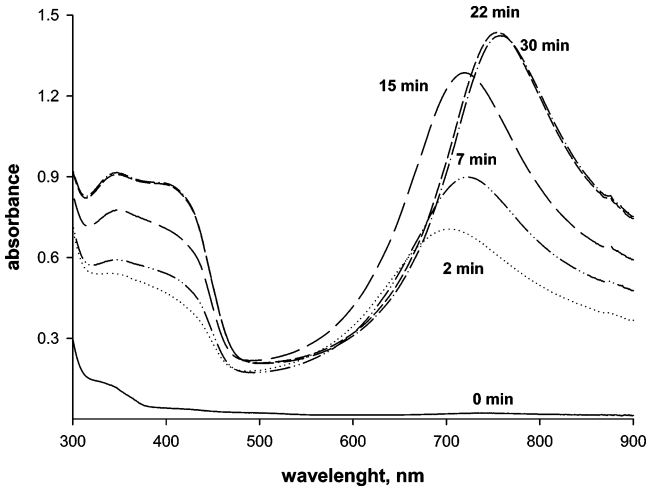
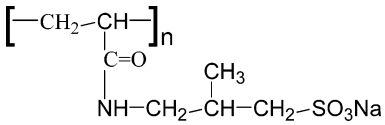


Figure 2. Kinetics of change of UV−vis spectra during the synthesis of PANI−PAMPS complex catalyzed by royal palm tree peroxidase at single addition of hydrogen peroxide. The experimental conditions: 0.1 M citrate−phosphate buffer, pH 2.8; [aniline] = [PAMPS] = 50 mM; [H_2O_2] = 0.25 mM; [RPTP] = 6×10^{-8} M. The UV−vis spectra were recorded 0, 2, 7, 15, 22, and 30 min after the initiation of the reaction.

Scheme 2. Chemical Structure of Poly(2-acrylamido-3-methyl-1-propanesulfonic acid)



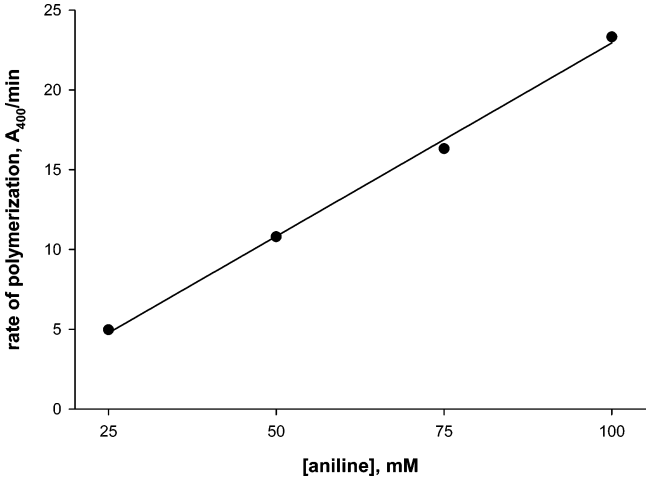


Figure 3. Effect of aniline concentrations on the rate of the enzymatic synthesis of polyaniline. Experimental conditions: 0.1 M citrate−phosphate buffer, pH 2.8; [aniline]:[PAMPS] = 1:1; $[H_2O_2]$ = 1 mM; [RPTP] = 6×10^{-8} M.

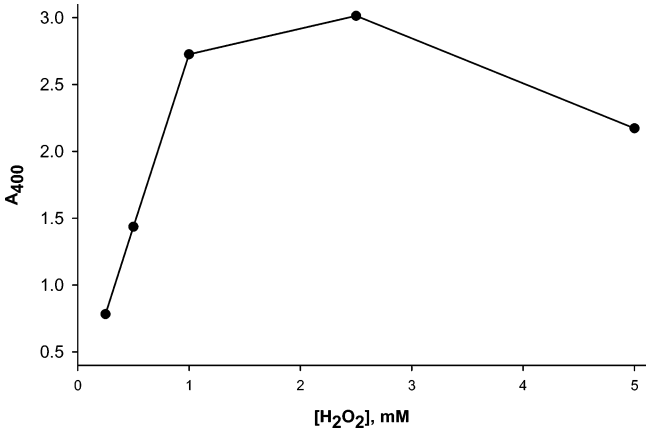


Figure 4. Effect of hydrogen peroxide concentration on the yield of polyaniline synthesis catalyzed by royal palm tree peroxidase. Experimental conditions: 0.1 M citrate−phosphate buffer, pH 2.8; [aniline] = [PAMPS] = 50 mM; [RPTP] = 6×10^{-8} M. The maximal absorbance at 400 nm was recorded 16 h after the polymerization initiation.

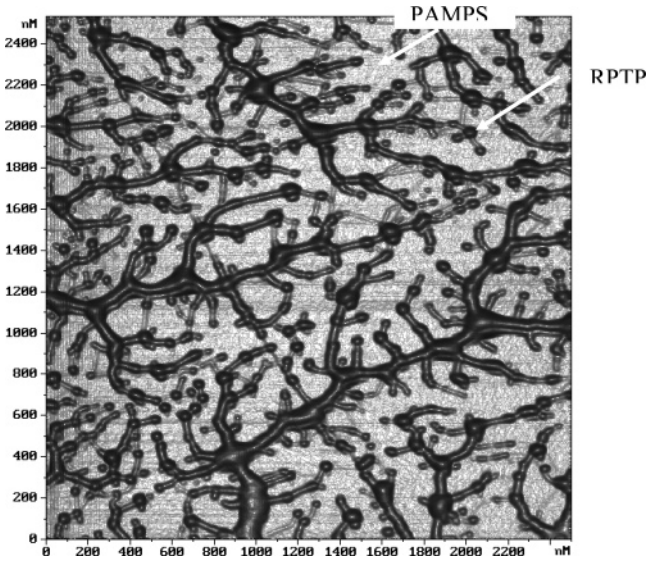


Figure 5. AFM image of film cast from a solution containing 50 mM PAMPS and 60 nM royal palm tree peroxidase.

To find favorite conditions for the enzymatic polymerization of aniline, the effect of aniline concentration on the reaction rate was evaluated. By varying the substrate concentrations from 25 mM to 100 mM in the reaction mixture, the rate of the aniline polymerization increased (Figure 3). Even at high concentrations of aniline, the substrate inhibition of RPTP was not observed, and, hence, the preparative synthesis can be carried out with maximal efficiency of the enzyme. Previously, a similar effect was observed in the enzymatic synthesis of PANI in the presence of sulfonated polystyrene.[11,12]

Polyaniline is produced by a mechanism of an oxidative polymerization.[15,16] Commonly, ammonium persulfate is used as an oxidant. A more attractive oxidant is hydrogen peroxide, whose product of degradation during the polymerization is only water and, hence, pure PANI is synthesized containing no contaminants. At the same time, H_2O_2 is a specific substrate of peroxidases. Therefore, with the use of H_2O_2 as an oxidant, acid-stable RPTP may be used as a biocatalyst in aniline polymerization.

At varying H_2O_2 concentrations in the reaction from 0.2 mM to 5 mM, it was shown that the maximal yield of PANI was observed at 2.5 mM H_2O_2 (Figure 4). At higher H_2O_2 concentration, the peroxidase activity drops. This fact is in good agreement with the classical mechanism of peroxidase catalysis[30] and was discussed by us previously in detail.[12]

In previous works,[11,12] we reported that a presence of sulfonated polystyrene in the feed decreased the rate of enzymatic polymerization of aniline. In contrast, varying the PAMPS concentration from 5 mM to 50 mM at fixed concentration of aniline (50 mM) did not affect the reaction rate. Because it is well-known that plant peroxidases are able to react only with low-weight molecular substrates, constancy of the polymerization rate at varying PAMPS concentration means that the concentration of aniline molecules capable to react with the enzyme is unchanged and, hence, aniline molecules do not form any high molecular weight complexes with PAMPS. Also, no precipitates were observed at storage of obtained complexes at ambient temperature for 1 year as a minimum.

At the same time, it was demonstrated that molecules of RPTP are bound with PAMPS. This fact was detected by atomic force microscopy (Figure 5). The nature of the formation of such complexes is not clear yet. Presently, it has only been noted that this complex is formed not because of ionic interactions but because under the reaction conditions (pH 2.8) both PAMPS and RPTP having pI 3.5[26] carry negative charges.

The variation of PAMPS concentration in feed and consequently in the product does not affect the spectroscopic characteristics of obtained PANI. Electronic spectra of all PANI−PAMPS complexes dispersed in 0.1 citrate−phosphate buffer, pH 4.0, were identical and exhibited three characteristic absorption bands for emeraldine salt of PANI (Figure 6A). The first absorption band at 350−375 nm is derived from $\pi-\pi^*$ electron transition within benzenoid segments. The second (400−415 nm) and third (800 nm) absorption bands are related to doping level and formation of polaron of the conducting form, respectively.[31−33] The former two peaks are combined into a single flat peak as

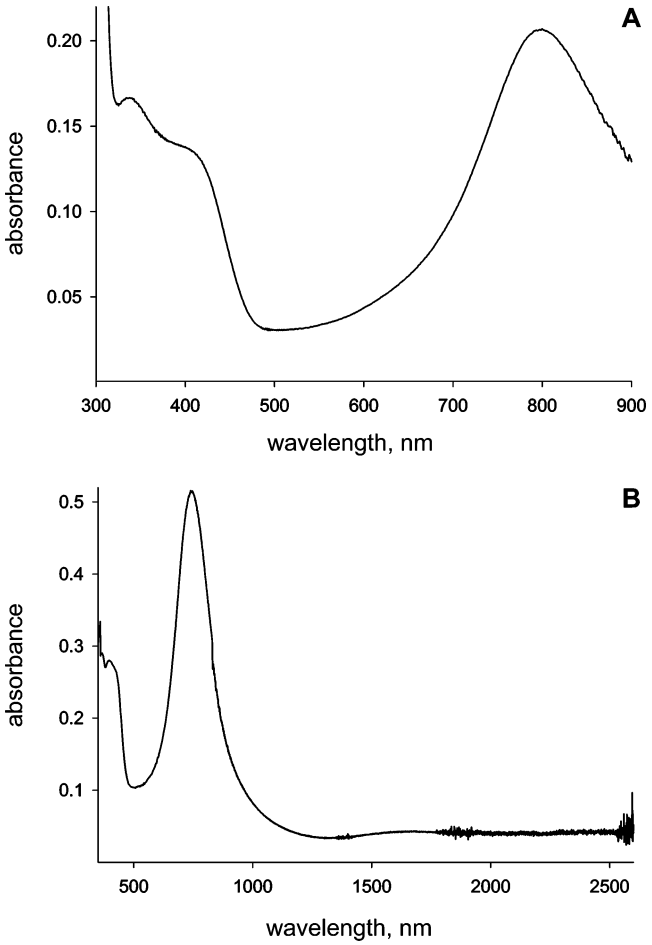


Figure 6. UV−vis spectrum of the dispersion (A) and NIR spectrum of film (B) of complex of polyaniline and poly(2-acrylamido-3-methyl-1-propanesulfonic acid).

described previously.[34] The existence of doped form of PANI confirms the formation of intermolecular complex between PANI and PAMPS. The relative narrow band at 800 nm indicates a formation of "compact coil" conformation of PANI chains in the complexes.[35] The narrow band at 800 nm was detected also for films cast from aqueous solutions of PANI−PAMPS complexes that in combination with low absorption near 2700 nm indicates that both in solid state and in dispersion state PANI exists in coil conformation (Figure 6B).

Previously, Rannou et al. showed that some fluorinated alcohols protonate PANI.[36] This protonation was accompanied by a strong broadening of NIR absorption band near 800 nm. We tried to use 1,1,1,3,3,3-hexafluoro-2 propanol to transfer PANI molecules in the polyelectrolyte complexes from coil conformation to extending one with simultaneous polaron delocalization. However, such effort resulted in failure. The spectra of PANI−PAMPS complexes were not changed after the treatment of PANI films by 1,1,1,3,3,3-hexafluoro-2-propanol.Therefore, the interaction between molecules PANI and PAMPS is too strong to destroy them by the fluorinated alcohol treatment. Other solvents should probably be used to extend PANI molecules in the complexes.

To evaluate the conformational variability, a spectroscopic study of the PANI−PAMPS complexes at different pHs was carried out. At pH values below 4.0, the UV−vis spectra of

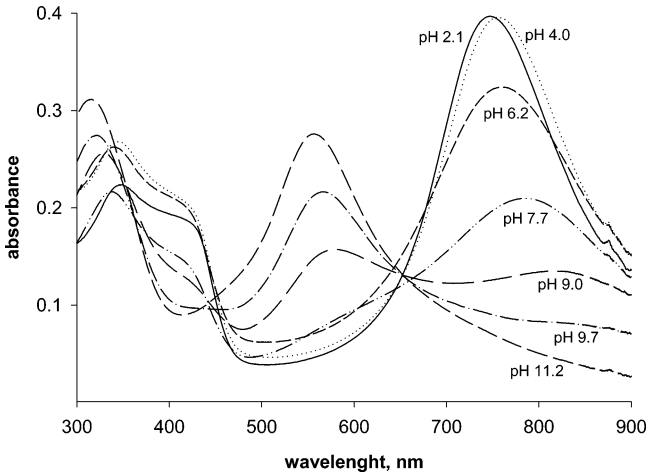


Figure 7. Electronic spectra of dispersion of PANI−PAMPS complex at different pHs.

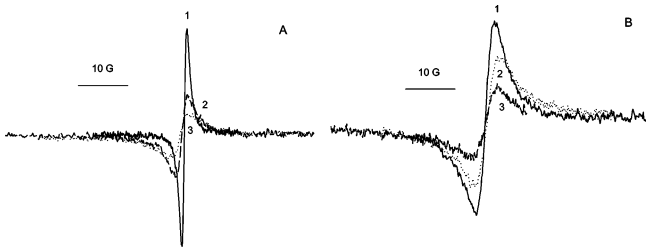


Figure 8. EPR spectra of dispersions (A) of PANI−PAMPS complexes containing different amounts of PAMPS and films (B) cast using the same dispersions. The preparations 1, 2, and 3 were produced at the molar ratio of the repeating unit of PAMPS and aniline of 2:5; 3:5, and 1:1, respectively.

the complexes are similar (Figure 7). This indicates that the pH change in this range does not affect the structure of the PANI−PAMPS complexes. At increasing pH in complexes' dispersion, the intensity of the band at 800 nm decreases gradually and disappears completely at pH 11.2. The missing of the polaron band and the band at 400−415 nm in 0.1 M NaOH coincides with an appearance of a new band at 557 nm. These changes reflect a cleavage of ionic bonds between PANI and PAMPS and, consequently, a transition of PANI−PAMPS complex from emeraldine salt form to emeraldine base form with the appearance of quinoid structures. Although under alkaline conditions the ionic bonds between PAMPS and PANI are disrupted, PANI stays in the dispersion state.

The EPR method allowed detection of unpaired or conducting electrons forming during the production and doping of PANI complexes. Analysis of EPR spectra of the PANI−PAMPS complexes dispersed in 1 mM HCl, pH 3.0, showed that a value of *g*-factor for the complexes synthesized enzymatically is 2.002 (Figure 8A). This value is similar to those reported previously for PANI produced by different methods[37−39] and is characteristic of EPR signal of free electron. The absence of hyperfine structure in EPR spectra of the complexes confirms an existence of delocalized free radicals in the complex backbone.

Comparison of EPR spectra of dispersed PANI−PAMPS complexes containing different amounts of PAMPS showed that at increasing PAMPS content peak-to-peak line width values (ΔH_{pp}) of EPR signals increased with simultaneous

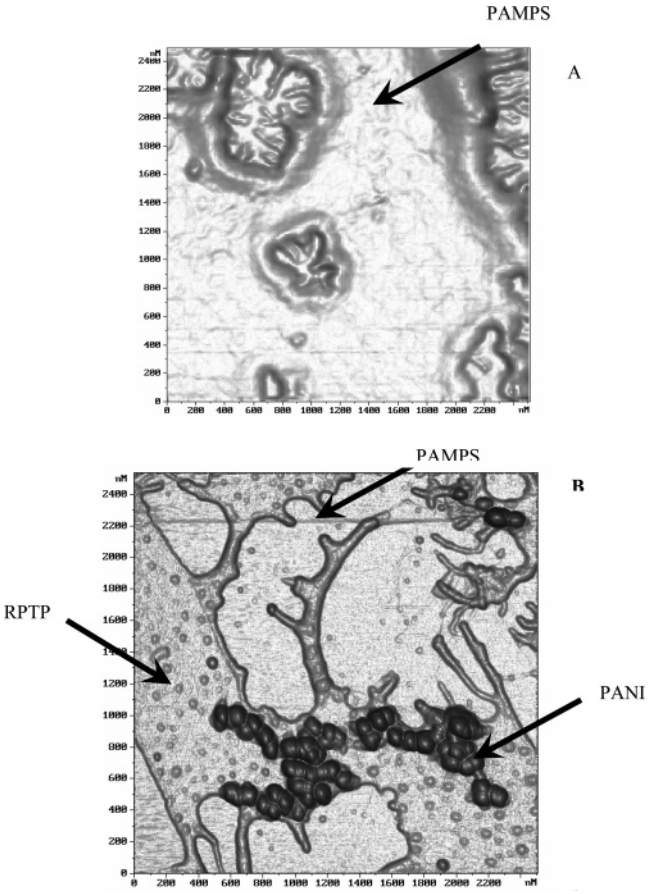


Figure 9. Morphology of PANI−PAMPS complex by atomic force microscopy. (A) PAMPS (control); (B) PANI−PAMPS complex produced enzymatically at the molar ratio of the repeating unit of PAMPS and aniline of 1:5.

increase of free-radical concentration (Figure 8A). These changes indicate that the template content affects delocalization and concentration of electrons in the PANI−PAMPS complexes in the dispersed state.

The opposite effect was observed for films cast from PANI−PAMPS dispersions. In the solid state, all EPR spectra of the complexes containing different PAMPS content had similar ΔH_{pp} values (3.3−4.5 G) (Figure 8B). This value was equal to those calculated for the complexes in the dispersed state containing the highest content of the template polymer.

The morphology of polyelectrolyte PANI−PAMPS complexes was investigated by atomic force microscopy. As shown in Figure 9A, PAMPS itself is absorbed on graphite forming flat film. For PANI−PAMPS complexes, we observed flat films of PAMPS with aggregated assemblies of PANI molecules (Figure 9B). These aggregates were localized on the PAMPS surface and did not form any PANI network, being a far distance from each other. The sizes of PANI nanoparticles depend on the PAMPS concentration used in the synthesis of each complex. The size of particles of complexes having the highest content of PAMPS (the molar ratio of the repeating unit of PAMPS and aniline 1:1) was 10−15 nm, the lowest content (the ratio 1:10) was near 25 nm, whereas at intermediate content of PAMPS (the ratio 1:5) the size of PANI particles was near 20 nm. Therefore, increasing the concentration of PAMPS in the reaction in

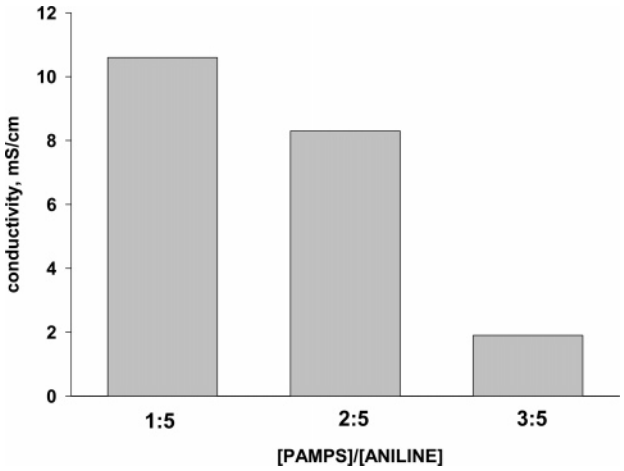


Figure 10. Effect of content of PAMPS in PANI−PAMPS complexes on their conductivity.

the synthesis of PANI probably forms a higher number of sites for initiation of formation of PANI particles that, in turn, results in decreasing the size of PANI aggregates. Thus, by a change of concentration of the polymeric anion, it is possible to modify the size of forming PANI particles that can affect the conducting properties of PANI.

This phenomenon was observed by us at determining conductivity of the PANI−PAMPS complexes containing different content of PAMPS. As shown in Figure 10, the dc conductivity of the complexes depends on the content of PAMPS, the higher conductivity being for the complexes containing a lower content of the polymeric template. The variability of the conductivity correlates with a change of morphology of PANI nanoparticules in the complexes. Although this magnitude is not high in comparison with the best samples of doped polyaniline (∼several hundreds S/cm),[3] it is similar to those measured previously for PANI complexes produced by other methods.[19,40] Thus, this work provides the conclusion that thermostable palm tree peroxidase is an efficient catalyst for the polymerization of aniline and production of processable conducting PANI complexes under green conditions.

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