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Peroxidase-catalysed synthesis of electroconductive polypyrrole

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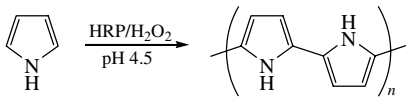
The enzymatic oxidative polymerization of pyrrole has been carried out in the horseradish peroxidase–H_2O_2 system to obtain electroconductive polymers in the presence of dopants.

Electroconductive polymers (organic semiconductors and metals), including polypyrrole (PPy), attract ever increasing attention due to their high conductivity and stability in an oxidised state.[1–4] Oxidative enzymatic polymerization[5–7] performed under mild biomimetic conditions (aqueous medium at ambient temperature) to ensure the effective conversion of a biosubstrate due to the high selectivity and recycling of the biocatalyst, is an alternative to the conventional chemical and electrochemical preparation of electroconductive polymers.

Nabid and Entezami[8] published the synthesis of water-soluble PPy by the oxidative polymerization of corresponding monomers with horseradish peroxidase (HRP) at an optimal concentration of hydrogen ions (pH 2). At pH > 4, the enzymatic polymerization of pyrrole does not occur. At the same time, it is known that an optimal performance of horseradish peroxidase is observed at pH 4–10.[9] Moreover, under low pH conditions this enzyme gets inactivated, while pyrrole is easily polymerised (according to a cation mechanism) to yield water-soluble oligomers.[10,11] Unfortunately, Nabid and Entezami[8] provided no data on the experiment in the absence of the enzyme; therefore, the possibility of the non-enzymatic cationic polymerization of pyrrole is not rejected. Thus, a further study of the synthesis of electroconductive pyrrole polymers catalysed by HRP remains an important research task.

The oxidative polymerization of pyrrole in the presence of HRP and H_2O_2 was carried out under standard conditions (pH 4–8). An optimum value for this process is pH 4.5.† The key role of HRP in the process was demonstrated by the fact that under similar conditions polymerization does not proceed in the absence of the enzyme.



The reaction at higher pH values leads to the formation of minor amounts of low-molecular-weight oxidation products. Non-enzymatic cationic oligomerization was observed at pH < 4.0, as predicted.[10,11] The non-enzymatic reaction mechanism was confirmed by a negative control experiment in the absence of HRP.

The polypyrrole obtained in the presence of the enzyme is a fine black powder insoluble in organic solvents, acids and alkalies. According to the elemental analysis data, the product probably contains oxygen that may be attributable to the presence of oxidised fragments of polypyrrole in the polymer due to either OH radical-induced chain termination or interaction of polypyrrole with atmospheric oxygen.[1] The presence of oxidised fragments is supported by an absorption band at 1700 cm^{-1} in the IR spectrum of the polymer obtained, which can be assigned to the C=O group stretching vibrations.

† Horseradish peroxidase (EC 1.11.1.7, Merck), enzyme activity determined with *o*-dianizidine at pH 5.9 is 235 units mg^{-1}. Pyrrole, polystyrenesulfonate (SPS) (molecular weight of 70 kDa) and buffer components from Aldrich were used. Pyrrole was further purified by distillation immediately before the reaction. The mediator was 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS) from Sigma.

The IR spectra were recorded on a Specord IR-75 instrument (Carl Zeiss, Jena) using KBr pellets. The ^{13}C NMR spectra ($CDCl_3$) were obtained on a Bruker DPX-400 spectrometer operating at 100.61 MHz. The ^{13}C NMR spectra were recorded with proton noise decoupling.

The ESR spectra were measured on a SE/X-2547 (Radiopan) radiospectrometer equipped with a magnetometer and a high frequency meter. The concentration of paramagnetic centres was calculated as previously described[20] using DPPH calibrated standards and a nomogram of double integration.[21]

The electric conductivity of polymer tablets pressed at 700 kg cm^{-2} was measured using a standard E6-13A teraohmmeter. The iodine doping of the polymers was carried out using a diffusion method in a gaseous phase.

The concentration of pyrrole in the reaction mixture was monitored by HPLC on a Milikhrom 1-A instrument equipped with a reverse-phase Separon C-18; the eluent was methanol–0.01 M KH_2PO_4 (2:3).

Enzymatic polymerization of pyrrole: pyrrole was oxidised by the HRP–H_2O_2 system in the absence of a mediator in a citrate buffer and in the presence of ABTS in a phosphate buffer, as well as in the presence of dopants (ABTS, SPS and lithium triflate). The synthesis of polypyrrole was carried out in a 0.1 M buffer (pH 4.5) at room temperature. The pyrrole concentration was 30 mM. The enzyme concentration was 0.1 mg cm^{-3} and the initial reaction volume was 100 ml. The molar ratios pyrrole:ABTS, pyrrole:SPS and pyrrole:lithium triflate were 8:1, 1:1 and 1:1, respectively. The reaction was initiated by the addition of a stoichiometric amount of H_2O_2 – in 0.1 ml aliquotes of 5% solution over an 8 h course under stirring. The polymer precipitate formed during next 24 h, then the resulting product was collected by filtration, washed with water and vacuum dried. The elemental composition of the polymers (%): PPy(citrate) C, 56.71; H, 5.13; N, 15.94; PPy(ABTS) C, 45.74; H, 3.89; N, 13.73; S, 7.14; PPy(SPS) C, 45.38; H, 3.94; N, 11.47; S, 6.48; PPy(TfLi) C, 54.44; H, 4.34; N, 15.44; S, 8.38.

The elemental analyses for C, H, N were carried out on a Flash EA 1112 Series (Thermo Finnigan) instrument. Sulfur was determined by titration with Ba ions. Ash content was determined gravimetrically. Oxygen content in the polymeric composite was determined from the mass difference after calculation of percentage of other components.

The molecular mass distribution of the soluble fraction of the polymers was determined using gel filtration on a Sephadex G-75 column. DMSO with addition of 0.03 M LiBr and 0.03 M H_3PO_4 was used as an eluent to avoid possible polyelectrolyte effects. Samples were applied as 0.2% (m/v) solutions in DMSO; flow rate, 0.15 ml min^{-1}. The column was calibrated using polystyrene standards with Mw 2500, 4750, 9500, 19100 and 33500 Da.

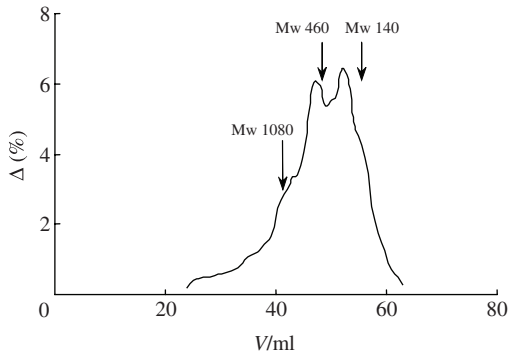


Figure 1 Gel-filtration curve of the polypyrrole fraction dissolved in $CHCl_3$.

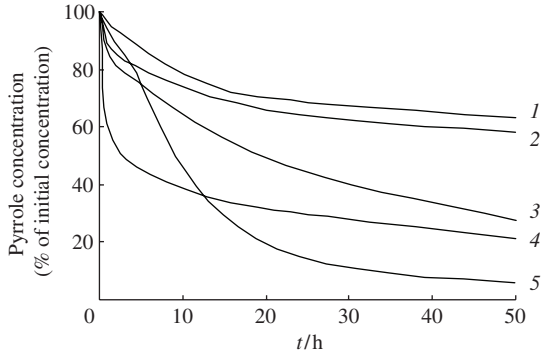


Figure 2 Monomer concentration dynamics during enzymatic polymerization: (*1*) phosphate buffer (pH 4.5), (*2*) citrate buffer (pH 4.5), (*3*) phosphate buffer (pH 4.5), ABTS, (*4*) phosphate buffer (pH 4.5), ABTS, SPS, (*5*) phosphate buffer (pH 4.5), ABTS, TfLi.

The ^{13}C NMR spectra demonstrated that the fraction obtained by chloroform extraction also contains pyrrole oxidation products (C=O group signals in the region 175–180 ppm). According to gel filtration analysis (Figure 1), this fraction contains an oligomer with a polymerization degree of 7–8, higher molecular weight components and lower molecular weight oxidation products.

The degree of pyrrole conversion is relatively low (30–35%), perhaps owing to the fact that pyrrole is not a specific substrate for HRP.

To optimise the procedure of polypyrrole preparation, the application of the oxidative enzymatic polymerization mediator 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)diammonium salt (ABTS)[7] was studied. The rate of enzymatic oxidation of ABTS is several orders of magnitude higher than that of pyrrole. That leads to the formation of ABTS radical cations at the initial reaction stage, which are capable of the further oxidation of pyrrole.

The use of ABTS in the reaction significantly enhanced the pyrrole polymerization rate and substrate conversion (Figure 2). The resulting polypyrrole samples were partially soluble in DMSO. The results of elemental analysis indicate that ABTS is incorporated into the structure of the polymer composite (pyrrole : ABTS ratio is 13 : 1).

In addition, to modify the polypyrrole properties (solubility and electric conductivity),[3,4] the polymer composites of polypyrroles were enzymatically synthesised using the ABTS mediator in the presence of electrolytes, lithium trifluoromethanesulfonate (TfLi) and sodium polystyrene-4-sulfonate (SPS), which are also incorporated into the polymer structure.

All the polymers prepared possess paramagnetic properties. Their ESR spectra show intense narrow singlets corresponding to the concentration of unpaired electrons of 10^{19}–10^{20} spin g^{-1} (Table 1). Analysis of the ESR spectral data indicates the presence of a branched system of conjugated bonds in the polymers.

The paramagnetic and conductivity properties of polypyrrole result from the availability of polarons (cation radicals) in its structure.[1,2,4,12–15] Depending on the polymer preparation method, either the citrate ion from a buffer solution or sulfate anions from the ABTS and SPS molecules or trifluoromethanesulfonate anions act as anion dopants. The more stable the anion used (*i.e.*, the stronger the corresponding acid) the higher the concentration of paramagnetic centres and the conductivity of the resulting polymer (Table 1). However, when SPS is used as a dopant, it probably acts as a matrix thus ordering the system and favouring oxidative polymerization through the α-position of the pyrrole ring. The PPy–ABTS–SPS composite has the best conductivity, which can be further enhanced by one order of magnitude by additional doping with iodine vapour (Table 1). Incomplete doping of the polymer during the synthesis can be explained by reduced charge diffusion among the growing chains due to their spatial separation by the SPS molecules.[5] Note that the doping of the PPy–ABTS–SPS composite with iodine does not significantly increase the concentration of unpaired electrons, but the ESR signal does broaden (Table 1). This could be explained by the formation of diamagnetic bipolarons in the polymer system,[1,4,15] which act as additional charge carriers thus increasing the conductivity of the iodine-doped PPy–ABTS–SPS.

Table 1 Electric conductivity of pyrrole polymers, the concentration of paramagnetic centres (*N*) and ESR signal characteristics.

Sample	Conductivity/ $S\ cm^{-1}$	N/spin g^{-1}	ΔH/mT	g-factor	Asymmetry parameter
PPy (citrate)	3.2×10^{-7}	5.2×10^{19}	0.470	2.0022	0.91
PPy (ABTS)	1.2×10^{-6}	3.3×10^{19}	0.400	2.0047	0.61
PPy (ABTS, TfLi)	6.0×10^{-5}	2.4×10^{20}	0.220	2.0028	0.86
PPy (ABTS, SPS)	2.0×10^{-4}	2.5×10^{20}	0.047	2.0028	0.87
PPy (ABTS, SPS, I_2)	5.1×10^{-3}	2.8×10^{20}	0.084	2.0038	0.83

The IR spectra of all of the synthesised polymers contain bands[7,16–19] corresponding to the main absorption bands of polypyrrole (ν/cm^{-1}): 3400 (3440) (ν_{N-H}), 1560 ($\nu_{C=C}$), 1180 (deformation vibrations of C–H and N–H bonds), 1030 (planar deformation vibrations of C–H and N–H bonds), 920 (the C–C bond vibration) and 790 cm^{-1} (out-of-plane deformation vibrations of the C–H bond). At the same time, the spectra of all polymers contain a band at 1700 cm^{-1}, which can be assigned to the $\nu_{C=O}$ vibrations that point to oxidation occurring in the polypyrrole synthesis under the given conditions.

The spectra of the polymers synthesised in the presence of ABTS and SPS show additional bands at 1020 and 650 cm^{-1}, which are characteristic of the sulfo group.[7,16]

Thus, HRP can be an effective catalyst for the oxidative polymerization of pyrrole. The reaction rate and substrate conversion can be controlled by adding a mediator. Enzymatic polypyrrole possesses electric conductivity properties, which strongly depend on the dopant nature.

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