STUDY OF POLYPYRROLE FUNCTIONALIZATION PARAMETERS

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Polipirolul a fost funcţionalizat prin reacţia cu glutardialdehida pentru inserarea centrilor de legatură pe suprafaţa polimerului, pentru imobilizarea covalentă a enzimei. Cinci parametri de reacţie au fost studiaţi: concentraţia de catalizator (acid sulfuric), raportul glutardialdehidă/apă, raportul polipirol/soluţie de funcţionalizare, temperatura de reacţie şi timpul de reacţie. Efectul fiecărui parametru a fost determinat, menţinându-i pe ceilalţi patru constanţi. Probele au fost analizate prin metode chimice şi spectroscopie în infraroşu cu transformată Fourrier.

The polypyrrole was functionalized by reaction with glutardialdehyde in order to insert further binding sites on the polymer surface for covalent immobilization of enzymes. Five parameters of functionalization reaction were studied: the catalyst (sulphuric acid) concentration, glutardialdehyde/water ratio, polypyrrole/functionalization solution ratio, temperature and time. The effect of each parameter was determined by keeping the other four constant. The samples were analyzed by chemical methods and Fourrier Transform Infrared Spectroscopy.

Keywords: polypyrrole, glutardialdehyde, functionalization

1. Introduction

Enzymes are preferred to conventional chemical catalysts because they have more specificity and efficiency [1]. The use of enzymes has many advantages because enzyme of high or low specificity can be selected to the desired function, little (or no) by-product formation is observed, optimal activity occurs under very mild reaction condition. But, at the same time, when working with enzymes there are some problems as the cost of enzyme preparation is often high, the enzymes are intrinsically unstable, are easily inhibited, the substrate or products which have low solubility in aqueous solution can pose difficulties [2].

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An immobilized enzyme is an enzyme whose movement in space has been restricted either completely or partially.

Among the advantages of using immobilized enzymes it can be mentioned: multiple or repetitive use for a single batch of enzymes, ability to stop a reaction by removing the enzyme from the reaction solution (or vice versa), product is not contaminated by enzyme, easy separation of the enzyme from the product [2]. Immobilization reduces the operation costs, facilitates an easy separation, speeds up recovery of enzyme and extends the stability of enzyme by protecting the active material from deactivation [3].

The method of immobilization should be gentle, in order not to inactivate the enzyme, and bind on the support as much enzyme as needed. Generally, for this purpose the polymers must be functionalized by introducing reactive groups able to react with COOH and NH groups of the enzyme protein [4].

The immobilized enzymes can be used to obtain biosensors [5]. Polypyrrole is thought to be one of the most suitable conducting polymers for obtaining sensors due to its good biocompatibility, stability and ease to polymerize at neutral pH. The polypyrrole based films can be easily obtained in aqueous solutions and have high stability [6].

Polypyrrole exhibits redox activity, it is a good protector against interfering materials (such as proteins from blood) and it gives the possibility to easily immobilize biomolecules [7, 8]. At the same time, polypyrrole is an inherent biocompatible polymer [7]. Polypyrrole proved to be an adequate matrix for electrochemical sensors [9, 10]. A wide type of biomolecules can be covalently attached to functional groups from polypyrrole [11, 12].

Functionalized polypyrrole films have been obtained by electropolymerisation in aqueous solution of pyrrole monomer in the presence of hydroquinone monosulfonate (HQS) as functional dopant in order to improve its electrical conductivity [13]. For the same reason, single wall nanotubes functionalized with polypypyrrole films doped with poly(*m*-aminobenzene sulfonic acid) were synthesized [14].

In the present paper, the functionalization was not realized during polymerization process as in the above works [13, 14], but after polymerization. The functionalization agent was glutaraldehyde, and the functionalization purpose was to create binding sites at certain distance from polymer surface. This is necessary in view of covalently immobilize enzymes on polypyrrole in order to prepare biosensors for monitoring the nitrite and nitrate ions in drinking water.

The aim of the work was to establish the effect of each parameter on functionalization reaction of polypyrrole with glutardialdehyde in order to find the optimal conditions. A Schiff's base was formed through this reaction. Some unreacted CHO groups remain free on the other side of the bonded aldehyde using a dialdehyde. Thus, functionalized polypyrrole is further able to covalently

immobilize enzymes by the reaction with the NH_2 or COOH of the protein enzyme.

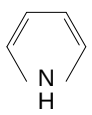
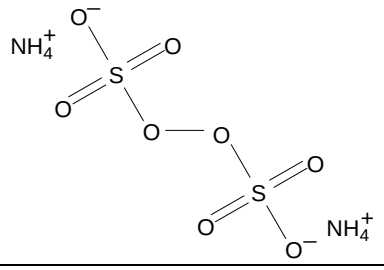
2. Experimental part

Materials

Pyrrole was supplied by Merck and distilled for further purification and then polymerized using ammonium persulphate as initiator in order to obtain polypyrrole (PPy). Ammonium persulphate (APS) was provided by Scharlau and used as received. Glutardialdehyde aqueous solution (50%) and sulphuric acid were received from Merck and used as received. The chemical structures of raw materials are shown in Table 1.

Table 1

Chemical structures of raw materials

		$OCH-(CH_2)_3-CHO$
Pyrrole (Py)	Ammonium persulphate-APS, $(NH_4)_2S_2O_8$	Glutardialdehyde

Sample synthesis

The PPy was synthesized by oxidative polymerization with ammonium persulphate using an adapted method, after [15]: 5mL pyrrole were dissolved in 250mL water; then, 16.43g APS were added stepwise during two hours under continuous stirring, at 5°C. The obtained slurry was filtered, washed and dried in oven at 50-60^{o}C for 6 hrs.

The functionalized samples were synthesized using various amounts from each component. The synthesis conditions are shown in Table 2. A liquid phase was prepared by mixing the specified amounts of water, glutardialdehyde and sulphuric acid, in this order. Then the liquid phase was poured onto polypyrrole solid phase. Polypyrrole was mixed with the liquid phase and heated for different times at the temperature. The final mixture was filtered and washed on the filter with water, then dried at about 40ºC.

Sample characterization

The functionalization degree represents the percent of polypyrrole NH groups, which react with CHO groups from glutaraldehyde. The amount of glutaraldehyde and the synthesis parameters do not allow the reaction of all NH groups. The functionalization degree was used as an output parameter in order to establish the effect of each input parameter. The functionalization degree was determined chemically by reaction of the formed imide groups with sulphuric acid 20%, followed by water vapour steaming of resulted aldehyde. This aldehyde was caught in a hydroxylamine clorhydrate solution. HCl forms and this was quantified by titration with 0.05 N NaOH solution, using green brome cresol as indicator. The titration was stopped when the color changed from yellow to green.

Table 2

The functionalization conditions

Sample	**Solid/liquid ratio**	**PPy** g	**H_2O** mL	**GA** mL	**Concentrated H_2SO_4** mL	**Reaction tempera -ture,** °C	**Reaction time** Min
Sulphuric acid amount variation							
PPy1	1:100	0.1000	8.00	2.00	0.109	70	30
PPy2	1:100	0.1000	8.00	2.00	0.122	70	30
PPy3	1:100	0.1000	8.00	2.00	0.136	70	30
PPy4	1:100	0.1000	8.00	2.00	0.149	70	30
PPy5	1:100	0.1000	8.00	2.00	0.163	70	30
Glutardialdehyde/water ratio variation							
PPy6	1:100	0.1000	9.75	0.25	0.136	70	30
PPy7	1:100	0.1000	9.50	0.50	0.136	70	30
PPy8	1:100	0.1000	9.00	1.00	0.136	70	30
PPy9	1:100	0.1000	8.50	1.50	0.136	70	30
PPy10	1:100	0.1000	8.00	2.00	0.136	70	30
Solid/liquid ratio variation							
PPy11	1:100	0.1000	8.00	2.00	0.136	70	30
PPy12	1:112	0.0893	8.00	2.00	0.136	70	30
PPy13	1:125	0.0800	8.00	2.00	0.136	70	30
PPy14	1:137	0.0729	8.00	2.00	0.136	70	30
PPy15	1:150	0.0667	8.00	2.00	0.136	70	30
Temperature variation							
PPy16	1:100	0.1000	8.00	2	0.136	70	30
PPy17	1:100	0.1000	8.00	2	0.136	60	30
PPy18	1:100	0.1000	8.00	2	0.136	65	30
PPy19	1:100	0.1000	8.00	2	0.136	75	30
PPy20	1:100	0.1000	8.00	2	0.136	80	30
Time variation							
PPy21	1:100	0.1000	8.00	2.00	0.136	70	20
PPy22	1:100	0.1000	8.00	2.00	0.136	70	25
PPy23	1:100	0.1000	8.00	2.00	0.136	70	30
PPy24	1:100	0.1000	8.00	2.00	0.136	70	35
PPy25	1:100	0.1000	8.00	2.00	0.136	70	40

The functionalization degree was determined with the following equation:

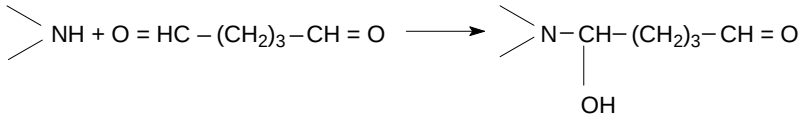
$$FD = \frac{(V - V_0) * F * 0.00215 * 200 * 100}{g_p * 50} - FD_p, \quad (1)$$

where V is NaOH solution volume used for the sample titration; V_0 is NaOH solution volume used for a blank titration (without polymer); F is NaOH solution factor (0.9860); g_p is the weight of the sample; FD_p is the FD value calculated with the first term in equation (1) for the pure polypyrrrole (PPy) sample (as result of aldehyde formation during PPy treatment with H_2SO_4). FDp was found to be 6.13%.

Fourier Transform Infrared Spectroscopy (FTIR) spectra were registered on Bruker VERTEX 70 equipment using 32 scans and 4 cm^{-1} resolution in 400-4000 cm^{-1} region. The samples were analyzed using ATR unit. FTIR spectra for the samples are compared to those obtained for pure PPy.

3. Results and discussion

Hydroxyl groups are formed through functionalization reaction between NH groups from polypyrrole and glutardialdehyde according to the following equation:



The second CHO group is able to react in the same way, leading to crosslinking. Reaction conditions must be chosen in order to leave enough unreacted CHO group for subsequent bonding of the enzyme.

The functionalization degree is useful to determine the amount of polypyrrole NH groups reacted with glutardialdehyde, because only a part of them react. The functionalization degree calculated for samples functionalized in different conditions is shown in table 3. Comparing the functionalization degrees (FD) for the 1st series of experiments, one may notice that it increases as the glutaraldehyde amount increases. From the 2nd series it can be seen that the solid/liquid ratio does not play an important effect (an increase of 0.003g PPy/mL liquid functionalization solution leads to only ~2% change in the functionalization degree).

The reaction temperature has an important effect (by increasing temperature from 60°C to 80°C, FD increases with ~ 25%). The reaction time is also important (at 25 min FD is only 7.53% and at 40 min FD it increases at 19.89%).

Table 3

Functionalization degree for the investigated samples

Sample	Parameter value	*FD* %
Glutardialdehyde/water ratio (mL/mL)		
PPy7	0.50/9.50	4.00
PPy8	1.00/9.00	8.41
PPy10	2.00/8.00	12.03
Solid/liquid ratio (g PPy/mL liquid)		
PPy14	0.0729/10	10.81
PPy12	0.0893/10	10.89
PPy11	0.1000/10	12.65
Temperature (◦C)		
PPy17	60	6.93
PPy16	70	12.36
PPy20	80	29.59
Time (minutes)		
PPy22	25	7.53
PPy24	35	12.74
PPy25	40	19.89

The results obtained by chemical determination of the functionalization degree were confirmed by FTIR spectra. They were registered and compared to the polypyrrole FTIR spectrum. Fig. 1 reveals the apparition of a peak at 3414.5cm^{-1} for the functionalized polypyrrole, which is absent in polypyrrole spectrum. This peak is assigned to hydroxyl groups formed through functionalization reaction between amino groups from polypyrrole and aldehyde group from glutardialdehyde (aldol condensation).

There are two peaks at 2918.1 cm^{-1} and 2848.9 cm^{-1}, which are assigned to the stretching vibration of CH_2 groups from bonded glutardialdehyde, they being absent in polypyrrole spectrum.

In Fig. 2 the peak for hydroxyl groups appears in the functionalized polypyrrole, and it is missing in the pure polypyrrole spectrum. There are also present the two peaks for the stretching vibration of CH_2 groups from bonded glutardialdehyde, which are absent in polypyrrole spectrum. The presence of CH_2 groups proves that functionalization reaction took place because polypyrrole does not contain these groups. They are due to the bonded glutardialdehyde, which introduces a greater mobility as a consequence of its flexible side chains.

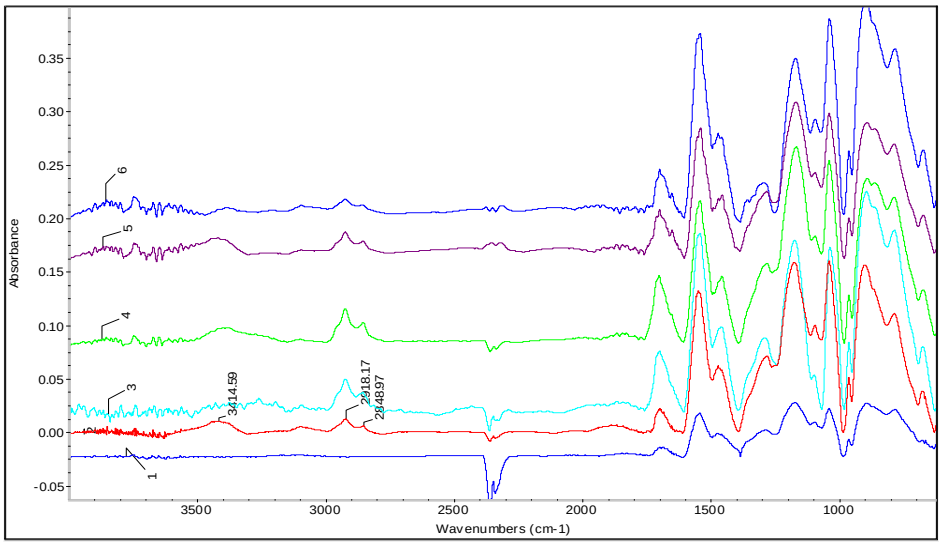


Fig. 1. FTIR spectra for pure polypyrrole PPy (1) and PPy1-PPy5 samples obtained with various catalyst (sulphuric acid) amounts: 0.109(2); 0.122(3); 0.136(4); 0.149(5); 0.163(6), respectively

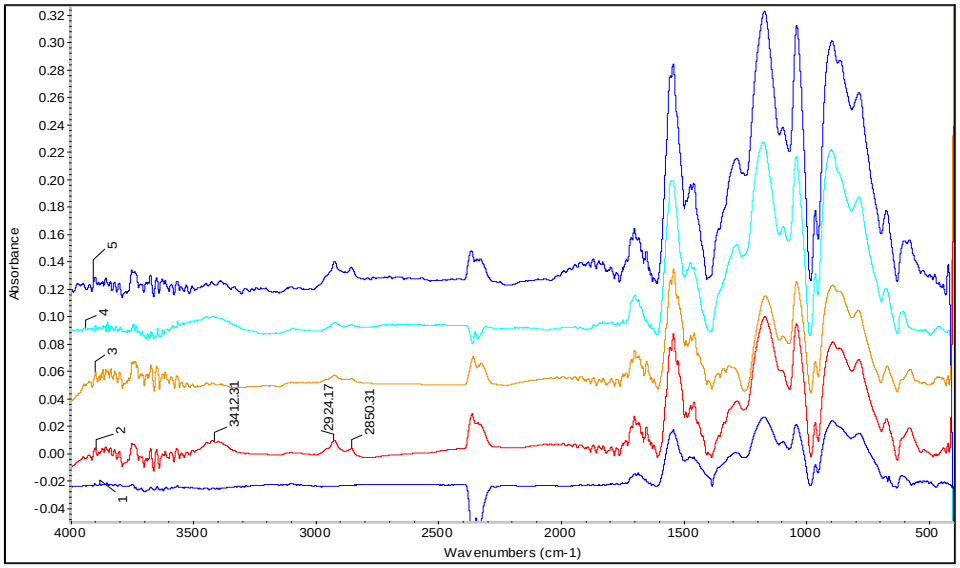


Fig. 2. FTIR spectra for pure polypyrrole (1) and PPy 6-PPy9 samples obtained with various glutardialdehyde/water ratios (2, 3, 4 and 5), respectively

The spectra shown in Figs. 3 – 5 show the presence of the peaks assigned to CH_2 groups which confirm the polypyrrole functionalization.

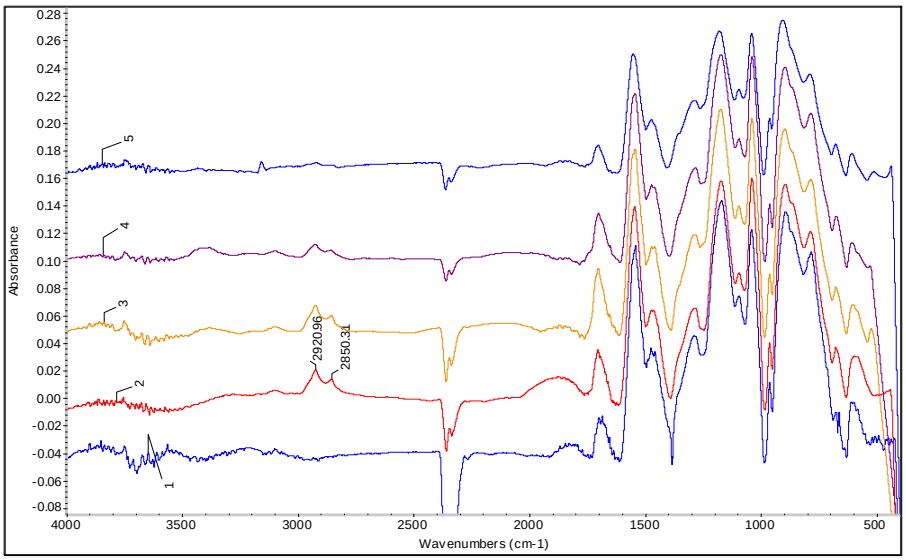


Fig. 3. FTIR spectra for pure polypyrrole (1) and PPy11-PPy13, PPy15 samples obtained with various solid/liquid ratios: 1:100(2), 1:112(3), 1:125 (4) 1:150(5), respectively

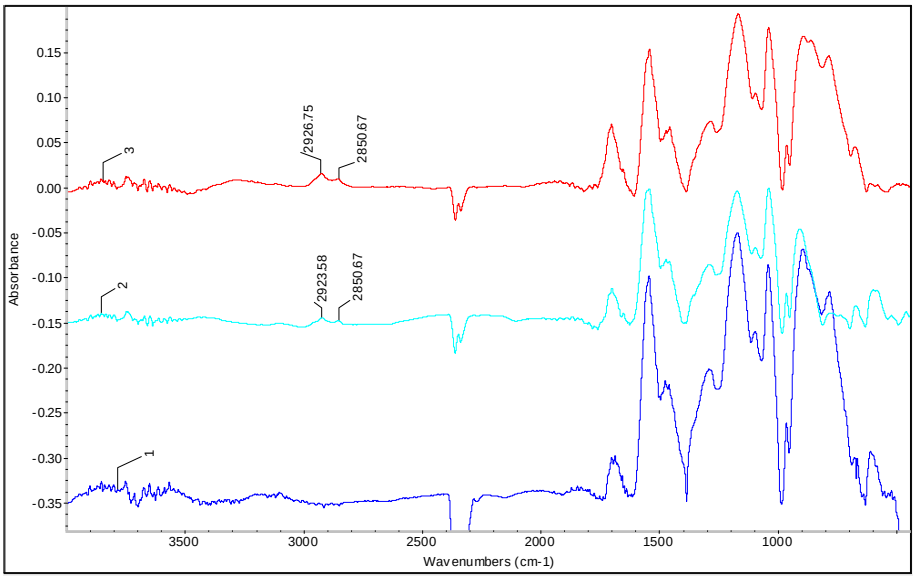


Fig. 4. FTIR spectra for polypyrrole (1) and PPy17, PPy 18 samples obtained at various temperatures 60 (2), 65 (3) ◦C, respectively

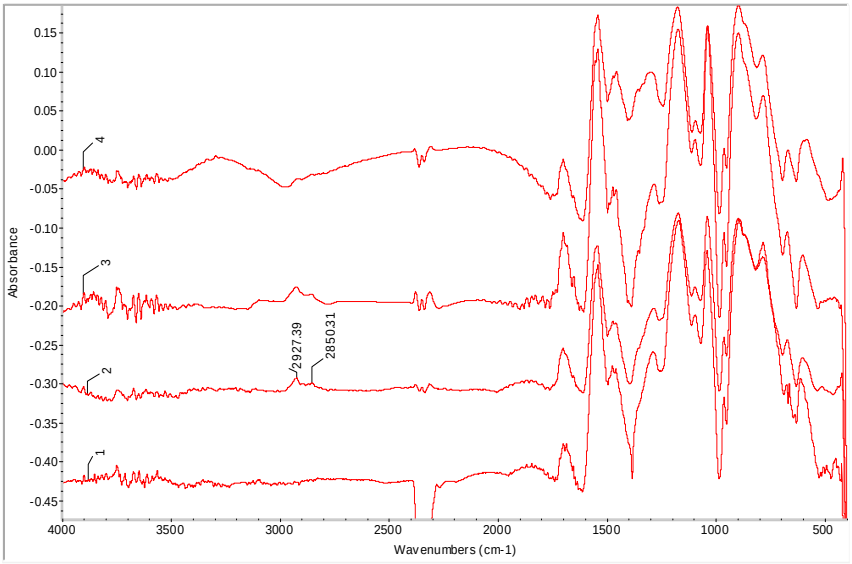


Fig. 5. FTIR spectra of polypyrrole (1) and PPy 21-PPy23 samples obtained at various reaction times 20(2), 25(3), 30(4) minutes, respectively

4. Conclusions

The study of polypyrrole reaction with glutardialdehyde in order to functionalize polypyrrole was performed. The functionalization degree determined by chemical analyses and FTIR proved that this reaction occurred.

For a higher functionalization degree of 0.1g functionalized polypyrrole it was necessary to use an amount of at least 2mL glutardialdehyde, a solid/liquid ratio of 0.01, and to perform the reaction at more than 60°C for a reaction time of at least 30 minutes.

The FTIR spectra of the functionalized polypyrrole are not significantly influenced by the functionalization degree. These spectra only confirmed that the chemical structure of polypyrrole was modified by functionalization. New peaks appeared at about: 3415 cm^{-1} (assigned to –OH groups), 2850 and 2920 cm^{-1} (assigned to $–CH_2–$ groups from bonded glutardialdehyde).

The obtained functionalized polypyrrole is able to covalently immobilize enzymes.

Acknowledgements

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