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Research Article

SYNTHESIS AND STUDY OF NOVEL PAPAIN -POLYPYRROLE COMPOSITE

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ABSTRACT

Papain enzyme and organic, conducting polymer polypyrrole, composite was synthesized by chemical oxidative polymerization technique, using pyrrole monomer and ferric chloride, as an oxidant in a ratio of 2:3. The papain-pyrrole composites was characterized by X-ray diffraction, which shows amorphous nature. *Scanning electron microscopy studies confirms a uniform granular structure of PPy-papain. Fourier transform infra red spectroscopy confirms all the characteristics absorption peaks of polypyrrole and papain.*

Key Words:

pyrrole, papain

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INTRODUCTION

Papain is a cysteine protease of the peptidase C1 family. Papain is obtained by cutting the skin of the unripe papaya and then collecting and drying the latex which flows from the cut. Papain consists of a single polypeptide chain with three disulfide bridges and a sulfhydryl group necessary for activity of the enzyme. papain is of crucial importance in many vital biological processes in all living organisms [1] The antibacterial activity of enzymes in combination with nisin was evaluated[2] polypyrrole is organic polymer formed by polymerization of pyrrole monomer.

PPy was first synthesized chemically in 1916 by the oxidation of the pyrrole monomer using hydrogen peroxide which yielded an amorphous black powder known as 'pyrrole black'. Generally, conjugated polymers are prepared by either chemical oxidation method or electrochemical oxidation methods. One of the main advantages of chemical oxidation method is no necessity of the complicated instruments.

The entrapping effectiveness of lipase in polypyrrole film was calculated, the quantity of entrapped enzyme is relative with the decrease of Open Circuit Potential, raise of pseudo capacitance and reduce of conductivity of film[3]. A biological route for the synthesis of water-soluble conducting polymer, polypyrrole was accomplished using an environmentally friendly process by horseradish peroxidase [4]. Conducting

polymers are a significant group of organic conductors, because of their mechanical and electronic properties such as flexibility and facile processability. These properties have made them very useful for biological sensor applications. [5] Among the conducting polymers, polypyrrole (PPy) is one of the mainly considered polymers because of its elevated electrical conductivity, steadiness and appealing technological applications. Nevertheless, to the best of our knowledge, papain- polypyrrole composite preparation is unavailable in the literature.

MATERIALS AND METHODS

Pyrrole and ferric chloride were of AR grade, obtained commercially from M/S Chemical International, Mumbai, enzyme papain was obtained from a supplier in kalyan. The X-ray diffraction pattern of the samples in this present case were recorded on X'pert PRO Panalytical X-ray diffractometer using $\text{CuK}\alpha$ radiation (1.54 Å).

Preparation of ppy and ppy-papain

PPY-Cl powder was obtained by pyrrole polymerization in the presence of ferric chloride as oxidant. 0.1 mol (16.23 g) of FeCl_3 , Dissolved in 300ml was stirred for 15 min, then 0.043 mol (3 ml) of pyrrole dissolved in 100 ml of distilled water was added to the stirred mixture. The oxidant/pyrrole molar ratio was 2:3 as recommended by Myers and Armes, which is the optimal value. With initial one hour moderate stirring,

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polymerization was carried out at room temperature. For ppy-papain composition, varied weight percent of papain was added to form polypyrrole-papain composites the polymerization was allowed to proceed for 6 hours at room temperature. Filtering and washing with distilled water obtained black precipitate. Obtained composites were dried at 60 °C for 24 hours further more; pure ppy was also prepared under the same reaction conditions for comparison.

RESULTS AND DISCUSSIONS

X-Ray Diffraction (XRD)

Figure 1. with XRD wide peak at around $2\theta = 24.978^\circ$, is attribute to the amorphous nature of pyrrole [6]. Wide peak are due to the scattering from PPy chains at the inter-planar spacing [7]. The average particle dimension of the compound polymer is premeditated by using the Scherrer's formula, $D = 0.94\lambda / \beta \cos\theta$

Where D is size of the particle, $\lambda = 1.5418$, the cosine of the Bragg angle, and β the full width at half height of angle of diffraction in radians. The above equation leads to particle size of the PPy-papain composite. There is no appreciable difference in XRD pattern for the ppy-papain composite.

The diffractograms were recorded in terms of 2θ . The maximum intensity occurs at 24.9788° corresponding to the d-spacing of 3.56193\AA for pyrrole and papain composite. The average particle size of X-ray peaks is found to be 15.57\AA for ppy-papain. A broad hump in the 2θ region 25° - 30° in the diffractograms of ppy-papain is due to the amorphous nature of the materials. The XRD of sample 4 intensity versus 2θ is shown in Figure 3.1. The average chain separation for composite calculated using formula, $S = 5\lambda / 8 \sin \theta$, Comes to 4.65\AA

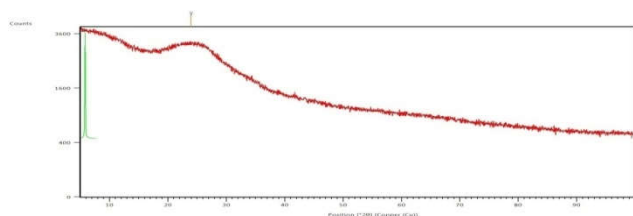


Figure 3.1 XRD of ppy-papain sample 4

Scanning Electron Microscope (SEM)

The surface morphologies of the PPy, and PPy-papain polymer composite were characterized using scanning electron microscopy. The microstructure of pure polypyrrole samples is shown in Figures fig 3.2a for ferric chloride as oxidizing agent. The sample has a uniform granular, porous morphology, with the characteristic cauliflower morphology of polypyrrole which was evident [14]. The amorphous nature seen in the SEM pictures is reflected in the XRD spectra previously shown. In fig 3.2b-d, shows increase in the size of granules as doping is increased for the PPy-papain polymer. For pure ppy the granule size is seen 260nm which increases as doping percentage increases 379 to 460nm. for fig d the connectivity in morphology seems to be better with bit decrease in porosity.

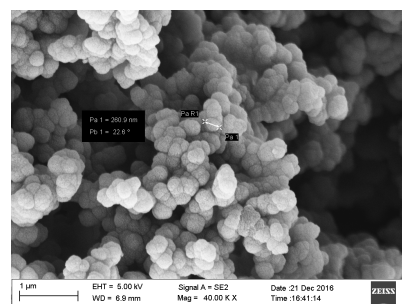


Fig 3.2a

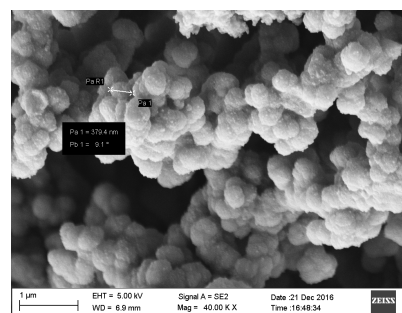


Fig 3.2b

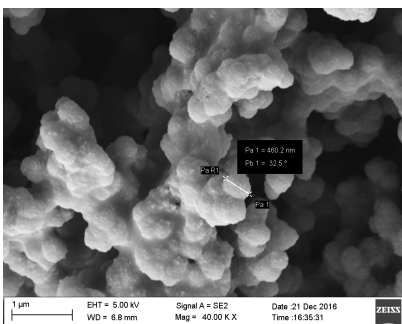


Fig 3.2c

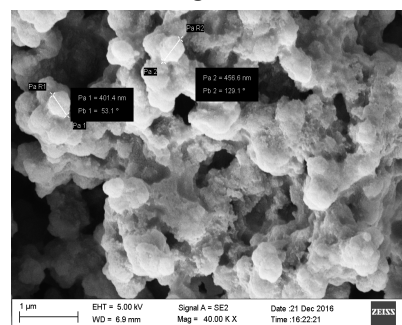


Fig 3.2d

Fourier Transform Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectra of pure and papain-pyrrole composite were recorded in the range of 4000 to 500 cm^{-1} to confirm polymerization [15]. FTIR investigations were carried out to confirm the polymerization of the monomer and absorption of papain content in the samples during the synthesis process. The principal characteristic peaks and existing functional groups in the pure and polypyrrole-papain composites fig 3.3a-d, are identified and assigned to various molecular vibrational groups. There are more bands in the medium towards low wavenumber region (1475 - 500 cm^{-1}) The wave number of the molecular groups and their assignments are shown in Table 3.3. The occurrence of the

amide I, amide II, sulphide and disulphide absorption bands infer the occurrence of papain into the polypyrrole as shown in table 3.3

Table 3.3

cm ⁻¹	functional groups
3096	2924 cm ⁻¹ is typically associated with -CH ₂ - asymmetric stretching [8]
1545	associated with -CONH amides-II[9]
1691	associated with -CONH amides-I[10]
1150,1078,852	be associated with sulphide and disulphide -CS stretching[11]
3424	a broad absorption band centred at 3424 matches the N-H stretching of the secondary amide bond [11] ascertains the formation of hydrogen bond by which papain is stabilized[12]

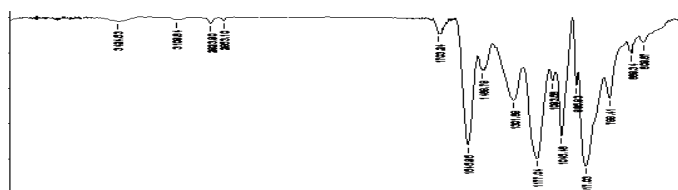


Fig 3.3a pure polypyrrole sample1

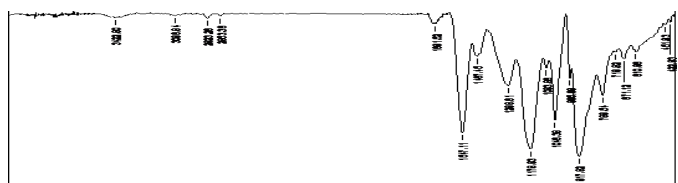


Fig 3.3b polypyrrole-papain sample2

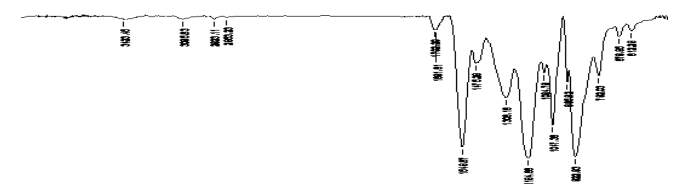


Fig 3.3c polypyrrole-papain sample3

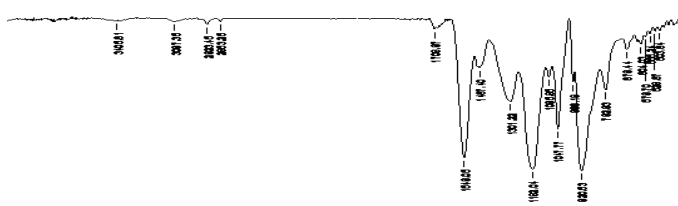


Fig 3.3d polypyrrole-papain sample4

CONCLUSIONS & RECOMMENDATIONS

PPy samples were synthesized using ferric chloride (FeCl₃) as an oxidant. FTIR, XRD, and SEM results have shown the formation of polypyrrole-papain composite. X-ray diffraction studies show that the peak occurs at 24.9788° corresponding to the d- spacing of 3.56193Å for pyrrole and papain composite. The average particle size of X-ray peaks is found to be 849nm for ppy- papain. The sample has a uniform granular morphology, with the characteristic cauliflower morphology of polypyrrole with size around 379 to 460nm. Fourier transform infrared (FTIR), spectra of the composite demonstrate the existence of papain into the polypyrrole.

Acknowledgements

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