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Full Length Article

Influence of gamma irradiation on polymerization of pyrrole and glucose oxidase immobilization onto poly (pyrrole)/poly (vinyl alcohol) matrix

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ABSTRACT

This paper describes the immobilization of glucose oxidase, GOx onto polymer matrix comprising of poly(pyrrole), PPy and poly(vinyl alcohol), PVA using gamma irradiation technique. Py/PVA-GOx film was prepared by spreading PVA:GOx, 1:1 solution onto dried pyrrole film and exposed to gamma irradiation from cobalt 60 source at doses ranging from 0 to 60 kGy. The films were subjected to structural and morphological analyses by using Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), Scanning electron microscope (SEM), Field emission scanning electron microscope (FESEM) and Atomic-force microscopy (AFM) techniques. Similar studies were also made on pristine pyrrole film which served as control. The SEM and FTIR spectra of Py/PVA-GOx film revealed that pyrrole has been successfully polymerized through irradiation-induced reactions. The results on the morphological properties of the samples characterize using FESEM, SEM and AFM further confirmed the occurrence of radiation-induced modification of Py/PVA-GOx film. The FTIR spectra showed the existence of intermolecular interaction between polymer matrix and GOx indicating that GOx had been successfully immobilized onto Ppy/PVA matrix by radiation-induced reactions. Results revealed that radiation induced reactions such as polymerization of pyrrole, crosslinking of PVA, grafting between the adjacent PVA and pyrrole molecules as well as immobilization of GOx onto Ppy/PVA matrix occurred simultaneously upon gamma irradiation. The optimum dose for GOx immobilization in the polymer matrix found to be 40 kGy. Therefore it is clear that this irradiation technique offered a simple single process to produce Py/PVA-GOx film without additional crosslinking and polymerization agents.

1. Introduction

In recent years, intrinsic conducting polymers with conjugated double bonds have been attracted much attention as advanced materials. Among other conducting polymers, polypyrrole (Ppy) was found to possess attractive applications as fundamental building materials [1,2]. It is an electro active polymer, that has been actively researched as a potential material for many applications because of its easy synthetization, rapid electron transport, chemical stability, environmental stability, good mechanical properties, low ionization potential, high electron affinity and promising biocompatibility under physiological conditions [3,4]. Ppy was used in this study to provide the optical and electrical properties which enables the development in technological application sensors and actuators. Although Ppy is capable of producing conducting polymers with high electrical conductivity and environmental stability [5,6], it still has some drawbacks such as poor processability and a lack of essential mechanical properties therefore a host polymer [6] has been combined to produce Ppy polymer composites that have the conducting properties with some superior mechanical properties of the host polymer. The most common method to prepare Ppy are either oxidatively chemical or electrochemical polymerization [7] in which oxidation agent is used for the polymerization process. Irradiation of conducting polymer for polymerization together with grafting process is not a well-established method, however,

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there was a study on the synthesis of Ppy by gamma radiation [8], which reported that its electrical conductivity is low as compared to the Ppy prepared by other methods due to the formation of α-β' bonding during Ppy formation and lack of oxidant and dopant during synthesis [8]. Gamma irradiation on polymers causes structural and chemical changes, which leads to variation in physical properties of the material [9]. The interaction of radiations with matters can induce free radicals which can initiate the polymerization, degradation and grafting reactions. The pyrrole free radicals react with each other to initiate the polymerization of monomer and thus forms the Ppy. Pyrrole dissociates into H and pyrrolyl radicals upon irradiation [8].

On the other hand, irradiation of poly vinyl alcohol (PVA) produces OH and H radicals which are unstable. These unstable reactive species react with each other to form intermolecular reaction between different polymers and crosslinking among the polymers. It is reported that high-energy radiation is a well-known technique for modification of polymers [10]. Polymers become electronically excited or ionized after absorption of energy. The excited molecules are able to induce chemical reactions in polymers which can lead to formation of radiation-induced cross linking [11,12]. Moreover, as reported by Tayel et al., the interaction of gamma with polymers leads to complex phenomena such as chain scission, radical composition, bond breaking, creation of unsaturated bonds, intermolecular crosslinking, free radicals formation, hydrogen release and some oxidation reactions [13].

Modification of polymer in conjunction with the immobilization of glucose oxidase (GOx) onto the polymer matrixes by gamma irradiation was investigated in this study. GOx is an enzyme commercially used in the pharmaceutical industry, as a biosensor for the enzymatic determination of glucose in the fermentation of liquor, and in the food industry for the removal of glucose and shelf life of various products [14–16]. It is also reported that the incorporation of enzymes onto a conducting polymer matrix provides very convenient and stable biocatalysing interfaces that have important bioanalytical applications [8,17]. Although glucose oxidase enzyme has attracted interest in the varying processes, this enzyme is unstable due to its complex molecular structure. Therefore, a number of immobilization techniques have recently been investigated to improve its stability [14,18]. There are many methods for enzyme immobilization: physical entrapment, adsorption [19,20], covalent binding [19,21], crosslinking and doping [19,22]. One problem with enzymes is that they are progressive and will lose their tertiary structure as temperature rises as reported by Ling and Aziz [23] who have investigated the possibility of potentially immobilizing enzyme on PVA at different temperature. However, although biomolecules are sensitive to high temperature and high doses of radiation, a study by Heineman [24] indicated that some enzymes including GOx are sufficiently resistant to gamma radiation.

Therefore, the present work aimed to study the gamma irradiation-induced polymerization of pyrrole and immobilization of GOx onto PPy/PVA matrixes which are believed to occur simultaneously. This *in-situ* process possesses many advantages, including no additives needed for the polymerization of pyrrole, crosslinking of PVA and immobilization.of GOx onto PPy/PVA matrixes. Additionally irradiation offer a simple single step with no harmful chemical residue.

2. Experimental set-up

2.1. Fabrication of pyrrole films

The pyrrole (Sigma Aldrich) was purified using distillation process performed at 130 °C. Pyrrole films were prepared by drop-casting directly on the glass slide surfaces. 200 μl of pyrrole was deposited onto 2.5 × 2.5 cm^2 glass slides and manually spread until the glass surface are covered with the monomer prior to films fabrication. The films were allowed to dry at ambient temperature overnight. The thickness is controlled by maintaining the amount of pyrrole (200 μl) onto measured glass substrate (2.5 × 2.5 cm^2). Some part of the obtained films were subjected to gamma irradiation, while the remaining were used as substratum for the fabrication of Py/PVA-GOx films.

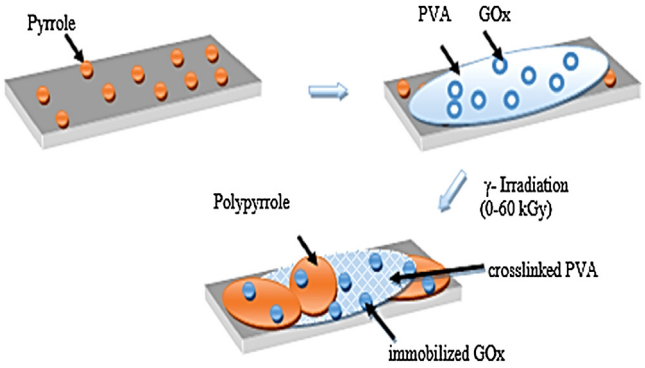

Fig. 1. The schematic illustration for polymerization of pyrrole, PVA crosslinking and immobilization of GOx onto polymer matrix.

2.2. Fabrication of pyrrole/PVA-GOx films

The un-irradiated pyrrole films were used as substratum for the fabrication of Py/PVA-GOx films. 1% PVA solution was prepared by dissolving 1 g of PVA (ACROS) in 100 ml of deionized water and autoclaved at 121 °C. 0.1% of GOx (Sigma Aldrich) solution was prepared by dissolving 1 g of GOx in 1000 ml of phosphate buffer (0.1 M, pH 7.4). For immobilization purpose, PVA solution was mixed with GOx solution with the ratio 1:1 and stirred vigorously. 500 μl of the mixture were then spread onto the pyrrole film substrates and allowed to dry at room temperature. The thickness is controlled by maintaining the amount of PVA-GOx solution (500 μl) onto measured glass substrate (2.5 × 2.5 cm^2).

2.3. Irradiation of pyrrole and Py/PVA-GOx films

The prepared pyrrole films and Py/PVA-GOx films were exposed to gamma irradiation from Cobalt-60 source by using JS 10000 IR219 (Nordion) gamma chamber. The irradiation doses for the pyrrole films were 20, 40, 60,100 and 150 kGy, meanwhile for the Py/PVA-GOx were 20, 40 and 60 kGy, respectively. The irradiation doses were calculated based on the dose rate:

$$\text{Dose} = \text{DoseRate} \times \text{Time}$$

From the dose rate (Gy/minutes), the exposure time was calculated and it is proportional to the radioactivity of Cobalt-60. The fabrication methods of Py/PVA-GOx films are schematically shown in Fig. 1 which illustrates the simultaneous polymerization, crosslinking and immobilization of pyrrole, PVA and GOx, respectively.

2.4. Thin film characterization

A scanning electron microscope (Model FEI Quanta 400, USA) was employed to observe the microstructure surface and cross section of pyrrole and Py/PVA-GOx films surfaces. Samples were prepared by fracturing the films into small sizes and mounted on stubs for surface and cross section analysis. The samples were than coated with a thin layer gold using a sputter-coater and allow to