IMMOBILIZATION OF PAPAIN IN NANOFIBROUS PVA MEMBRANES BY ELECTROSPINNING

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Abstract In this paper, papain enzyme (E.C. 3.4.22.2, 1.6 U/mg) was successfully immobilized in poly (vinylalcohol) (PVA) nanofibers prepared by electrospinning technique. Morphology of the electrospun nanofibers was characterized through scanning electron microscopy (SEM), obtaining diameters distribution in the range of 80-170 nm. The presence of the enzyme in the PVA nanofibers was confirmed through infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). Maximum enzyme catalytic activity was reached at 13% of enzyme loading. The catalytic activity of the immobilized papain was 88% of the crude enzyme and retained its catalytic activity after six cycles of reuse. Crosslinked samples maintained 40% of its initial activity after 40 days storage.

Introduction. Papain is a thiol protease commonly found in many plants and fungi organisms. The kinetics and structure of papain has been well studied; being the enzyme par excellence to compare the efficiency of various immobilization methods [1]. Papain has been used as catalyst to oligomerize hydrophobic aminoacids [2]. The potential uses of papain include the development of assays and biosensors. The past two decades, electrospinning technique has been studied as a useful tool for the production of polymer fibers with diameters in the nanometer to sub-micrometer. The process of nanofiber electrospinning can be successfully controlled resulting in long, uniform nanofibers, which can be deposited forming non-woven mats and membranes with open porosity, high surface area and good mechanical properties. Such distinctive characteristics and superiority made electrospun fibers excellent candidates for protein immobilization.

In this work, we studied electrospun poly (vinyl alcohol) (PVA) nanofibers to immobilize the papain. The morphology of the electrospun PVA fibers embedded with papain, the loading efficiency, the crosslinking process, the catalytic activity and the recyclability of the immobilized enzyme was studied.

Experimental part. The encapsulated papain samples were prepared using a PVA solution of 8.0 wt %. The enzyme was added to the polymer solution until the targeted concentration of enzyme was reached. Once the enzyme was dissolved in the polymer solution, the electrospinning process was carried out. The immobilization of the papain encapsulated in the nanofibers was achieved through PVA crosslinking using glutaraldehyde. The amount of encapsulated enzyme on the PVA membrane was measured by the Bradford protein assay. The amidase activity of the papain was measured using the Earlanger et al. method adapted to the papain [3], where Nα-Benzoyl-L-arginine 4-nitroanilide (BAPA) is used as substrate. PVA nanofibers and PVA nanofiber with encapasulated papain were analyzed by Scanning Electron Microscope (SEM), FTIR and X-ray photoelectron (XPS) spectostroscopies.

Results and discussion. Figure 1 shows the effect of the enzyme concentration upon the final morphology of the electrospun nanofibres. Our approach to produce PVA nanofibers was successful, since no bimodal diameter distribution, indicative of polymer phase separation was found. PVA nanofibers, without enzyme (Figure 1a), showed defect free morphology, and 81 nm average diameter. After GA treatment, the PVA/papain membranes appear densely packed in comparison to their uncrosslinked forms (Figure 2). FTIR and XPS analysis were used to prove the presence of the papain in the PVA electrospun nanofibers. The IR spectra of electrospun papain loaded PVA nanofibers are shown in Fig. 3. The absorption band at 1650 cm^{-1} is induced from amide I bond (O=C-NH), characteristic of proteins [4].The surface chemistry composition of PVA and PVA/papain membranes was analyzed by XPS. We can observe the presence of nitrogen element in the PVA/papain membrane

due to the papain, whereas this element is absent in the control PVA non-woven membrane. The maximum enzymatic activity reached by the papain immobilized in electrospun PVA membranes was approximately 88% of that of the free papain (1.22 X 10^{-2}U/mg). The reusability of immobilized papain was studied by measuring the activity during several cycles., the relative activity of the immobilized papain decreased along with the reusing times (Fig.7). The immobilized enzyme retained over 12% of its initial activity after six cycles of reuse. The catalytic behavior of the papain at different storage times was improved by the immobilization process (Fig. 8). The immobilized papain retains nearly 40% of its initial activity after 14 days of storage, which is much better than the native enzyme.

References

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Figures

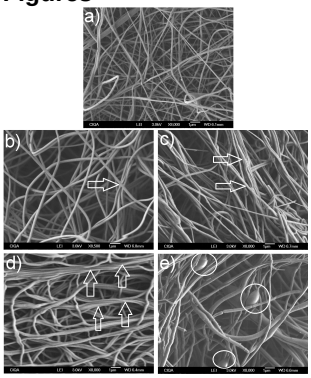

Fig.1. SEM images of PVA nanofibers with different enzyme concentrations: (a) PVA without enzyme, (b) 5%, (c) 10%, (d) 16%, (e) 33%.

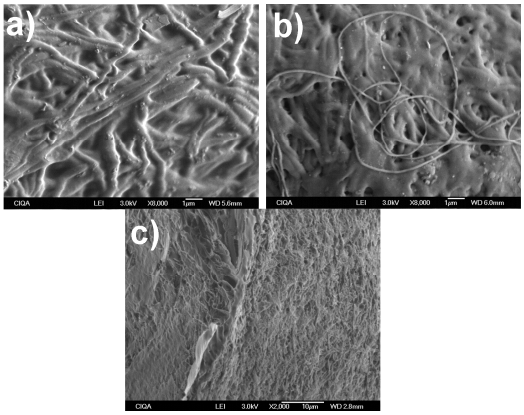

Fig. 2. Crosslinking effect over the morphology of PVA nanofibers: (a) 15 minutes, (b) 30minutes, (c) 60 minutes.

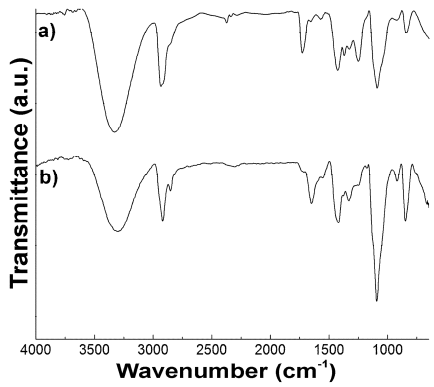

Fig. 3. FTIR spectra of PVA alone (a), and encapsulated enzyme

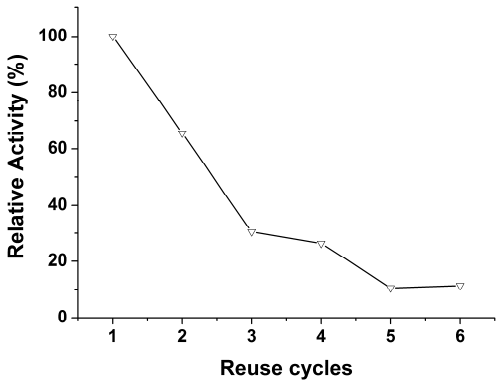

Figure 8. Relative activity of the immobilized after several cycles of reuse

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