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Semi-interpenetrating polymer networks (IPNs) for entrapment of glucose isomerase

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Abstract

Glucose isomerase (GI) was entrapped in three different hydrogels such as poly(acrylamide), semi-interpenetrating poly(acrylamide)/κ-carrageenan, and poly(acrylamide)/alginate polymer networks. The values for pH optimum and temperature for free and immobilized glucose isomerase were found to be the same as 7.5 and 60 °C, respectively. The K_m values for free and immobilized enzyme in poly(acrylamide), poly(acrylamide)/κ-carrageenan and poly(acrylamide)/alginate matrices were determined as 18.87, 1.22, 2.78, and 4.54 mg/mL, respectively, while the V_{max} values for the same systems calculated as 2.51, 0.63, 0.72, and 0.82 mg/mL min, respectively. The storage stability values of immobilized enzyme systems were observed as 81%, 33% and 32%, respectively, after 42 days. In addition to this, it was observed that, after 25th use in 5 days, the retained activities for immobilized enzyme in poly(acrylamide), and semi-interpenetrating polymer networks of poly(acrylamide)/κ-carrageenan and poly(acrylamide)/alginate matrices were found as 98%, 71% and 72%, respectively.

Keywords: Glucose isomerase; Entrapment; Acrylamide; κ-Carrageenan; Alginate; Semi-interpenetrating polymer networks

1. Introduction

Uses of alginate, κ-carrageenan and other biopolymers as support materials for immobilization of various molecules, proteins, and cells have received considerable attention in recent years. Biopolymer supports have certain advantages over other polymeric materials such as low cost, ease of enzyme accessibility, hydrophilic character, and presence of hydroxyl groups on the surface capable of interaction with proteins. Enzyme immobilization on these supports is quick and apparently irreversible and provides nontoxic and biocompatible microenvironment conducive to the catalytic activity and stability of the enzyme. Hydrogels of natural polymers such as gelatin, chitosan, xanthan, and agarose were used conveniently in both wet and dried states, although, these supports suffer from low mechanical strength and ease of microbial degradation [1,2].

There are many different macromolecular hydrogel structures formed by physical and chemical

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interactions. They include the followings: cross-linked or entangled networks of linear homopolymers, linear copolymers, block or graft copolymers, polyion–multivalent ion, polyion–polyion or H-bonded complexes, hydrophilic networks stabilized by hydrophobic domains, interpenetrating polymer networks (IPNs) or physical blends. IPNs are mixtures of two cross-linked polymers. If one polymer is cross-linked and the other is linear, the structure is called semi-IPN. In general IPNs are formed either by simultaneous parallel reactions according to various mechanisms or by swelling one network in monomers from which the second network may be produced [3]. IPNs are preferred in a number of biotechnological and biomedical applications because of their certain unique biophysical properties such as ease of fabrication to various geometrical forms, soft and rubbery texture, unusual stability to biofluids, minimum mechanical irritation to surrounding tissues, etc. [4].

High fructose corn syrup (HFCS) production is considered as one of the most important industrial enzymatic processes in the industry. Isomerization of glucose to fructose by the enzyme glucose isomerase (GI) is the last step in the manufacture of HFCS, which is traditionally carried out in a packed bed reactor using immobilized GI [5–11].

The aim of this work is to investigate the use P(AAm) and semi-IPNs prepared from P(AAm)/κ-carrageenan and P(AAm)/alginate as supports for immobilization of GI. For this purpose, the effect of pH, temperature, re-use numbers and storage stability on the activity of GI for free and entrapped forms were investigated.

2. Experimental

2.1. Materials

Glucose isomerase (E.C. 5.3.1.5) was purchased from Genencor International Company. D(+) Glucose, magnesium sulfate, acrylamide (AAm), ammonium persulphate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TEMED), bisacrylamide, L-cysteine, carbazole, calcium chloride, boric acid were obtained from Merck (Germany). κ-Carrageenan, sodium alginate, acetic acid and phosphoric acid were obtained from Fluka (Germany). Perchloric acid and sulfuric acid were obtained from Sigma (Germany). All reagents were commercially obtained and of technical grade.

B-R buffer solution was prepared in such a way that 2.3 mL glacial acetic acid, 2.7 mL phosphoric acid, and 2.4720 g boric acid were dissolved in triple-distilled water in a total volume of 1.0 L. Portions of this solution (50 mL) were taken and the pH was adjusted between 5.0 and 9.0 by addition of an appropriate amount of 2.0 M NaOH.

2.2. Entrapment of glucose isomerase into P(AAm) and semi-IPNs

Glucose isomerase was entrapped into P(AAm), semi-IPNs of P(AAm)/κ-carrageenan and P(AAm)/alginate. In the first part of this study, GI was entrapped by using P(AAm) hydrogels. P(AAm) hydrogels were synthesized by free-radical crosslinking polymerization of AAm in aqueous solutions. APS and TEMED were used as the redox initiator system. The AAm (2.85 g) APS (10 mg) and bisacrylamide (0.15 g) were dissolved in distilled water (20 mL). After the addition of TEMED (1 mL) and enzyme solution (1 mL), the solution was poured into a flat bottom petri dish. P(AAm) hydrogels were cut into equal size cubes.

In the second part of this study, in 20 mL distilled water; AAm (2.85 g), bisacrylamide (0.15 g), APS (10 mg), TEMED (1 mL), κ-carrageenan (1.0 g) were mixed and 1 mL of enzyme solution was added. This mixture was mixed well and poured into a flat bottom petri dish. After polymerization, the semi-IPN gels were cut into equal size cubes ($5 \times 5 \times 5$ mm^3). P(AAm)/alginate semi-IPNs were prepared in the same way by using Na–alginate (0.2 g) instead of κ-carrageenan. The gel cubes were immersed in distilled water and kept in refrigerator for 1 h. Gel cubes were washed thoroughly three times with distilled water and used in the further experiments.

2.3. Determination of glucose isomerase activity

The glucose isomerase activity was determined according to Tomas's cysteine-carbazole method for fructose [12]. One unit of glucose isomerase activity was defined as the amount of enzyme required to produce one μmol fructose in one minute under the reaction conditions. As this method, fructose concentrations were determined with UV–Vis spectrophotometer (Unicam UV/Vis spectrophotometer UV2) at 566 nm, and with the help of fructose calibration curve.