Fabrication of Fructose Biosensor Based on Single Mode Planer Optical Wavelength

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Abstract

In present studies, the new optical sensing platform based on optical planar waveguide (OPWG) for fructose estimation was reported. The OPWG sensor with short response time (110s) was charactrised using the 0.1M acetate buffer, pH 4.5. the fabricated fructose sensor showed concentration dependent linear response in the range 1×10^{-9}to1×10^{5}M of fructose. Lower limit of detection of this novel AG-INV-GOD cladded OPWG sensor was found to be 1.5×10^{-11} M fructose, which indicates that the developed biosensor has higher sensitivity towards fructose as compared to earlier reported sensor using various transducer systems. These fructose sensing biochips showed good for 8 cycles. The proper confinement of acid invertase and glucose oxidase in hyadrogel composite was confirmed by scanning electron microscopy (SEM) images.

Introduction

The analysis of fructose is of great importance. Therefore different types of biosensers based on immobilized glucose oxidase and invertase, for the estimation of glucose (Mohammaddi et al., 2004; Yagci et al., 2003; Hsieh et al., 2000) as well as fructose (Salgado et al., 1998; Alcazar et al., 2005) have been reported. The first optical waveguide (OWG) sensor was described by Smock et al. (1979). Optical waveguided light spectroscopy (OWLS) is powerful method for monitoring the adsorption of macromolecules on sensor surface. In most applications the sol-gel material is used to provide a micro-porous support matrix in which analyte-sensitive species are entrapped and smaller analyte molecules may diffuse. The sol-gel method is a low-temperature process used to synthesise optically transparent, amorphous bulk glasses and thin films. Fabrication of planar waveguide sensors via sol-gel method is advantageous for several reasons: the method is technically simple, inexpensive and thus amenable to mass production. The optical quality of the waveguiding and sensing layer is excellent.

Materials and methods

1. Planer waveguide preparation and characterization

The planer optically single mode waveguide were fabricated by thermal exchange of Na^+ by K^+ ions, in soda-lime glass. A microscopic glass substrate (4.0cm×2.2cm×0.1cm) containing sodium oxide (Na_2O) was kept in molten potassium nitrite (KNO_3) salt at 300-400^{0}C. the samples were treated for 3 hr to obtain a sufficient diffusion of potassium, in the 2µm guiding layer, for a monomode excitation. The diffusion

constant for single mode optical guide in the exchanged layer was calculated from the equation (Ansari et al., 1997):

$$D=2(Dt)^{1/2}$$

The light transmitted through planar optical waveguide, was focused on a silicon photovoltic detector. The signal of detector was processed using computer-based multimeter. The detector output voltage was used as the sensor output, with various changes in chemical inputs.

Planer optical waveguides were first cleaned with de-ionized water and dried under dust free conditions. The individual gel solution of agarose and guar gum polysaccharide were prepared and checked for clarity and POWG mode intensity.

2. Experimental setup

A layer of agarose-guar gum (AG) entrapped INV-GOD coated on sensor chip surface was used as a sensitive film. The thickness of AG-enzymes film was measured and found to be 10µm. the cladded planer optical waveguides were characterized using prism-film coupling method. The planer waveguides with and without cladding were mounted on a spectrophotometer Table and adjusted for field pattern (m-line, m=0). Without cladding, the insertion loss of the waveguide was observed to be 0.78dB, calculated by measuring the intensity of direct light and transmitted light through the waveguide. The cladded waveguide gives a TM polarized mode, varied by an external polarized sheet. The fructose solution was added on the surface of enzyme cladded waveguide biochip and output was measured. Data was collected via an analogue to digital converter with a high speed parallel link to computer. Fructose solution prepared in acetate buffer was used throughout the measurements. An agarose-guar gum film was used as the waveguiding layer in the constructed biosensor. Initially, the response was recorded only in presence of 0.1M acetate buffer pH 4.5. then the response of the sensor was checked for the blank (AG without enzyme) cladding and also for AG with enzymes in absence of fructose. The fabricated biosensor was calibrated using different concentrations of buffered fructose.

Result and Discussion

A number of natural agar variants exist which involve substitution with 0-methyl, 0-sulphate and pyruvic acid ketals.The important factor here appears to be the degree of substitution by negatively charge groups. The charge density of the polysaccharide chain alerts the degree of aggregation of the double helices in the gel network when mixed with guar gum or other brittle (dea, 1989). The special feature of guar galactomannan is its very high viscosity, so we have choosen the guar gum to increase mechanical strength of composite gel. Gracia and Andrade (1997) reported on a comparative investigation of the gel-setting temperature and mechanical properties of agarose gel at several concentrations and 1:1 agarose guar gum gels of total polymer concentration. It is a unique carbohydrate polymer because it gives the highest viscosity at a equivalent concentrations compaired to other carbohydrate polymers (seaman et al.,

1980). In present studies, we optimized the composite combination for proper imprisonment of the enzymes without leaching. The 3.1 agarose guar gum found to be the best blend, which retain enzymes without leaching (bagal and karve, 2006).

Both agarose and guar gum are hydrophilic biopolymers involved in formation of hydrogen bonding with optical waveguide surface which results into non detachable films of AG sol-gel. The co-immobilization efficiency for both invertase and fructose oxidase was found to be 90% and 81%, respectively. The 3% (w/v) agarose and 1% (w/v) guar gum sol-gel containing 8 units of invertase and 12 units of GOD showed negligible leaching, proving the fine-tuning of the matrix. Further this observation was supported by physical characterization of cladding (SEM images).

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