

## ABSTRACT

The high prevalence of *Diabetes mellitus* (DM) in Indonesia and the high price of biosensor as a diagnosis tool drive any research for preparing biosensor. Enzyme Immobilization that produces a high stability is the key to success in preparing biosensor. This study aims to develop an enzyme immobilization techniques that can produce high stability and its application to the manufacture of biosensors. The *Glucose oxidase* (GOx) enzyme immobilized with covalent binding in chitosan-based composite membranes. Chitosan-based membranes prepared by phase separation method with EIPS, NIPS and combination each method. Membranes characterized include water permeability, morphology and chemical composition. Immobilized enzyme was prepared by immersing the membranes that have been activated by Glutraldehyde (GA) 1% in *Glucose oxidase* (GOx) enzyme solution. Immobilizing GOx enzymes on the membranes that activated provides greater concentration of bound enzyme (CS 37.4  $\mu\text{g}/\text{cm}^2$ , CS-CNT 38.4  $\mu\text{g}/\text{cm}^2$ ) and longer stability than without activation (CS 17.7  $\mu\text{g}/\text{cm}^2$ , CS-CNT 27.4  $\mu\text{g}/\text{cm}^2$ ). pH 5 was the optimum pH for immobilizing GOx. The concentration of enzyme solution affects the concentration of enzyme-bound.  $K_m$  and  $V_{max}$  obtained 0.36 mM and 105 mM/min respectively. Immobilization techniques that have been developed applied to the preparing glucose biosensors and its electrochemical behavior was observed. Third configuration of working electrode (EM-K & CNT-Ez) produces the highest oxidation peak that have 2.3 mA currents.

**Keywords:** enzyme immobilization, *Glucose oxidase*, glucose biosensor, chitosan membrane