

Polypyrrole/glucose oxidase electrode for electrochemical determination of glucose

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INTRODUCTION

A growing interest in biosensors for use in medical, environmental and food analysis has been recognized. Biosensors are devices that transform chemical information, usually the concentration of a specific sample component, into an analytically useful signal. Their selectivity depends on the characteristics of enzyme and biosensors' response rate and sensitivity on electroconducting polymer used.

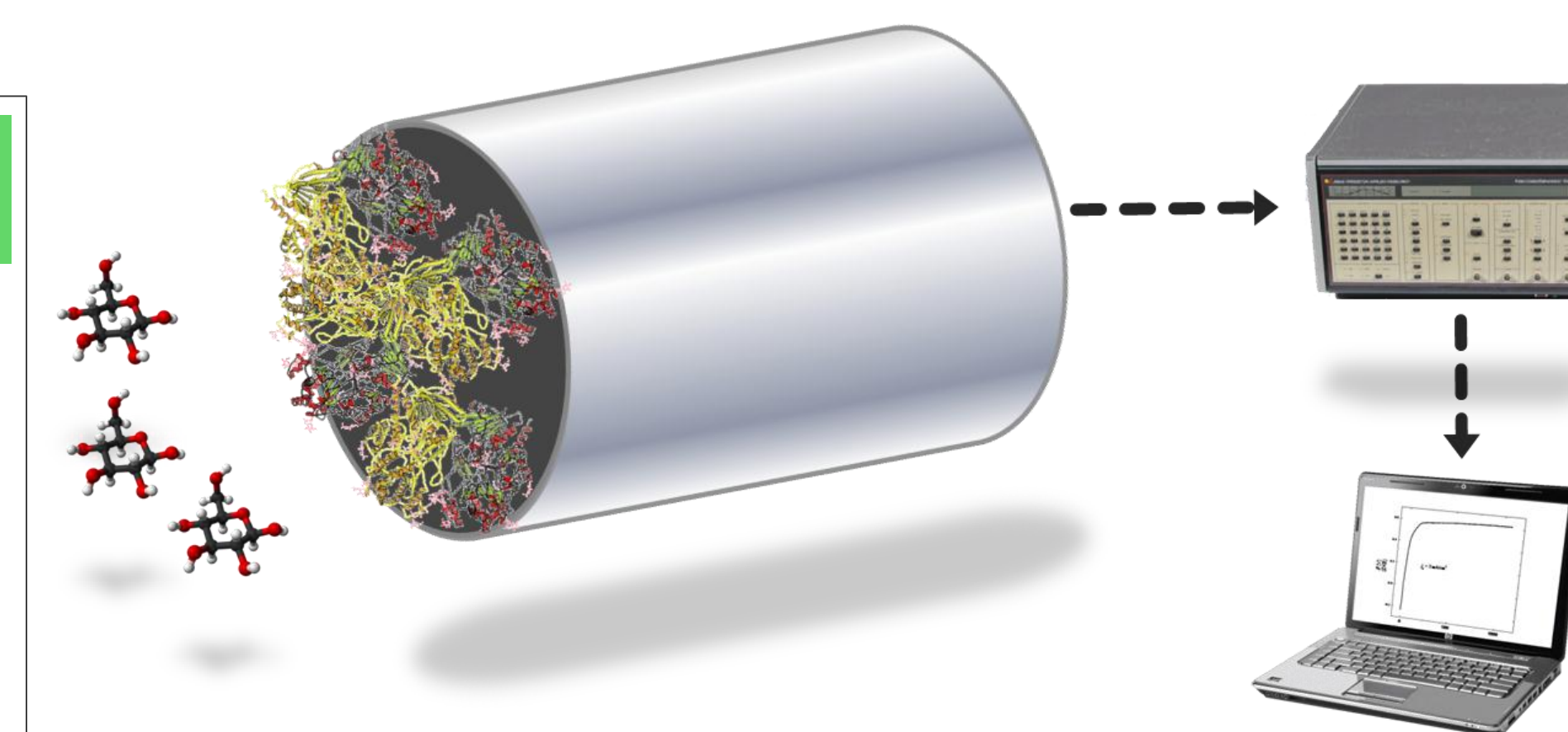
Glucose oxidase (GOx) is the most widely used enzyme in the field of biosensors because of its high specificity for a commercially important analyte, high turnover number and high stability. On the other side, among the conducting polyheterocyclic polymers, polypyrrole (PPy) is of particular interest because the relatively low oxidation potential of the monomer enables films to be grown from aqueous solutions that are compatible with most of biological elements.

The aim of this study was to investigate the possibility of glucose determination using enzyme electrode obtained by immobilization of GOx into polypyrrole electrochemically polymerised on platinum electrode.

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EXPERIMENTAL

Electrochemical polymerization of PPy on platinum electrode was performed at constant current density of 2.0 mA/cm² from aqueous solution of 0.5 mol/dm³ HCl containing 0.2 mol/dm³ pyrrole. Immobilization of GOx from *Aspergillus niger* was performed via glutaraldehyde (1.2% (w/w)). PPy electrode was first left in glutaraldehyde during 1 h, and then immersed in phosphate buffer solution (pH 5.5; 0.1 M) containing 15 mg/cm³ GOx during 24 h. Determination of enzyme amount before and after immobilization was performed by measurement of protein concentration using Bradford method with BSA as standard. Enzyme electrode was investigated at constant current density of 42 nA/cm² in solutions containing different glucose concentration. In order to determine storage stability of PPy enzyme electrode, the electrode was left in phosphate buffer (0.1 mol/dm³, pH=5.5) at 8 °C. Potential-time curves for glucose concentration of 0.1 mmol/dm³ were recorded after 5, 10, 15 and 20 days.



RESULTS

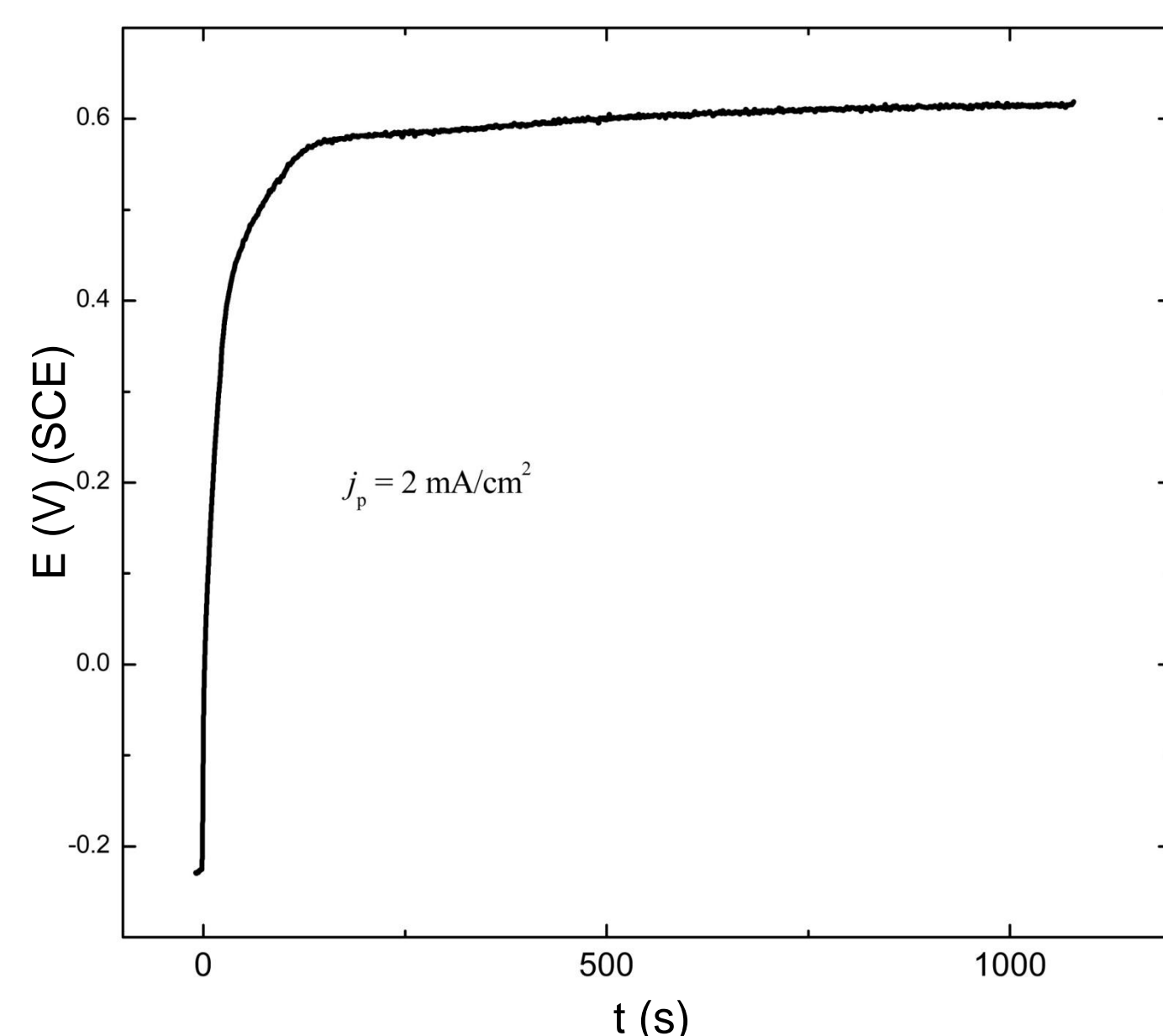


Fig 1. Electrochemical polymerization of PPy from 0.5 mol/dm³ HCl and 0.2 mol/dm³ pyrrole, $j_p=2.0$ mA/cm².

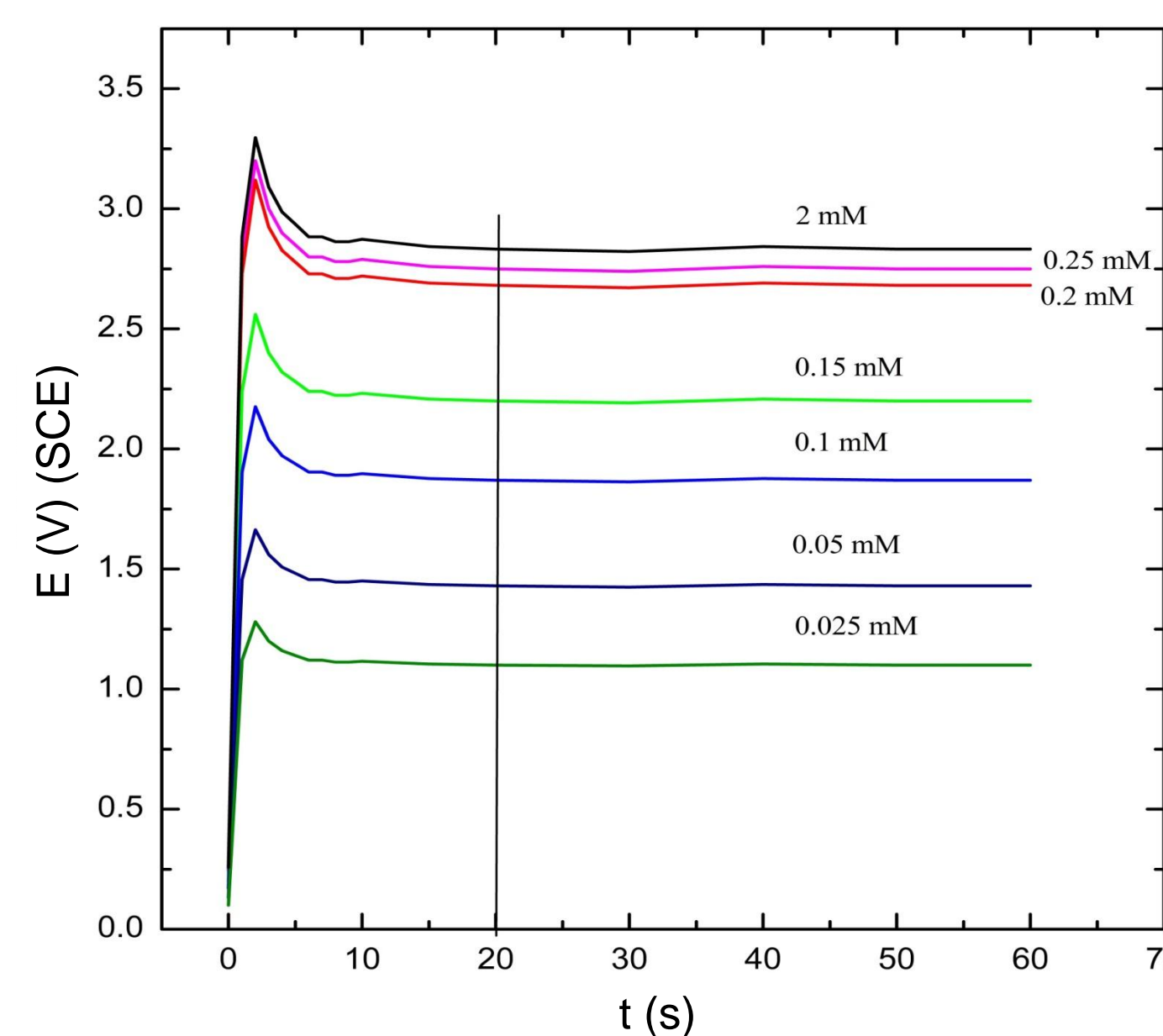


Fig 2. Chronopotentiometric curves of PPy enzyme electrode in glucose solution of 0.025, 0.05, 0.1, 0.15, 0.2, 0.25 and 2 mmol/dm³.

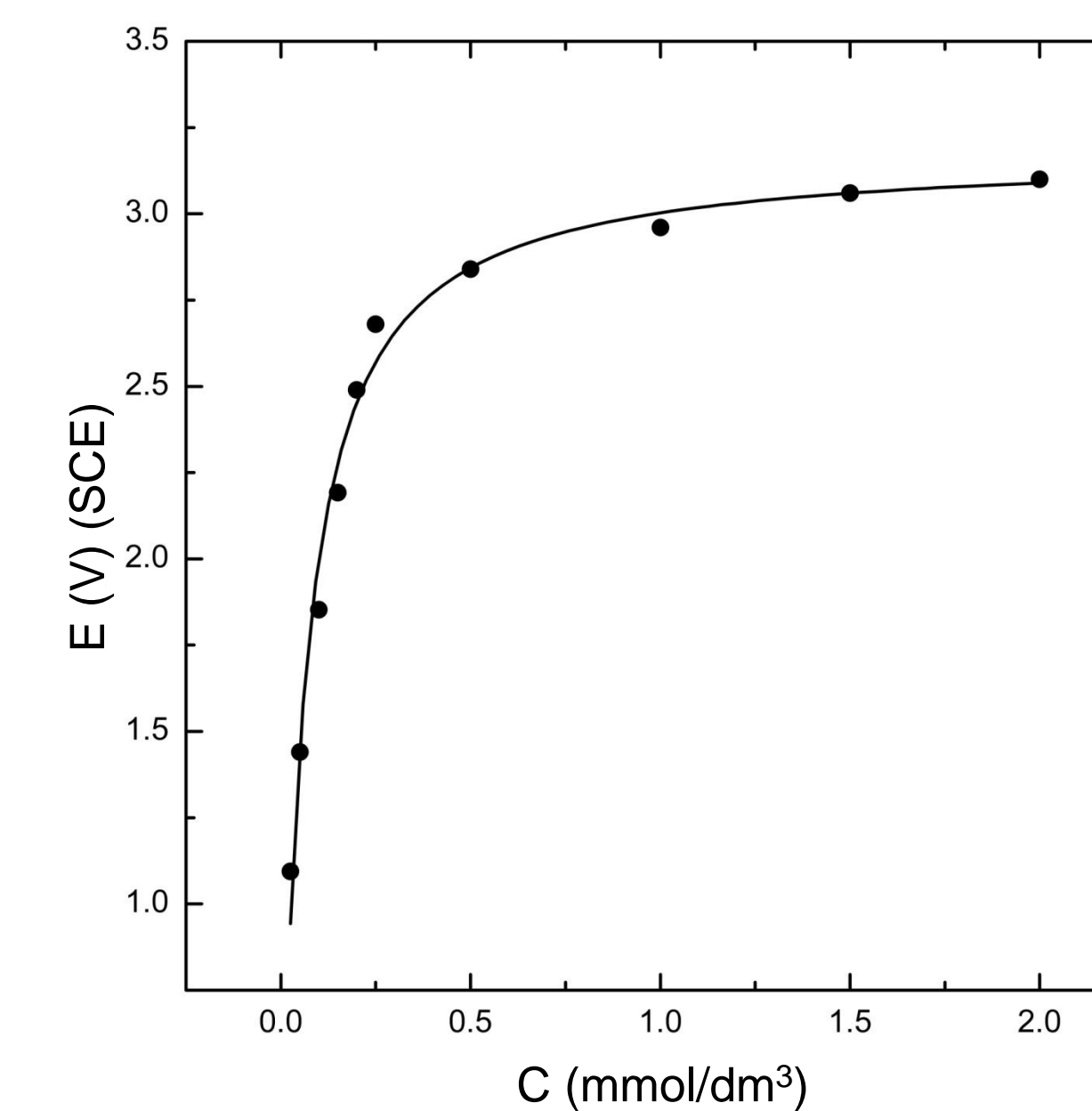


Fig 3. Dependence of PPy enzyme electrode potential on glucose concentration.

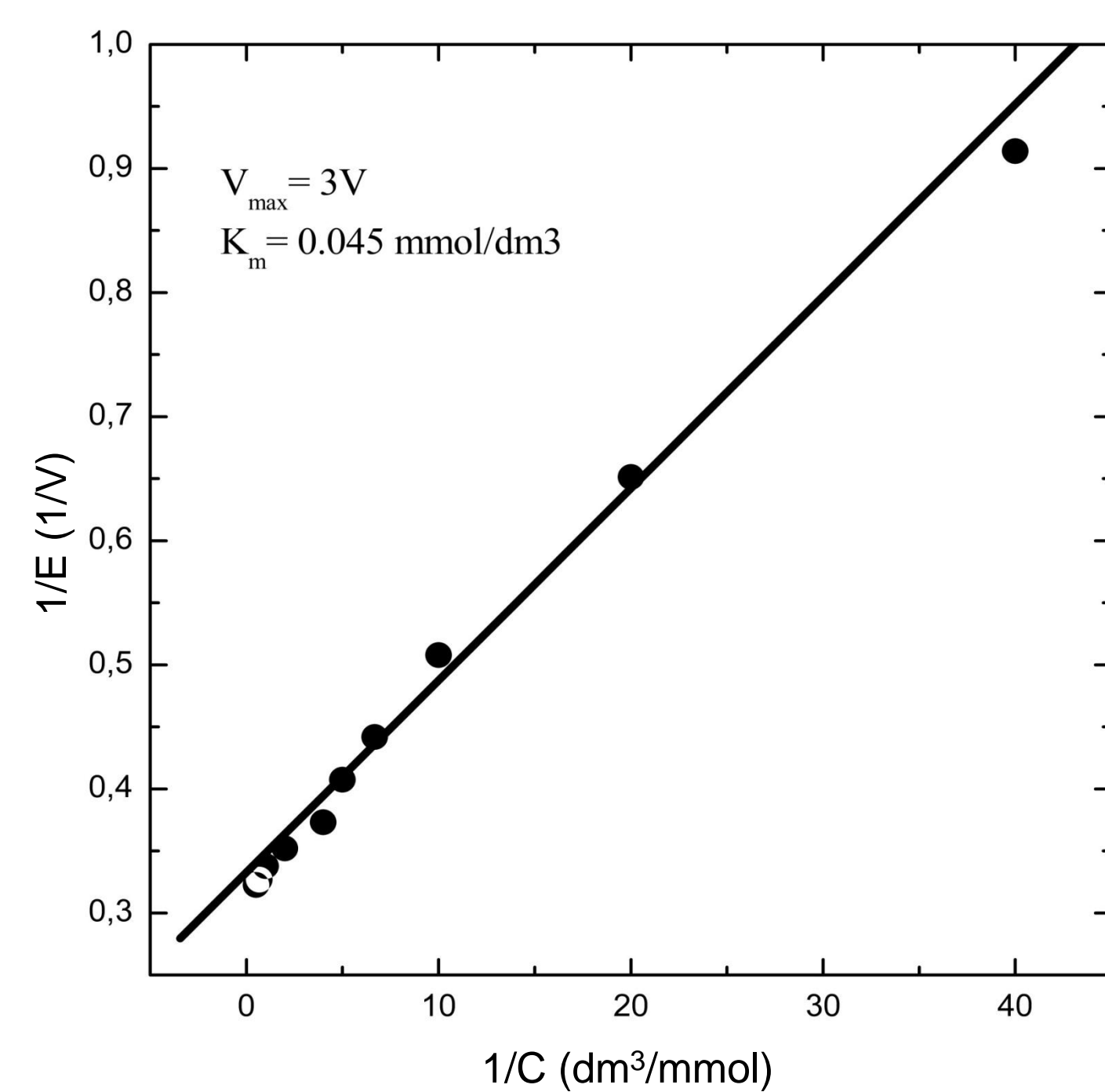


Fig 4. Determination of Michaelis-Menten kinetics parameters.

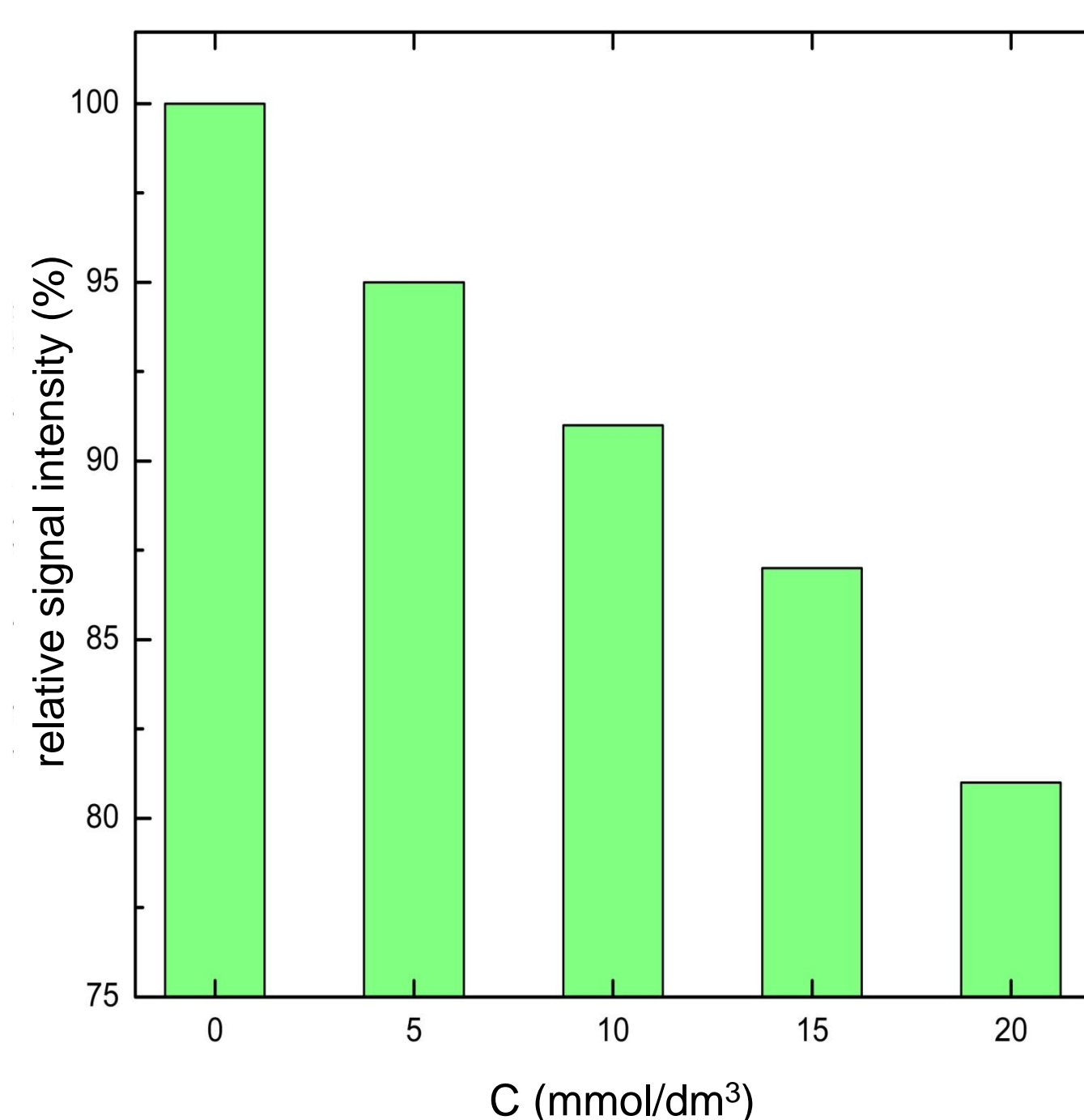


Fig 5. Storage stability of PPy/GOX electrode.

CONCLUSION

PPy enzyme electrode was formed by immobilization of GOx in PPy, electrochemically polymerized on platinum electrode. It was estimated that 93% of proteins were immobilized in PPy enzyme electrode. Apparent Michaelis constant was determined and it was found to be 0.045 mmol dm⁻³, which is much lower than that of free enzyme indicating enhanced enzyme efficiency when it is immobilized into polymer electroconducting matrix. This low value for apparent Michaelis constant is probably the result of excellent three-dimensional structure of PPy that prevents diffusion limitations and allows favorable orientation of bound enzyme and, because of that, high accessibility to substrate. PPy/enzyme electrode lost 5% and 18% of its initial signal after 5 and 20 days, respectively. Loss of the electrode signal could be related to enzyme leaking and PPy degradation during storage which led to lost in polymer conductivity.