Development of Optical Biosensors Based on Oxidases and Hydrogels Performing in Organic Phase and Aqueous Phase Solvents

WU Xiaojun

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Principal Supervisor: Dr. Martin M. F. CHOI

Hong Kong Baptist University

May 2002
ABSTRACT

Six types of optical biosensors have been developed for response to glucose, cholesterol, alcohols and phenolic compounds by immobilising five kinds of oxidases, those are glucose oxidase, cholesterol oxidase, alcohol oxidase, horseradish peroxidase and tyrosinase. The oxidases are entrapped within different supporting matrices with various multiple immobilisation methods. Oxygen-sensitive silica gel particles or silicone films based on a ruthenium(II) complex are employed as oxygen transducers. The quenching effectuation of the optical transducer is quantified by the amount of oxygen consumption in the enzymatic oxidation so as to determine the contents of the analytes. This study emphasised the fabrication of the enzyme immobilisation using supporting materials including silica sol-gel, hydroxyethyl carboxymethyl cellulose sodium salt (HECMC), poly (vinyl alcohol) (PVA), octadecylsilica (ODS) and silica gel particles, the biosensor characterisation, as well as some applications. The multiple architecture of the enzyme immobilisation and the biosensor were designed to protect the immobilised-enzymes, increase the rate of mass transfer processes and enhance their apparent biocatalytic activities.

All biosensors acquired their notable achievements. The glucose biosensor displayed its shelf-time longer than three years. One of the cholesterol biosensors, of which the enzyme was heavily encapsulated within the sol-gel ODS matrix, continuously and fully realised its response in organic-aqueous micelle solution. The other cholesterol biosensor, of which the enzyme was encapsulated in the PVA-HECMC-ODS matrices, well performed in both water-free and water-saturated hydrophobic organic flowing carriers. One of the alcohol biosensors was arranged as a biphasic reactor working in n-hexane or a solvent mixture of acetonitrile (90 % v/v) and water (10 % v/v). The other alcohol biosensor was fabricated into many micro-heterogeneous biphasic reactors working in n-
hexane and it was free from the inhibition of benzyl alcohol and benzyl aldehyde. A cyclic chemical amplification phenolic compounds biosensor was also developed by the combination of shreds of entrapped tyrosinase and adduct of L-ascorbic acid and PVA (AsA-PVA). It worked in hydrophobic organic solvent and efficiently suppressed the polymerisation of o-quinone.

The analytical features of all biosensors were investigated in detail. They all exhibited satisfactory analytical features in terms of sensitivity, response time, shelf and operation lifetimes, repeatability and specificity. Some common issues closely related to biosensors such as enzyme immobilisation, role of water and solvent are also discussed. The glucose biosensor and one of the cholesterol biosensors have been successfully applied to analyse the glucose contents in commercial beverage samples and the free cholesterol contents in commercial butter samples, respectively.
TABLE OF CONTENTS

DECLARATION i
ABSTRACT ii
ACKNOWLEDGEMENTS iv
TABLE OF CONTENTS v
LIST OF TABLES xi
LIST OF FIGURES xii
LIST OF ABBREVIATION xvi

Chapter 1 Introduction 1

1.1 Biosensor 1

1.1.1 Biosensor and composition 1
1.1.2 Enzyme biosensor performed in aqueous phase 2
1.1.3 Enzyme biosensor performed in organic phase 3

1.2 Enzyme immobilisation 4

1.2.1 Methods of enzyme immobilisation 4
1.2.2 Materials of enzyme immobilisation 5

1.3 Transducers 6

1.4. Scope of study 8

1.4.1 Enzyme immobilisation and configuration 8
1.4.2 Optical oxygen transducer 10
1.4.3 Analytical characterisation of biosensors 12

1.5 Objective of the study 15
Chapter 2 Experimental section

2.1 Chemicals

2.2 Fabrication of oxygen transducer

2.2.1 Preparation of oxygen-sensitive particles

2.2.2 Preparation of oxygen-sensitive membrane

2.3 Enzyme immobilisation

2.3.1 GOx in sol-gel-HECMC hydrogel network

2.3.2 ChOx in silica sol-gel-ODS hydrogel network

2.3.3 ChOx in PVA-HECMC-ODS hydrogel network

2.3.4 A0x in HECMC-silica gel water pool

2.3.5 Stabilised A0x and HRP in HECMC-ODS pieces

2.3.6 Tyrosinase in PVA-HECMC co-polymer-ODS materials coupled with AsA-PVA pieces

2.4 Assembly of biosensing system

2.5 Instrumentation

2.6 Response behaviour of oxygen transducer

2.6.1 Response to dissolved oxygen in aqueous phase

2.6.2 Response to dissolved oxygen in organic phase

Chapter 3 A silica sol-gel glucose optical biosensor

3.1 Introduction

3.2 Results and discussions

3.2.1 Response mechanism and dynamic range

3.2.2 Enzyme immobilisation

3.2.3 Effect of dissolved oxygen
3.2.4 Effect of pH
3.2.5 Effect of temperature
3.2.6 Response time
3.2.7 Repeatability and stability
3.2.8 Interference test
3.2.9 Glucose determination in beverage
3.2.10 Preliminary glucose determination in urine sample
3.3 Brief summary

Chapter 4 Cholesterol optical biosensor
4.1 Introduction
4.2 Cholesterol oxidase in ODS-sol-gel matrices
  4.2.1 Results and discussions
    4.2.1.1 Response mechanism and dynamic range
    4.2.1.2 Enzyme immobilisation
    4.2.1.3 Effect of working solution
    4.2.1.4 Effect of pH
    4.2.1.5 Effect of temperature
    4.2.1.6 Response time, repeatability and lifetime
    4.2.1.7 Interference
  4.2.2 Brief summary
4.3 Cholesterol oxidase in PVA-HECMC-ODS network matrices
  4.3.1 Results and discussion
    4.3.1.1 Response mechanism and dynamic range
    4.3.1.2 Enzyme immobilisation
4.3.1.3 Effect of organic solvent
4.3.1.4 Response time, repeatability and stability
4.3.1.5 Effect of pH
4.3.1.6 Lifetime
4.3.1.7 Interference test
4.3.1.8 Free cholesterol analysis in butter samples
4.3.2 Brief summary

Chapter 5 Alcohol optical biosensor
5.1 Introduction
5.2 Alcohol oxidase in silica gel particles and HECMC water pool
5.2.1 Results and discussions
5.2.1.1 Response mechanism and dynamic range
5.2.1.2 Enzyme immobilisation
5.2.1.3 Selectivity
5.2.1.4 Response time and stability
5.2.1.5 Effect of pH
5.2.1.6 Lifetime
5.2.1.7 Interference
5.2.2 Brief summary
5.3 Alcohol oxidase and peroxidase within novel cellulose matrices
5.3.1 Results and discussions
5.3.1.1 Response mechanism and dynamic range
5.3.1.2 Enzyme stabilisation, immobilisation and free-inhibition
5.3.1.3 Effect of oxygen
5.3.1.4 Response time and repeatability
5.3.1.5 Effect of pH
5.3.1.6 Lifetime
5.3.2 Brief summary

Chapter 6 Organic phase phenolic compounds optical biosensor coupled with cyclic chemical reaction

6.1 Introduction
6.2 Results and discussions
6.2.1 Response mechanism and dynamic range
6.2.2 Immobilisation of enzyme
6.2.3 Configuration of cyclic amplification reaction
6.2.4 Effect of oxygen
6.2.5 Effect of pH
6.2.6 Response time and repeatability
6.2.7 Lifetime
6.3 Brief summary

Chapter 7 Discussion

7.1 Role of water
7.2 Effect of solvent

Chapter 8 Conclusion

8.1 Enzyme immobilisation
8.2 Mass transfer
8.3 Analytical feature