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Electrochemical biosensing using hydrogel nanoparticles

Mohammad Hasanzadeh [a,*], Nasrin Shadjou [b,*], Miguel de la Guardia [c]

[a] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran
[b] Department of Nanochemistry, Nano Technology Center, Urmia University, Urmia, Iran
[c] Department of Analytical Chemistry, University of Valencia, Dr. Moliner 50, Burjassot, Valencia 46100, Spain

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ABSTRACT

Biology and medicine have seen great advancements in the development of nanobiosensors capable of characterizing and quantifying biomolecules. This review reports a systematic study of the usefulness of hydrogel nanoparticles (HNPs) in the different steps of the electroanalytical process developed in electrochemical biosensing systems. We illustrate the advantages offered by HNPs in detection of analytes with representative recent examples that highlight the scientific interest in widening the use of HNPs in electrochemical biosensing methods. We review different types of HNP-based electrochemical biosensors, such as enzyme, protein, and nucleic acids, in terms of their sensing performance and their potential for future development.

Contents

1. Introduction

1.1. Why hydrogel nanoparticles?

Nanotechnologies and biotechnologies offer opportunities to design complex and optimized soft structures with synergistic properties. The possibilities for controlling chemical and physical properties *via* the design of 3D gel structures provide a powerful strategy for incorporating versatility into engineering gels of the nanometer scale. Generally, nanocomposite polymer hydrogels may be defined as cross-linked polymer networks swollen with water in the presence of nanostructures or nanoparticles (NPs), which add unique physical properties to these architectures, such as responsiveness to mechanical, optical, thermal, magnetic, and electric stimulation. All these unique properties enable these matrices to be applied in electronic, optical, sensor, actuator and microfluidic systems as well as in catalysis, separation devices, drug delivery, and many other biotechnological areas [1]. Methods for immobilizing biomolecules in different matrices are continually being developed, and such biocomposites have important applications in chemical and biological analyses.

* Corresponding authors. Tel.: +98 914 3619877; Fax: +98 41133632312 (M. Hasanzadeh).
E-mail addresses: mhmmd_hasanzadeh@yahoo.com (M. Hasanzadeh), Nasrin.shadjou@gmail.com (N. Shadjou).

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1.2. Why hydrogel nanoparticles in electrochemical biosensing?

Designing a sensor with hydrogel NPs (HNPs) is a successful approach to obtain superior sensor performance and to minimize the overall cost of a sensor. We therefore summarize below examples of applications of HNPs in electrochemical biosensing reported so far in the literature, with their advantages and limitations, and stress their potential for future developments in this field. Also, we focus on the current surface-modification strategies with HNPs, the influence of polymeric properties on biosensor performance, and the applications of HNPs in conjunction with different transducers for the detection of a variety of analytes. In this review, we selectively review recent advances of HNPs for electrochemical biosensing. Also, we include illustrative examples and compare and contrast enzyme biosensors based on HNPs. We also discuss the feasibility of HNP usage in electrochemical biosensing, for which these technologies might be implemented to produce sensitive assays for clinical diagnostics of genetic and infectious disease. Based on the latest research articles (2009 to January 2014), we summarize HNP-based electrochemical biosensors comprehensively in this review. More importantly, we discuss in detail several outstanding properties of electrochemical immunosensors and opportunities in their research, and the potential and the prospects for their development.

2. Application to glucose and H_2O_2

The determination of glucose concentration is very important in biology, chemistry, food processing and fermentation, as well as in clinic for diagnosing diabetics [2]. Amperometric biosensors based on electron transfer between an electrode and immobilized glucose oxidase (GOD) are especially promising because of their practical advantages, such as operational simplicity, low fabrication cost and suitability for real-time detection.

Recently, Odaci *et al.* [3] prepared a new HNP-based electrochemical biosensor for detection of glucose. In this report, HNP-based electrochemical biosensors were prepared using *in-situ* synthesis of poly(ethylene glycol) (PEG) hydrogels containing gold NPs (AuNPs). In this method, photo-induced processes involving reduction of metal salts to NPs and polymerization of difunctional monomers to cross-linked polymeric matrix occur simultaneously. However, the linear range (0.1–1.0 mM) and the limit of detection (LOD) (0.06 mM) of this work were not acceptable.

Also, a novel strategy was proposed by Chen *et al.* for fabricating a glucose biosensor based on electrodeposited platinum NPs and three-dimensional porous chitosan (CS) membranes [4]. Due to its huge surface area and porous structure, it was a promising matrix for fabricating an efficient biosensor, which had a good response time (within 5 s) and a linear response from 6 μM to 4.2 mM glucose with an LOD of 2 μM. Moreover, the methodology can be applied for the immobilization of other enzymes.

In another report, a new matrix was designed and produced, entailing the co-electropolymerization of pyrrole-biotin (Py-B) with pyrrole-alginate (Alg-Py) [5]. In this work, the sensor construction consisted of the conjugation of biotinylation to Py-B through avidin bridges, followed by copolymerization with Alg-Py. When this set-up did not include the pyrrole-modified alginate but unmodified alginate, its performance values were significantly less (Fig. 1). During measurement of the concentration of the model glucose analyte via hydrogen-peroxide oxidation, the current was observed to be conducted faster to the electrode surface, so providing a slower response time, and a higher sensitivity value, using a relatively low enzyme load. Sensitivity of this sensor was 1.5 μA/mM.

From this report, we conclude that incorporating enzymes into hydrogels *via* electropolymerization of polypyrrole is an effective means of imparting bioactivity to a transducer, but the biotransducer becomes more sensitive to the mass-transport limitations imparted by the over-oxidized polypyrrole. GOD was shown to be stabilized and to increase activity over time within the electroconductive hydrogel.

Another configuration for constructing hydrogel-based electrochemical sensors is the hydrogel-carbon nanotube (CNT). Cui *et al.* [6], demonstrated that redox hydrogel-modified CNT electrodes could be developed for a sensitive amperometric sensor. In this work, a redox polymer, poly(vinylimidazole) complexed with Os (4,4-dimethylbpy) 2Cl (PVI-dmeOs) was electrodeposited on Ta-supported multi-walled CNTs (MWCNTs) (Fig. 2). The resultant PVI-dmeOs thin film did not change the surface morphology of the CNTs, but turned the CNT surface from hydrophobic to hydrophilic. The PVI-dmeOs/CNT electrodes sensed rapidly, sensitively and specifically to model redox enzymes (i.e., GOD and lactate oxidase) in amperometric experiments in electrolyte solutions containing the substrates of the measured redox enzymes. The results obtained by this group suggested that the PVI-dmeOs film may enhance the sensing sensitivities by wiring the enzyme molecules through the redox centers tethered on the mobile redox polymer backbones to the CNT electrodes.

The determination of hydrogen peroxide (H_2O_2) is of great importance in clinical, food, pharmaceutical and environmental analyses. An electrochemical biosensor based on a horseradish peroxidase (HRP) electrode has been extensively employed for H_2O_2 determination due to the procedural simplicity, intrinsic sensitivity and selectivity [7]. To improve the performance of the enzyme electrode, effective immobilization of HRP within a biocompatible material using a simple, controllable procedure is of great significance.

As a biocompatible polymer, CS with a highly porous surface with orderly three-dimensional network was a focus of study in recent years due to its cheapness, hydrophilicity, non-toxicity, excellent film-forming ability and remarkable biocompatibility [8]. These attractive characteristics have prompted its application as one of the most promising matrices for enzyme immobilization.

Xi *et al.* [9] prepared a novel biosensor using *in-situ* formation of CS-CNT-Nile blue-HRP (CS-CNT-NB-HRP) biocomposite film on electrode surface by electrodeposition. This proposed procedure for preparing the biosensor offered a simple, convenient methodology for *in-situ* incorporation of NB, CNTs and HRP into three-dimensional structures of CS hydrogel. The linear response of the reagentless biosensor for the determination of H_2O_2 was in the range 1.0–2.4 μM with an LOD of 0.12 μM.

Recently, Feng *et al.* [10] fabricated a biohybrid hydrogel by integrating HRP with polyhydroxyl cellulose (PHC), which is prepared by mixing poly(vinyl alcohol) and carboxymethyl hydroxyethyl cellulose. PHC has a large number of hydroxyl groups and can create a stable platform for the enzyme to maintain its natural configuration; thereby, HRP exhibits its enzymatic activity while entrapped in the hydrogel. Compared with free HRP, the hybrid hydrogel displays excellent peroxidase activity due to the biocompatibility of the hydrogel. Immobilizing the biohybrid hydrogel onto the surface of an indium tin oxide (ITO) electrode, the direct electrochemistry of HRP was obtained and a third-generation H_2O_2 biosensor was developed successfully. Under optimized conditions, the H_2O_2 biosensor showed a linear response over the range 1.0 μM–1.0 mM with an LOD of 0.5 μM.

A novel mediator-free, third-generation H_2O_2 biosensor has been fabricated by Tan *et al.* [11], based on an Hb-entrapped poly(vinyl alcohol) (PVA)/titanium dioxide (TiO_2) hybrid material. MWCNTs were then dispersed into the composite matrix. It was found that such a hybrid material could retain the native biocatalytic activity of the entrapped Hb in electrochemical experiments. In addition, MWCNTs enhanced the catalytic performance of H_2O_2 and promoted direct electron transfer of Hb immobilized onto the surface of a glassy-carbon electrode (GCE).

Seamless integration of biological components with electrochemical sensors is critical in the development of microdevices for cell analysis. Recently Yan *et al.* [12] described the integration of