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# Biocomposite of cobalt phthalocyanine and lactate oxidase for lactate biosensing with $MnO_2$ nanoparticles as an eliminator of ascorbic acid interference

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**Abstract**

We report on the formation of colloidal biocomposites resulting from the electrostatic self-assembly of negatively charged lactate oxidase (LOD) and oppositely charged nanoscaled cobalt phthalocyanine (NanoCoPc) colloid under enzyme-friendly conditions. The biocomposites were further used to fabricate lactate biosensors due to their excellent film-forming ability and strong adsorbability on the surface of glassy carbon electrode. The electrochemical assays of such films revealed a large capacity of NanoCoPc for the retention of LOD. Here, NanoCoPc colloid was not only used as carriers for immobilization of LOD, but also displayed intrinsic electrocatalytic activity for the oxidation of $H_2O_2$, a product of enzymatic reaction. A chitosan film containing $MnO_2$ nanoparticles was further electrodeposited as an external layer, which could effectively eliminate the interference from ascorbic acid. Under optimal conditions, the biosensor showed a wide linear response to lactate in the range of 0.020–4.0 mM, with high sensitivity (3.98 $\mu A\,cm^{-2}\,mM^{-1}$), as well as good reproducibility and long-term stability. The biosensor has been used for the determination of lactate in real samples with an acceptable accuracy.


*Keywords:* Cobalt phthalocyanine; Nanoparticle; Lactate oxidase; Biocomposite; $MnO_2$

## 1. Introduction

The interest of fabricating lactate biosensors has been widely increasing due to the importance of measurement of lactate in the food analysis, sports medicine and metabolic studies. One of the most important strategies is to use only lactate oxidase (LOD) because of its accessibility and independence of external cofactors [1]. Many approaches have been adopted to develop LOD immobilization techniques, including mixing within the bulk of composite electrode materials or screen-printable pastes [2–4], physical entrapment or encapsulation using polymeric [1,5,6], sol–gel or hydrogel [7,8] matrixes, and covalent binding or cross-linking using functional reagents [9,10]. While offering an effective interface, some of these procedures are tedious, require expensive reagents, and result in poor stability and perturbed function, or environmentally unattractive solvents. For example, the conventional sol–gel process requires the use of strong acid or base as a catalyst, and such strong acid and base as well as organic alcohols formed during the hydrolysis of the silane precursor can cause deactivation of biomolecules [11]; in physical entrapment methods, co-immobilized electron mediators (such as ferrocene derivatives and osmium complexes, etc.) are harmful due to their leakage when the enzyme electrode is used as an implantable biosensor [12]; as a preferable immobilization technique, the covalent bonding of enzymes to a modified substrate, which offers the benefits of stable enzyme attachment, may also interfere with enzyme activity [13], e.g., LOD is completely deactivated upon cross-linking with glutaraldehyde unless the extent of the cross-linking is well controlled [9]. Thus, new immobilization schemes and advanced sensing materials are still highly desired for immobilizing enzymes in an enzyme friendly environment.

Our recent work [14] has developed a novel and stable electrocatalytic system in which cobalt phthalocyanine (CoPc) colloid

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from non-derivative CoPc is prepared by using a conventional ultrasonic dispersion re-precipitation in the surfactants solution. This system provides a kind of enzyme-friendly environment. Under the neutral condition, the biocomposite can be formed between the negatively charged enzyme and the oppositely charged nanoscaled cobalt phthalocyanine (NanoCoPc) colloid. This provides not only an easy method for enzyme immobilization, but also a high activity of the immobilized enzyme by ionic bonding [15]. This kind of the biocomposite material also possesses an excellent film-forming ability and a strong adsorbability, providing a possibility to fabricate a long-term stable biosensor.

As we know, in the presence of oxygen, LOD can catalyse the conversion of lactic acid into pyruvic acid and $H_2O_2$. The amount of $H_2O_2$ formed can be measured amperometrically. However, direct amperometric detection of $H_2O_2$ requires a relatively high oxidation potential at the unmodified electrode, and thus it may suffer severe interference from readily oxidizable species, such as ascorbic acid, in real samples. In order to eliminate this interfering substance, two most common strategies were used. One was to employ a permselective membrane that minimized the access of the interfering substance to the electrode surface. The permselectivity of the membranes is based on either size selectivity [5,16] or charge exclusion [2,17]. The other strategy was to employ an additional modification of the electrode by catalysts [3,5,6], mediators [17], or more complicated multi-enzyme systems [4,18,19] that allowed detection of $H_2O_2$ at lower potentials and therefore reduced the current responses of interfering species. However, some concomitant problems are still found with eliminating the interferents. For instance, in the former method, the permselective membrane may decrease the biosensor sensitivity and only partially eliminate the interferents [2,5,17]. These poor performances may be caused by the same charge of lactic acid as that of ascorbic acid under normal conditions. While in the latter method, a rather complicated procedure or expensive reagent is required for the fabrication of the biosensor [19]. Recently, as an alternative strategy to eliminate the interferent, our group has approved that the pre-oxidation of the interfering substances with chemical oxidants ($MnO_2$ nanoparticles) contained in the membrane can effectively eliminate the interference from ascorbic acid in the determination of glucose [20]. The merit of this strategy may be more obvious in the system where the substrate and the interferent possess of the same charge.

In this work, a simple approach to LOD immobilization is described to fabricate a lactate biosensor. The method relies on the formation of a biocomposite, LOD–NanoCoPc, between LOD molecules and NanoCoPc colloid through electrostatic interactions. The experimental conditions are investigated in relation to the components of the films and the performance of the biosensors is characterized in detail. In addition, a strategy is introduced to eliminate the interference from ascorbic acid, in which the interferent is pre-oxidized by the chitosan and $MnO_2$ nanoparticles co-deposited as an external layer on the biosensor. The resulting biosensor can be used in the analysis of real samples.

## 2. Experimental

### 2.1. Chemicals and apparatus

Lactate oxidase from *Pediococcus* species (EC 1.1.3.2, 37 U $mg^{-1}$), lithium L-lactate, uric acid, β-D-glucose, 4-acetamidophenol and Chitosan from crab shells (85% deacetylated) were purchased from Sigma, and a Nafion (5%, v/v) ethanol solution and cobalt phthalocyanine were obtained from Aldrich. The chemicals were all used as received without further purification. All other reagents were commercially available and of analytical grade. All the solutions were prepared with doubly distilled water.

Chitosan solutions were prepared by adding chitosan flakes to water and gradually adding 2.0 M HCl to the solution to maintain the pH near 3.0, followed by filtration. The pH of the filtrate was adjusted to about pH 5.0 by using 1.0 M NaOH.

### 2.2. Preparations of the NanoCoPc colloid and biocomposite suspension of LOD–NanoCoPc colloid

The NanoCoPc colloid was prepared similarly as described previously [14] with a little modification. Briefly, 0.10 g CoPc was first dissolved in 5 mL 98% concentrated sulfuric acid, then the solution was added drop by drop into a 100 mL aqueous solution containing 0.60 g hexadecyltrimethyl ammonium bromide in an ice-water bath under ultrasonic and violent stirring conditions. The resulting transparent blue colloidal solution was filtrated by a semipermeable membrane until it became neutral, and was diluted to 150 mL with water as a stock solution. The biocomposites of LOD–NanoCoPc colloid were prepared by adding a LOD solution (1.4 mg $mL^{-1}$) to CoPc colloidal stock solution at a ratio of 1:2 under ultrasonic condition.

### 2.3. Preparation of the modified electrodes

Prior to modification, the glassy carbon electrode (GCE, 3 mm diameter) was first polished with sand paper followed by 1.0, 0.3, and 0.05 μm alumina slurry, respectively, then sonicated in a water bath to remove any residual. Then the nanoscaled CoPc colloid modified GCE (NanoCoPc/GCE) or the LOD–NanoCoPc colloids modified GCE (LOD–NanoCoPc/GCE) were prepared by coating a 15 μL NanoCoPc stock solution or biocomposite colloid solution on the GCE, followed by air-drying about 2–3 h and rinsing with water several times for use.

In order to eliminate the influence of interfering species, two strategies were designed: (i) a Nafion film and (ii) a chitosan film containing $MnO_2$ nanoparticles as an external layer of the biosensor. In the former, the Nafion film was deposited according to the literature [17]. Briefly, a LOD–NanoCoPc film was covered with a layer of Nafion by applying 2× 5 μL of 0.3% Nafion (a commercial 5% Nafion was diluted with ethanol and neutralized with 25% ammonia), followed by evaporation of the solvent and then rinsing with water. The Nafion coated electrode was denoted as Nafion/LOD–NanoCoPc/GCE. In the second strategy, the preparation method of $MnO_2$ nanoparticles