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# Development of label-free electrochemical lactose biosensor based on graphene/poly(1,5-diaminonaphthalene) film

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ABSTRACT

In this work, a lactose biosensor was developed by co-immobilizing β-galactosidase (β-Gal) and glucose oxidase (GOx) on microelectrodes pre-modified with Pt/graphene/P(1,5-DAN) for estimation of lactose in dairy products to prevent lactose intolerance. The Pt microelectrode was modified with graphene and 1,5-polydiaminonaphthalene film. Graphene was synthesized by chemical vapor deposition on copper tape and manually transferred to the electrode surface. Polymeric P(1,5-DAN) was grafted on top of the graphene film by electropolymerization. Modified surface of the electrode was characterized by Raman spectra analysis, FE-SEM, AFM and cyclic voltammetry. The results indicated that deposition graphene film on electrode surface induced considerable enhancement in current signal, over 20 times as high as the uncoated electrode surface. The developed sensor was successfully used to determine lactose in model samples with sensitivity, correlation coefficient ($R^2$) and limit of detection (LOD) estimated to be 1.33 μA/(μgml$^{-1}$), 0.995 and 1.3 μg/ml, respectively. The combined graphene and conductive P(1,5-DAN) could serve as a novel sensing platform on electrochemical sensors with superior sensitivity.



## 1. Introduction

Conductive polymers, due to their unique physicochemical properties, have attracted considerable attention in several areas [1]. Recently, polydiaminonaphthalene (PDAN), a conductive polymer synthesized from aromatic diamine, has been extensively used as electrocatalysts, adsorbents and especially as electroactive membrane in chemical and biological sensors [2–5]. PDAN possesses a variety of interesting characteristics such as electroconductivity, electroactivity, electrochromism, and electrocatalysis. In addition, the chemical reactivity of functional amino groups on polymeric structure was reportedly related to chelating or reduction ability of PDAN [6]. The compatibility and conducting properties of P(1,5-DAN) have been explored to facilitate the binding of biomolecules or to shorten the distance between electrode and enzyme active sites [5].

On the other hand, in electrochemical researches, chemical modification of the electrodes is vital for improving performance and capabilities of the sensors. Depending on applications, chemical, electrochemical, optical, electrical and transport properties of the electrodes can be controlled and manipulated in a rational manner [7]. Some authors reported that coating the electrode surface with polymeric films could enhance the power and scope of electrochemically modified electrodes [8–12]. In addition, such modifications decrease the over potential, increase reaction rate, improve electrocatalytic properties of substrates, stability and reproducibility of the electrodes [13]. The unique

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properties of P(1,5-DAN), therefore, make it an excellent material for modifying electrochemical electrodes. Numerous studies have applied P(1,5-DAN) in electrochemical sensors for detection of cholesterol, hydrogen peroxide ($H_2O_2$), domoic acid, and water [5,14–18]. Electrodes modified with polymeric films can be prepared via different methods. However, electropolymerization is widely preferred due to its convenience. In addition, the deposition can be easily manipulated by adjusting the electrochemical parameters [19]. Nevertheless, the performance of P(1,5-DAN)-based modified electrode is hindered by its compactness, low specific areas and restrictive sites which limit the diffusion of substrate from solution to the electrode surfaces [5]. In addition, P(1,5-DAN) has very low conductivity at neutral pH which strongly affects the sensitivity of the P(1,5-DAN) film [2]. To enhance the performance of P(1,5-DAN), various nanomaterials such as iron oxide ($Fe_3O_4$), zinc oxide (ZnO), carbon nanotube (CNT) have been included in the P(1,5-DAN) membrane [5,6,20–22]. For example, the nanocomposite P(1,5-DAN)/CNT was successfully used in detection of ascorbic and uric acids in voltammetric analysis of heavy metals with significantly improved in sensitivity, selectivity and reproducibility [23].

Graphene, a carbon-based nanomaterial, is the subject of countless researches, especially on its electrochemical behavior. The high specific surface area and excellent electron conductivity of graphene are useful in various applications such as fuel cells, supercapacitors, batteries and electrochemical sensors [24–30]. Recently, chemical vapor deposition (CVD) has been proven to be an effective method to produce high quality graphene at low production cost. In addition, CVD graphene has great potentials in electrochemical sensing applications [31,32]. However, the combination of CVD graphene film and PDAN as the materials for modifying electrochemical electrodes has not been reported.

The objective of this study was to investigate the effects of combined CVD graphene film and P(1,5-DAN) on sensing performance of electrochemical electrode. Electrochemical behavior and nanostructure of the modified electrode were characterized by cyclic voltammetry, Field Emission Scanning Electron Microscopy (FE-SEM) and Atomic Force Microscopy (AFM). The prepared electrode was used as a lactose biosensor to evaluate its performance in real sensing conditions.

## 2. Experimental

### 2.1. Chemicals

Monomer 1,5-diaminonaphthalene (1,5-DAN), β-galactosidase (β-Gal, EC 3.2.1.23, from *Aspergillus oryzae*, 8000 U/g) and Glucose oxidase (GOx, EC 1.1.3.4, from *Aspergillus niger*, 245,900 U/g) enzymes were purchased from Sigma Aldrich (Germany). The D-lactose (MW = 360.31) was purchased from Fluka (Switzerland). Deionized water with resistivity of about 18 MΩ cm was used for all the experiments.

### 2.2. Microelectrode fabrication

The electrochemical microelectrodes was fabricated on $Si/SiO_2$ 4-inch wafer by the standard CMOS technology. The 100 nm-thickness working electrode (WE) and counter electrode (CE) was patterned and deposited by Pt sputtering (with Cr adhesion layer). The reference electrode (Ag/AgCl) was fabricated by chlorination process of 100 nm Ag layer in 50 mM $FeCl_3$ solution for 15 s. The diameter of WE was 200 μm whereas the width of CE and RE was 100 μm [33].

### 2.3. Graphene synthesis

The graphene film was synthesized by thermal CVD method at 1000 °C in Argon (Ar) environment (1000 sccm) [34]. Briefly, copper (Cu) tape having thickness of 35 μm was used as substrate for graphene-film growth. To reduce the native copper oxide and to facilitate the growth of Cu grains on Cu tape surface, the samples were annealed at CVD temperature (1000 °C) for 30 min under the flow of Ar (1000 sccm) and hydrogen (300 sccm). After 30 min, methane ($CH_4$, 30 sccm) was introduced to initiate the synthesis of graphene film. After a preset time, the samples were cooled rapidly under the flow of Ar (1000 sccm) [34]. The transfer of graphene to working electrode of the sensor was described by Nguyen et al. (2012) [35]. In brief, a layer of polymethyl methacrylate (PMMA) was coated on top of the graphene film. The underlying Cu tape was chemically etched by iron (III) nitrate solution. Residual Cu was removed by transferring the suspending graphene film to deionized water. Then, the graphene/PMMA film was transferred to working electrode and the PMMA layer on the top was dissolved by acetone. The modified electrode was washed several times by deionized water before use.

### 2.4. Electropolymerization of P(1,5-DAN) film on the surface of Pt/graphene working electrode

The P(1,5-DAN) film was electrochemically synthesized on the Pt/graphene electrode surface by cyclic voltammetry (CV). The electrodes were immersed in 1 M $HClO_4$ solution containing 1.00 mM 1,5-DAN monomer. Electrochemical polymerization of P(1,5-DAN) was performed in 20 cycles with scan rate of 50 $mV^{-1}$ and potentials ranging from −0.02 to +0.95 V. The electropolymerization of P(1,5-DAN) film was conducted using Autolab PGSTAT30 potentiostat (Ecochemie, Netherlands) [23].

### 2.5. Immobilization of enzymes on Pt/graphene/P(1,5-DAN) modified electrode

To facilitate the immobilization of enzymes onto the electrode surface, the obtained electrodes were incubated in 25% glutaraldehyde vapor for 1 h. Glutaraldehyde served as a cross-linker for enzyme binding (Scheme 1). The immobilization of 80 IU β-galactosidase (β-Gal) + 22131 IU glucose oxidase (GOx) in 1 mL PBS 1× solution was conducted by dispersing one drop (10 μl) of the mixture onto the surface of the as-prepared Pt/graphene/P(1,5-DAN) working electrode. The electrode was let dried overnight at 4 °C in the air before use.

### 2.6. Characterization of the modified electrode

The electrode surface was characterized by FE-SEM (Hitachi S-4800), operating at an acceleration voltage of 10 kV. Atomic force microscopy (AFM) images were obtained by scanning probe microscope (PicoScan 2500, Agilent, USA) in the tapping mode. Single lever silicon probe (force constant of 46 $Nm^{-1}$ and resonance frequency of 354 kHz) was used as cantilever for all measurements. Raman spectra of graphene and PDAN were measured by LabRAM Raman spectroscopy (Horiba, Japan) under ambient condition with excitation laser of He–Ne at 632.8 nm.

### 2.7. Electrochemical measurements

All electrochemical measurements were conducted by Autolab PGSTAT 30 electrochemical analyzer (EcoChemie, the Netherlands). Electrochemical behaviors of the modified electrodes were characterized by cyclic voltammetry (CV) method with scan rate of