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# Development of a sensitive and selective kojic acid sensor based on molecularly imprinted polymer modified electrode in the lab-on-valve system

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ABSTRACT

In this work, a kojic acid electrochemical sensor, based on a non-covalent molecularly imprinted polymer (MIP) modified electrode, had been fabricated in the lab-on-valve system. The sensitive layer was synthesized by cyclic voltammetry using *o*-phenylenediamine as the functional monomer and kojic acid as the template. The template molecules were then removed from the modified electrode surface by washing with NaOH solution. Differential pulse voltammetry method using ferricyanide as probe was applied as the analytical technique, after extraction of kojic acid on the electrode. Chemical and flow parameters associated with the extraction process were investigated. The response recorded with the imprinted sensor exhibited a response in a range of 0.01–0.2 $\mu$mol L$^{-1}$ with a detection limit of 3 nmol L$^{-1}$. The interference studies showed that the MIP modified electrode had excellent selectivity. Furthermore, the proposed MIP electrode exhibited good sensitivity and low sample/reagent consumption, and the sensor could be applied to the determination kojic acid in cosmetics samples.

## 1. Introduction

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone) has many applications in cosmetics. Many consumers use products containing kojic acid and its derivatives to lighten freckles and other dark spots on the skin. In the area of food production, kojic acid is used to preserve food color and kill certain bacteria because it can inhibit the formation of dihydroxyphenylalanine from tyrosine in the process of melanin biosynthesis [1–3]. In addition, kojic acid plays an important role in monitoring fermentation process. However, because of the 4-pyrone in kojic acid molecules, it may have adverse effect on human health like benzene [4,5], so it is necessary to develop an assay method for the analysis of kojic acid.

Current methods used to determinate kojic acid are high performance liquid chromatography (HPLC) [6,7], ion pair liquid chromatography (IPLC) [8] and electrochemistry [9]. Among these methods, chromatography analysis is commonly adopted for specific determination of kojic acid, nevertheless this requires relatively expensive and complicated instruments. Electrochemistry shows attractive characteristics of high sensitivity, relatively inexpensive instrument and multi-element detection. Reports can be found for kojic acid electrochemical determinations with different modified electrode [10–12].

In recent years, the development of highly selective sensors for diverse applications has been the target of great research efforts. The demand of selectivity can be qualified by the specific interaction between analytes and chemical matrix of the sensor. Therefore, molecularly imprinted polymer (MIP) with excellent recognition ability appears as promising candidates to accomplish such requirements [13–15]. In particular, MIP offers important advantages such as the possibility of synthesizing polymers with a redetermined selectivity for a particular analyte. The imprinting process is obtained by polymerizing in the presence of a template molecule. After polymerization, the template is removed by washing, and then MIP demonstrates recognition properties towards the target analyte, due to the shape and chemical functionality considerations in the sites in the polymer matrix [16–18]. For the fabrication of the MIP based sensor, electrochemical methods are very often used both at the stages of preparing and using the MIP to detect the analytes. However, these methodologies are restricted to batch mode operations, which were laborious and large sample/reagent. From this respect, coupling of the on-line flow system using MIP as selective determination can be used as an excellent alternative for the development of electrochemical detection. Although some flow injection procedures based on amperometric detector using MIP as a selective solid phase extraction sorbent for sample preconcentration were described, due to

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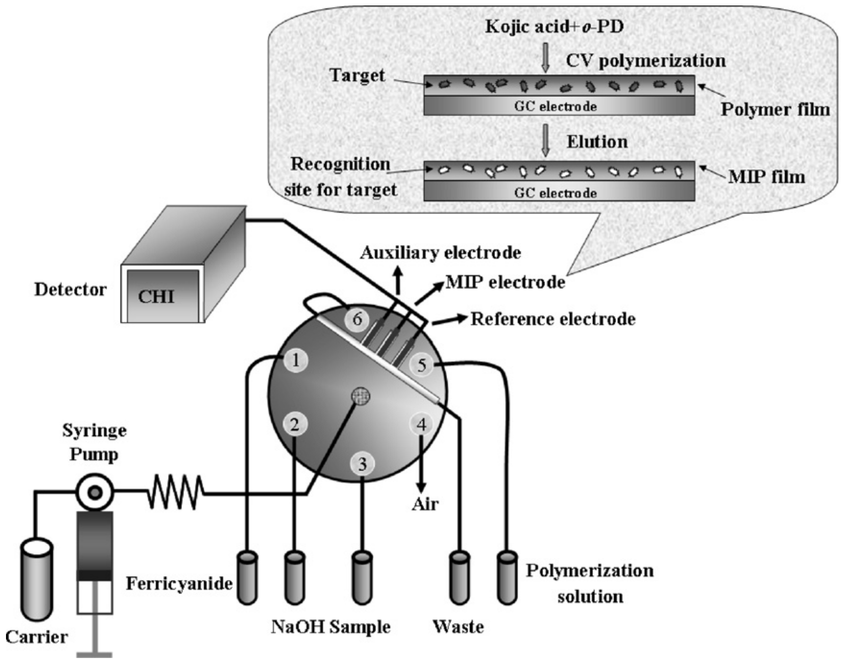

**Fig. 1.** Illustration of the SI-LOV manifold for the determination of kojic acid with MIP modified electrode.

their continuous flow character, still needed large amounts of reagent [19–21].

The sequential injection lab-on-valve (SI-LOV), as the third generation of flow injection, integrated all necessary laboratory facilities for fluidic handing, such as connecting ports, working channels and a multi-functioned flow cell [22–24]. It has been employed in a variety of applications of chemical and physical processes with detection by electrothermal atomic absorption spectrometry (ETAAS) [25], inductively coupled plasma mass spectrometry (ICPMS) [26], atomic fluorescence spectrometry (AFS) [27] and liquid chromatography (LC) [28]. In previous literatures, SI-LOV manifold coupled with electrochemical methods have been reported [29–31], which highlighted the advantages of electrochemical detection technique coupled with on-line operation systems. In this research we firstly developed a kojic acid sensor for sensitive and selective determination of kojic acid levels in cosmetics using a combination of molecular imprinting technology and electrochemical method in the SI-LOV system. The assay protocol comprised the following steps, including the electrode preparation, electrode washing, analyte extraction and electrochemical measurement. The characteristics of the MIP sensor were studied in details, and the proposed method was used successfully for kojic acid determination in cosmetics samples.

## 2. Experimental

### 2.1. Apparatus

Electrochemical studies were performed using a CHI660A electrochemical workstation (Chenhua Instrument, Shanghai, China). The SI-LOV system consisted of the following components: a FIAlab-3000 sequential injection system (FIAlab Instruments, Bellevue, WA, USA) and a home-made LOV unit. The sequential injection system equipped with a 2.5 mL syringe pump (Cavro, Sunnyvale, CA, USA) was employed for sample and reagent delivery. FIA software for Windows 5.0 was used to control the instrument. The LOV unit, incorporating an electrochemical flow cell (EFC) with a volume of 200 μL, was mounted on the multiport valve. A glassy carbon electrode modified with MIP was used as working electrode. A platinum wire and an Ag/AgCl electrode were used as the counter and reference electrodes, respectively. The scanning electron micrographs (SEM) images were obtained by scanning electron microscope (Hitachi S-4800, Japan). All externally used tubes were made of 0.8 mm i.d. PTFE tubing (Upchurch Scientific, OakHarbor, WA, USA).

### 2.2. Reagents and materials

Kojic acid and *o*-phenylenediamine (*o*-PD) were purchased from Aladdin Reagent Company (Shanghai, China). Other chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 1 mmol $L^{-1}$ ferricyanide solution that serves as probe was prepared by dissolving ferricyanide in 0.1 mol $L^{-1}$ KCl medium. 1.2 mol $L^{-1}$ NaOH solution was used for template removal. 5 mmol $L^{-1}$ *o*-PD containing 10 mmol $L^{-1}$ kojic acid in 0.2 mol $L^{-1}$ acetate buffer solution (pH 5.2) was used for electropolymerization. 0.2 mol $L^{-1}$ acetate buffer solution (pH 5.2) was employed as carrier stream. The chemicals and reagents were at least of analytical reagent grade and used without further purification. Double de-ionized water (18 MΩ $cm^{-1}$) was used throughout the experiments. Nitrogen gas (99.999%) was used for purging oxygen.

### 2.3. Operating procedure

Before determination, the glassy carbon electrode surface was polished manually to obtain a fresh surface. The proposed flow system for the determination of kojic acid was depicted in Fig. 1. The central port was connected to the syringe pump and also to any of the six ports of the multiport valve, at the same time, allowing the aspiration of ferricyanide (port 1), NaOH (port 2), sample (port 3) and polymerization solution (port 5) into the holding coil. The procedure consisted of the following steps, as described below: 200 μL of polymerization solution was aspirated by syringe pump and stored in the holding coil. Then the solution was reversed, and dispensed into the EFC while a potential range between 0 and 0.8 V was applied to the glassy carbon electrode during nine cycles (scan rate: 50 mV/s). After the electropolymerization process, 200 μL of NaOH solution was drawn pass the EFC to remove the template inside the polymer. Thereafter, 200 μL of sample solution was aspirated