Talanta 158 (2016) 134–141

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Droplet-based glucosamine sensor using gold nanoparticles and polyaniline-modified electrode

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ARTICLE INFO

Article history:
Received 14 February 2016
Received in revised form
16 May 2016
Accepted 16 May 2016
Available online 16 May 2016

Keywords:
Droplet-based microfluidics
Glucosamine
Carbon paste electrode
Central composition design,
chronoamperometry

ABSTRACT

A droplet-based electrochemical sensor for direct measurement of D-glucosamine was developed using carbon paste electrodes (CPEs) modified with gold nanoparticles (AuNPs) and polyaniline (PANI). Central composition design (CCD) was employed as a powerful method for optimization of parameters for electrode fabrication. The optimized amounts of AuNPs and PANI obtained from the response surface were determined as 300 and 3000 mg L^{-1}, respectively. Coupled with a droplet microfluidic system, the analysis of glucosamine was performed in a high-throughput manner with a sample throughput of at least 60 samples h^{-1}. In addition, the adsorption of the analyte on the electrode surface was prevented due to compartmentalization in droplets. Linearity of the proposed system was found to be in the range of 0.5–5 mM with a sensitivity of 7.42×10^{-3} A mol^{-1} L cm^{-2} and limits of detection and quantitation of 0.45 and 1.45 mM, respectively. High intraday and interday (evaluated among 3 days) precisions for the detection of 50 droplets containing glucosamine were obtained with relative standard deviation less than 3%. The system was successfully used to determine the amounts of glucosamine in supplementary products with error percentage and relative standard deviation less than 3%. In addition, the amounts of glucosamine measured using the developed sensor were in good agreement with those obtained from a CE method. These indicate high accuracy and precision of the proposed system.

1. Introduction

Osteoarthritis (OA) or a degenerative joint disease is a group of mechanical abnormalities of joints, which especially occurs in the elderly and overweight people as well as athletes [1]. D-Glucosamine (Glc*N*), an amino monosaccharide found in connective tissues and gastrointestinal mucosal membranes [2], is widely used to alleviate the symptoms of OA because it has been believed that Glc*N* prevents the deterioration of the cartilage and surrounding fluid in the joints [3]. Therefore, Glc*N* is manufactured as supplementary products and marketed to people suffering from OA [4]. Nowadays, the use of Glc*N* supplements for treatment of OA is growing rapidly, leading to a variety of commercial brands of Glc*N* supplements distributed into the markets. Therefore, a simple and rapid method for quantitative analysis of Glc*N* is required to control the quality of Glc*N* products during manufacturing processes and the products distributed into the markets.

Previously, the analysis of Glc*N* was achieved using several methods, including fluorescent sensors [5], capillary electrophoresis with fluorescence detection [6] and high-performance liquid chromatography with a variety of detection systems, such as electrochemical detection [7,8], mass spectrometry [9–11], refractive index [11,12], fluorescence [2,6,13] and UV detection [3]. Since Glc*N* is a monosaccharide derivative of glucose, quantitative determination using fluorescence detection was required a derivatization step using a fluorescent dye. Therefore, electrochemical detection was favorably performed by taking advantages of sugar compounds exhibiting electrochemical properties. Accordingly, the analysis of Glc*N* using electrochemical detection was easily achieved with its native structure. Therefore, we present herein a facile and reliable method using an electrochemical sensor as an alternative for quantitative determination of Glc*N* supplements. Since less matrix effect is generally found in supplementary products when compared to biological fluids, an electrochemical sensor was developed for a direct measurement of Glc*N* without a separation step. In addition, a Glc*N* sensor was coupled

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http://dx.doi.org/10.1016/j.talanta.2016.05.052
0039-9140/

with a droplet microfluidic system in order to perform the analysis in a high-throughput manner with small sample consumption. Moreover, compartmentalization in droplets prevented the adsorption of analytes on the electrode surface. Accordingly, no washing and drying steps between consecutive runs were required, resulting in minimization of analysis time compared with batch-wise electrochemical sensing methods. Therefore, advantages of a droplet system, including rapid mixing, no sample dispersion and adsorption, low sample consumption, portability and high throughput analysis, enhance the analytical performance of the chip-based Glc*N* sensor.

In this work, carbon paste electrodes (CPEs) were selected to fabricate a chip-based Glc*N* sensor. CPEs are easily to fabricate into a chip-based format and possess a wide working potential and withstand electrode fouling [14]. Gold nanoparticles (AuNPs) were used to modify chip-based CPEs because it was previously reported that gold electrodes were able to catalyze the oxidation of carbohydrates [7] and AuNPs modified carbon electrodes were used to monitor Glc*N* during the synthesis of glucosaminic acid [15]. The advantages of AuNPs are that they are inexpensive and possess more surface area when compared to gold-pad electrodes. In addition, polyaniline (PANI), a well-known conducting polymer, was also employed for electrode modification to enhance electrochemical signal due to its excellent electrochemical properties, good environmental stability and non-toxicity [16]. Central composition design (CCD) was used in this work to optimize pH of the working medium and the amounts of PANI and AuNPs for electrode modification. The box-like design of experiments was carried out and a responsive surface was then plotted to formulate an equation to find the optimized conditions of each parameter (pH, the amounts of PANI and AuNPs) [17,18] Under the optimized conditions, the analytical performance of the developed system for determination of Glc*N* was evaluated. Finally, the proposed system was employed for quantitative analysis of Glc*N* in supplementary products. This approach offers a fast, low cost, simple and reliable platform for determination of glucosamine products. In addition, the proposed system could be an ideal route for screening product quality in both markets and manufacturing processes by further developing the whole system to be fully automated. This could make manufacturing quality control far more efficient.

2. Experimental

2.1. Materials and instrumentation

All chemicals are analytical grade. Gold nanoparticles were synthesized from $HAuCl_4$ (Sigma-Aldrich, Singapore) and 1% sodium citrate (Sigma-Aldrich, Singapore) using a conventional chemical reduction method [19]. Polyaniline was grounded with (+)-camphor-10-sulfonic acid and dissolved in 2-methyl-N-pyrrolidone (NMP) (Sigma-Aldrich, Singapore). Materials and chemicals were used as received: D-(+)-glucosamine hydrochloride (Fluka Chemica CH-9471, Switzerland), sodium chloride (NaCl: Merck, Thailand), disodium hydrogen phosphate (Na_2HPO_4: Merck, Thailand), potassium dihydrogen phosphate (KH_2PO_4: Carlo ERBA, Thailand), potassium chloride (KCl: Ajax Finechem, Thailand), noujol mineral oil (Perkin Elmer, Thailand), silver paint (SPI supplies, USA), graphite powder (≤ 20 μm, Sigma-Aldrich, Singapore), ethanol (Merck, Thailand) and Sylgard 184 elastomeric kit (Dow Corning, USA). Electrochemical measurements were conducted using a commercially available potentiostat (eDAQ, ED410, 410–088, Australia). For microfluidic experiments, all solutions were contained in 1 mL plastic syringes (Nipro, Thailand) connected to the inlets of a microfluidic device using polyethylene tubing (0.38 mm I. D., 1.09 mm O.D., PORTEX, Belgium). The solutions were driven through the device using syringe pumps (PHD 2000, Harvard Apparatus, USA).

2.2. Preparation of solutions

All aqueous solutions were prepared using Milli-Q water (18.0 MΩ cm, Milli-Q Gradient System, Millipore, Thailand). Phosphate buffer saline (PBS) pH 7.4 at a concentration of 0.1 M was prepared by dissolving 2.0 g NaCl, 0.05 g KCl, 0.36 g Na_2HPO_4 and 0.06 g KH_2PO_4 using Milli-Q water in a 250 mL volumetric flask. To adjust pH of the PBS buffer, 0.1 M phosphoric acid and 0.1 M NaOH were used to obtain a desired pH. A stock solution of D-glucosamine at a concentration of 100 mM was prepared by dissolving 21.56 mg of D-glucosamine in 1.0 mL of 0.1 M PBS in a safe-lock tube (Eppendorf, Thailand). A mixture of AuNPs and PANI was prepared by dissolving AuNPs and PANI in NMP in a safe-lock tube. The mixture was vortexed before use. For droplet-based microfluidic experiments, an oil solution for droplet generation was a 10:2 (v/v) mixture of perfluorodecalin (mixture of *cis* and *trans*, 95%, Sigma-Aldrich, Germany) and 1*H*, 1*H*, 2*H*, 2*H*-perfluoro-1-octanol (97%, Sigma-Aldrich, Germany). D-Glucosamine supplementary products were purchased from a local drugstore (Thailand). All samples were prepared in 0.1 M PBS to have a final concentration of 3.0 mM.

2.3. Design and fabrication of microfluidic devices

There were two designs of PDMS microfluidic devices; one was a device consisting of a circular reservoir with a diameter of 0.8 cm (a well-like device) for measurements using cyclic voltammetry and the other was a device with microchannels for electrochemical measurements in droplets. The design of microchannels followed the microfluidic layout presented in previous work [14]. PDMS devices were fabricated using traditional soft lithography [20]. Each device was composed of two PDMS layers. The top layer was a microchannel- or reservoir-patterned plate and the bottom layer was an electrode-patterned plate. The microchannels consisted of a major channel (500 μm wide) and a confined channel (50 μm wide) and all channel depth was 100 μm. The electrode-patterned PDMS plate consisted of three microchannels with 500 μm width and 100 μm depth for fabrication of three microelectrode bands.

Chip-based CPEs were fabricated by means of screen printing [14]. Graphite powder, nujol oil and PDMS were mixed and then filled into the microchannels of the electrode-patterned PDMS plate. A rubber scraper was used to spread the carbon paste onto the electrode area to smooth out the surface of the electrodes. The excessive paste was cleaned up using Scotch Magic Tape™. Subsequently, for electrode modification, 1 μL of the mixed solution of AuNPs and PANI microfibers was applied onto the middle electrode using the drop-casting technique and the electrodes were then left in a 65 °C oven for 1 h.

To make a well-like device with a circular reservoir, a square piece of PDMS was punched using a 0.8 cm diameter puncher. For device assembly using plasma treatment, only the punched PDMS or microchannel PDMS was exposed to plasma, not the electrode-patterned PDMS. This was because the nujol oil component from the electrodes could be decomposed upon plasma treatment [21], resulting in low efficiency of the CPEs. Electric wires were then attached at the end of each electrode using silver paint. In order to reduce the noise, epoxy glue was applied over the silver paint area and left to be cured for 1 h. Thereafter, the devices were ready to use. The complete devices are shown in Fig. 1(a) and (b).

2.4. Central composition design (CCD) for electrode modification

The amounts of AuNPs and PANI used for electrode modification were optimized to achieve maximum current signal. Stock solutions of 1000 mg L^{-1} AuNPs and 10,000 mg L^{-1} PANI were prepared in NMP and the desired amounts were used for electrode modification. Effect of pH of the working buffer was studied because both AuNPs and PANI are pH dependent. CCD was used for optimization