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Full Paper

Design and optimisation of enzymatic glucose assay in meso-fluidic chemiluminescence device with glucose oxidase immobilised on pencil lead

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Abstract: A simple meso-fluidic chemiluminescence device was developed for glucose determination using an enzymatic assay. A gold nanoparticles/glucose oxidase bioreactor was constructed by immobilising glucose oxidase enzyme on a piece of pencil lead and packing it into a channel. Poly(methyl methacrylate) and poly(dimethylsiloxane) were used for the fabrication of a sandwich-type meso-fluidic device. A chemiluminescence measurement set-up was employed to monitor the oxidation processes in the meso-fluidic chip. The enzymatic glucose assay involves the oxidation of glucose to generate hydrogen peroxide followed by luminol/Co^{2+} chemiluminescence detection. Reagent concentration, flow rate, sample volume and photomultiplier tube applied voltage were thoroughly optimised. A linear relation exists for glucose concentration between 0.5×10^{-5} - 1.2×10^{-3} M ($r^2 = 0.9946$). The detection limit (3σ) was found to be 1.0×10^{-6} M. The proposed system was successfully applied for quantitative analysis of glucose in biological fluids and energy drink samples. The meso-fluidic data were validated with standard spectrophotometric or HPLC methods.

Keywords: glucose biosensor, glucose oxidase, meso-fluidic device, chemiluminescence detection

INTRODUCTION

D-Glucose, a monosaccharide, is the central carbohydrate in human physiology. It functions as a primary source of energy for the body, and is a vital compound in biochemistry and clinical chemistry. Blood glucose level is an indicator with a high predictive value in such disorders as diabetes mellitus, coronary heart disease and arterial hypertension [1]. Several techniques such as

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classical titration, spectrophotometry and high performance liquid chromatography (HPLC) have been developed and used for glucose determination in different sample matrices. However, these methods are generally time-consuming and require high-cost equipment. In order to eliminate these drawbacks, electrochemical biosensors have been used widely for daily glucose monitoring due to their high accuracy, low cost, rapidity and simplicity. However, there is still a great demand for further development of glucose biosensors because the determination of glucose concentration is very important in clinical applications and industrial food analysis.

The development of enzyme-based glucose biosensors has been the subject matter of a large number of research lines in the past few decades [2, 3]. Various chemical compounds with high chemical stability and good biocompatibility such as Nafion® or PANi [4, 5] have been widely used as a protective and selective coating material and as a support material for enzyme immobilisation in the enzymatic-biosensor construction. In recent years nano-sized particles of noble metals, especially gold nanoparticles (AuNP), have played a significant role in the preparation of biosensors due to their electronic, chemical and physical properties. They can also provide a biocompatible micro-environment that is non-toxic to biological systems and greatly increase the number of immobilised biomolecules on the electrode surface, thus improving the biosensor sensitivity [6-8].

Meso-fluidic technology in general seeks to improve analytical performance by reducing the consumption of reagents, decreasing analysis time, increasing reliability and sensitivity through automation, and integrating multiple processes into a single device. The materials used to construct meso-fluidic immunoassay devices vary depending on the application, although the vast majority are constructed of glass, silicon or polymers. One particular polymer that has recently been used extensively is poly(dimethylsiloxane) (PDMS). It is a transparent, elastomeric polymer that can be fabricated rapidly, with dimensions as small as 10 nm. The elastomeric nature of PDMS makes it a great sealant since the adhesion due to conformal surface contact with a smooth, flat surface is often enough to seal meso- or even micro-channels for low pressure applications [9, 10].

The miniaturisation of bioanalytical assays and sample pretreatments by exploiting meso-fluidic lab-on-valve configurations was reviewed by Chen and Wang [11]. The application of several optical sensing systems incorporated into a micro-fluidic system was reviewed by Kuswandi et al. [12]. The review illustrates a successful application of chemiluminescence (CL) detection in micro-fluidic approaches and its beneficial techniques for the quantitative determination of specific analytes in real sample matrices.

As mentioned earlier, the detection procedure for a glucose biosensor is commonly based on electrochemical techniques. However, a chemical detector based on CL as a sensitive indirect detection for glucose has also generated interest. In 2005 Marle and Greenway [13] applied the luminol CL reaction within a micro-fluidic device to produce a miniaturised analytical system for the determination of hydrogen peroxide in rainwater. In another study an ultrasonic-flow injection CL manifold for determining hydrogen peroxide was proposed, where CL obtained from luminol-H_2O_2-cobalt(II) reaction was enhanced by the application of ultrasound [14].

Some CL flow-based methods for the determination of glucose based on an enzymatic mini-column bioreactor using simple flow-injection analysis have been described, including that using a nylon tubular reactor containing immobilised glucose oxidase (GOx) incorporated with sequential injection analysis [15, 16]. The CL detection involved an enzymatic oxidation of glucose to D-gluconic acid and H_2O_2, after which the generated H_2O_2 oxidises luminol to produce CL emission in the presence of an alkaline medium. The solid support for enzyme immobilisation could be done on

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the inside surface of the CL flow-through cell or even on an eggshell membrane with glutaraldehyde as a cross-linker [17]. Lan et al. [18] also reported that AuNP can remarkably enhance the CL response of the glucose biosensor.

In the present investigation we report on the fabrication of a meso-fluidic biochip with an incorporation of a gold nanoparticles/glucose-oxidase (AuNP/GOx) bioreactor and a CL assay. The GOx is immobilised on pencil lead and the AuNP facilitate the binding of GOx with the carbon surface substrate and prolong the life of the enzymatic biosensor when used for quantitative analysis via the flow-based system.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used were analytical reagent grade and used without further purification. Distilled deionised water was used for preparing standard and reagent solutions. The chemicals and reagents used were: luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma-Aldrich), gold (III) chloride trihydrate ($HAuCl_4.3H_2O$, Sigma-Aldrich), glucose oxidase (GOx) (Sigma-Aldrich), D-glucose (Ajax), cobalt sulphate (Merck), disodium hydrogen phosphate (BDH), trisodium citrate dihydrate (Sigma-Aldrich), potassium hydroxide (Merck), L-ascorbic acid (Merck), 4-acetamidophenol (Sigma-Aldrich), uric acid (Hopkins & Williams), salicylic acid (Ajax), D-galactose (Merck), citric acid (Merck), cholesterol (Fluka), oxalic acid (Ajax) and caffeine (Sigma-Aldrich). Stock standard solutions of glucose were prepared daily in 0.05M phosphate buffer solution (pH 7.4). The GOx solution (2.0 mg mL^{-1}) was prepared by dissolving GOx in 0.05M phosphate buffer (pH 7.4).

A stock solution of luminol (0.10 mol L^{-1}) was prepared in 0.06M KOH solution and stored in the dark at 4°C. A working solution of 4.0×10^{-4}M luminol was prepared daily by diluting the stock solution with 0.06M KOH. The stock solution of Co^{2+} (3.5×10^{-3} M) was prepared in deionised water (from a Milli-Q system). A working solution of 1.0×10^{-7}M Co^{2+} was prepared by diluting the stock solution with deionised water.

Preparation of AuNP

The preparation of AuNP was done according to the method of Tangkuaram et al. [6]. Gold (III) chloride trihydrate and trisodium citrate dihydrate were used as a gold precursor and a reducing agent respectively. Five mL of 0.01% $HAuCl_4.3H_2O$ was initially microwaved with a commercial microwave oven (MS103HCE Samsung, Korea) at a power level of 200 W. Then 98 µL of 1.0% trisodium citrate dihydrate was added and the mixture was microwaved at the same power level for 2 min. The AuNP formed was subjected to a UV spectrophotometric validation with a double-beam UV spectrophotometer (U2900, Hitachi, Japan) and a scanning electron microscope (JSM5410-LV, JEOL, Japan) to determine the morphology and particle size.

GOx Immobilisation on Pencil Lead

The surface of a 5H pencil lead (2-mm diameter, Steadtler®) was polished by hand with fine abrasive paper (Buehler, USA) to remove the contaminants until a mirror-like finish was obtained with a size of 20 mm long, 2 mm wide, 0.5 mm thick. Before use the pencil lead was rinsed with distilled water followed by a 5-min. sonication in distilled water to remove residual abrasive particles. The AuNP/GOx bioreactor was prepared by drop-casting the AuNP solution onto the

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surface of the pencil lead, followed by GOx and glutaraldehyde activation. The treatment of the immobilised AuNP/GOx bioreactor with glutaraldehyde was necessary to activate all the primary amino groups of the enzyme to cross-link with supports containing primary amino derivatives [19]. The AuNP/GOx was washed with phosphate buffer (0.025 M, pH 7.4) and stored at 4°C until use.

Preliminary investigations were carried out by cyclic voltammetry using a potentiostat (CHI1230A, CH Instrument, USA) to investigate whether the immobilised AuNP/GOx bioreactor functioned properly by giving a successful enzymatic reaction of D-glucose with the immobilised GOx. A three-electrode system was used, with a platinum wire as the auxiliary/counter electrode, an Ag/AgCl reference electrode (3M KCl) (CHI111, CH Instrument, USA) and the immobilised AuNP/GOx glassy carbon electrode as the working electrode.

For the cyclic voltammetric scanning, 2.0 mL of 0.10M phosphate buffer solution (pH 7.4) as supporting electrolyte was transferred to a measuring cell. Cyclic voltammograms were obtained for the immobilised electrode at a potential range of 0.20-1.20 V at 100 mV s^{-1}, with and without successive addition of 10 μL of 2.0M D-glucose solution.

Design and Fabrication of Meso-fluidic Device

The meso-fluidic CL flow-through cell was designed by Adobe Illustrator 10 software (Adobe Systems, USA) and a designed meso-channel is illustrated in Figure 1(A). The meso-channels were engraved by a CO_2 laser, which was used to carve the channels into poly(methyl methacrylate) (PMMA) or Perspex (A. C. S. Xenon Co., Thailand), following the desired pattern. The flow conduit was comprised of a carrier, reagent stream inlets, and a spiral coil (2.5-cm diameter) of a channel that was 500 μm wide, 200 μm deep and 52.0 cm long. The AuNP/GOx bioreactor was loaded into a channel 20 mm long, 2 mm wide and 0.5 mm deep in the laser-engraved planar acrylic carrier channel of the flow design. The meso-fluidic device cover plate was made from a PDMS sheet formed from a 10:1 mixture of prepolymer and curing agent (Sylgard 184, Dow Corning, USA). The mixture was stirred thoroughly and degassed in a vacuum for 15 min., then poured onto a glass-slide template and cured at 70°C for 1 hr. After curing, the PDMS replica was peeled from the template and cut to the respective sheet size. Finally the device was sealed by tightening the PMMA sandwich sheets with screws. The completed set-up of the meso-fluidic device was a sandwich type as illustrated in Figure 1(B).

Instrumental Set-up

The meso-fluidic CL set-up, used in all experiments, is illustrated in Figure 1(C). It consisted of a four-channel peristaltic pump with a rate selector (Minipuls 3, Gilson, France), a sample injection valve (V-450, Upchurch Scientific, USA) and the PTFE connection tubing (0.5-mm i.d., Supelco, USA). The CL signal was monitored in a custom-built flow-through luminometer consisting of a meso-fluidic chip mounted against a red sensitive photomultiplier tube (PMT) (Thorn-EMI 9828SB, Electron Tubes, UK). The operational potential for the PMT was provided by a stable power supply (Thorn-EMI model PM20, Electron Tubes, UK) at a voltage of 880 kV. The output of the PMT, proportional to the CL intensity, was monitored continuously and displayed by a personal computer via a digital multimeter interfaced with a voltage divider (C637BFN2, Electron Tubes, UK). The UNI-T® UT60D AC/DC software was used to determine the peak maximum.

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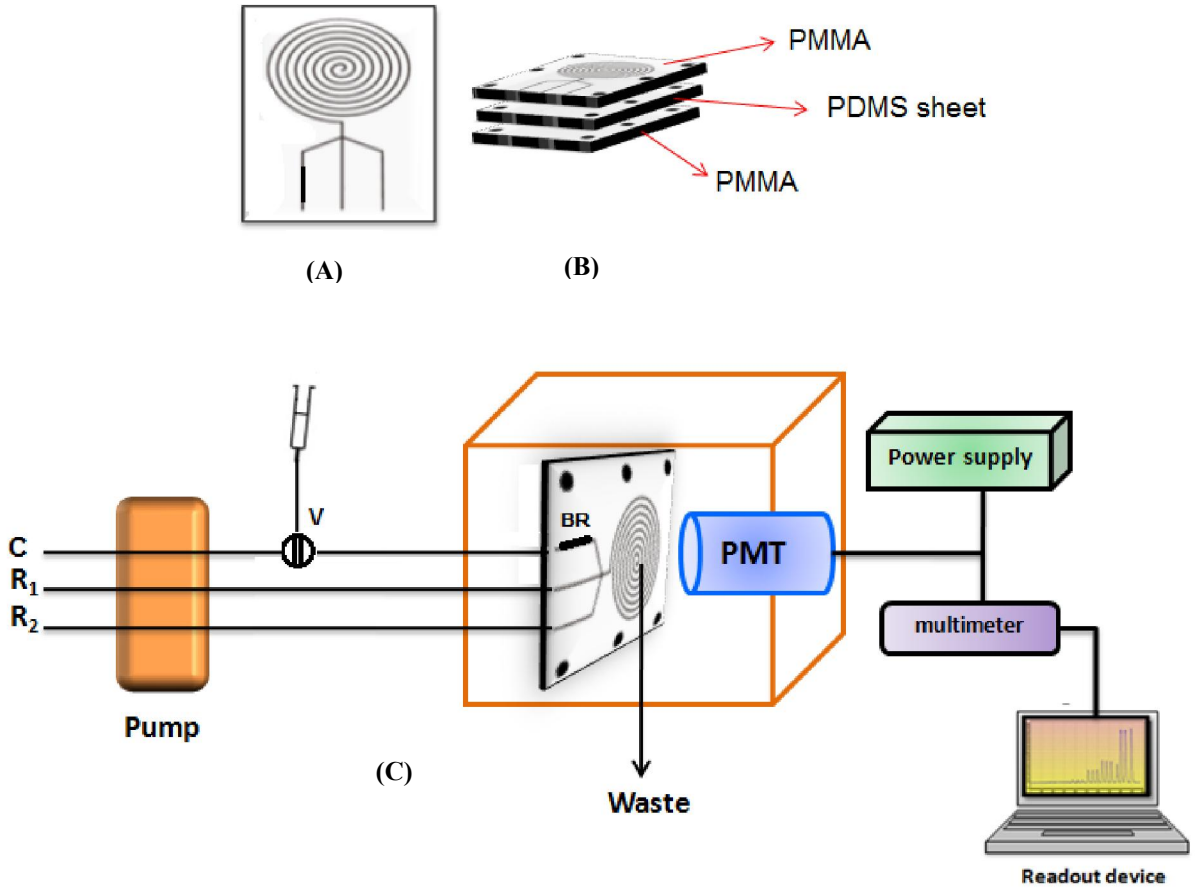

Figure 1. (A) Illustration of laser-engraved flow lines of meso-fluidic platform; (B) a sandwich-type meso-fluidic device; (C) schematic diagram of meso-fluidic chip coupled to AuNP/GOx bioreactor in the CL luminometer (BR = AuNP/GOx bioreactor; C = 0.05M phosphate buffer solution (pH 7.4); R_1 = 4.0×10^{-4}M luminol solution in 0.06M potassium hydroxide solution; R_2 = 1.0×10^{-7}M Co^{2+} solution)

Evaluation of the Meso-fluidic Manifold

When a sample containing D-glucose is introduced into the meso-fluidic device, it generates hydrogen peroxide through an enzymatic reaction in the AuNP/GOx bioreactor. The generated H_2O_2 is then detected by the enhanced CL reaction of luminol/Co^{2+} in alkaline medium. A peristaltic pump was used to pump the carrier and both reagent solutions. The performance of the manifold was evaluated by H_2O_2, an oxidation product of glucose in the meso-channel. The luminol solution, the Co (II) solution and the carrier stream of the phosphate buffer were pumped at an equal flow rate of 0.4 mL min^{-1}. They were mixed and passed through the spiral flow-through micro-conduit, where the CL intensity was detected by the PMT. The output of the PMT, which is proportional to the CL intensity, was monitored continuously. Henceforward, various factors influencing the performance of the meso-fluidic bioreactor with CL reaction of luminol/Co^{2+} were thoroughly examined.

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Preparation of Samples

The developed meso-fluidic CL method with AuNP/GOx immobilised on pencil lead was applied to the determination of glucose in a commercial oral rehydration powder, some energy drink samples and biological fluid samples. This was performed in order to evaluate the reliability of the proposed meso-fluidic CL system.

A stock solution of the rehydration powder was prepared by dissolving 1.0 g of the powder in 50 mL of a 0.05M phosphate buffer (pH 7.4) carrier solution. A 20-µL aliquot of the stock solution was diluted to 10 mL with the carrier solution and served as the blank standard. The analysis was performed by the standard addition method.

Drug-free human blood obtained from healthy volunteers (after obtaining their signed consent) was immediately centrifuged (5000 rpm) for 30 min. at room temperature to remove particulate matter and cell debris. The pellets were discarded and the separated supernatant serum was stored frozen until use in the assay. The blood serum samples used in any given experiment were from the same batch, collected at the same time and centrifuged for the same duration of time. An aliquot volume of the blood serum sample was fortified with phosphate buffer solution to achieve the desired concentration. The quantification was performed by means of a calibration method with the related calibration equations.

RESULTS AND DISCUSSION

Characterisation of AuNP and Preliminary Study

An image of AuNP from the scanning electron microscope is shown in Figure 2. It can be seen that their average particle size was about 10 nanometers, which agrees well with the result obtained from the UV-visible spectrum, where the maximum wavelength was observed at 521 nm, giving a sherry colour [6].

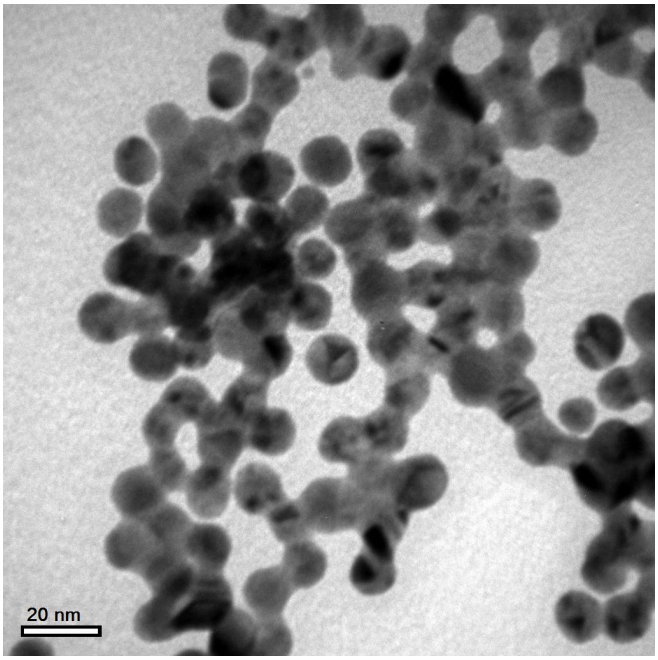

Figure 2. Scanning electron micrograph of AuNP

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The validation of the AuNP/GOx immobilised on the pencil lead electrode was carried out by cyclic voltammetry. The GOx enzyme immobilisation on a graphite sensor was first investigated by Ianniello and Yacynych [20] in an attempt to construct an enzyme-immobilised, chemically modified electrode as an amperometric sensor for glucose. However, the immobilisation technique gave a relatively low response in glucose measurements. Moreover, it was found that direct immobilisation of GOx onto the surfaces of graphite is difficult to achieve due to the hydrophobic property of graphite. Thus, AuNP were used in this study to mediate the immobilisation of GOx on the graphite surface. Colloidal AuNP were specifically used since it has been reported that these nanoparticles can adsorb redox enzymes and proteins without the loss of their biological activity [21].

The electrochemical behaviour of D-glucose was investigated using the AuNP/GOx-immobilised glassy carbon electrode by cyclic voltammetry. Figure 3 shows the cyclic voltammograms of D-glucose in the concentration range of 5×10^{-3}-25×10^{-3} M. The magnitude of the current detected by the electrode increases as the concentration of D-glucose in the supporting electrolyte is increased. The enhanced anodic current indicates the enzymatic reaction of D-glucose with GOx at the electrode surface. Glucose, with the aid of atmospheric oxygen, reacts enzymatically with GOx, producing gluconolactone and hydrogen peroxide as end products [22]. The presence of AuNP in the electrode is essential for an effective biomolecular immobilisation of GOx and gives a longer lifetime stability of the bioreactor [6]. The hydrogen peroxide generated during the enzymatic reaction is the essential factor that drove the CL reaction.

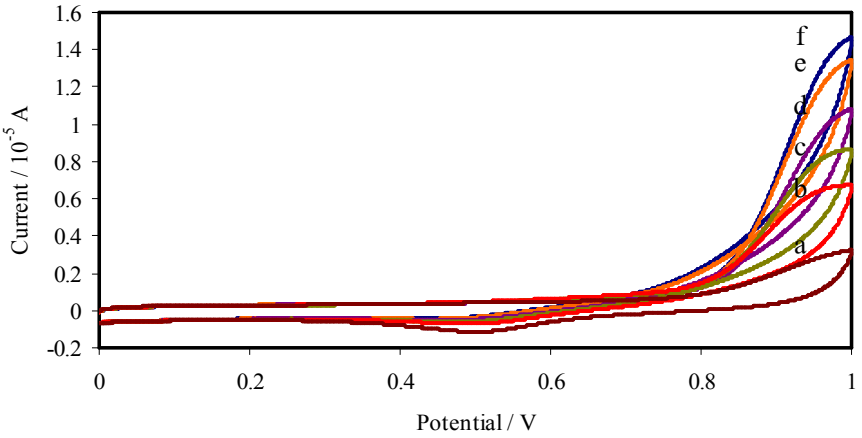

Figure 3. Cyclic voltammograms of AuNP/GOx-immobilised glassy carbon electrode in (a) blank supporting electrolyte solution, and in the presence of (b) 5×10^{-3} M, (c) 10×10^{-3} M, (d) 15×10^{-3} M, (e) 20×10^{-3} M and (f) 25×10^{-3} M of D-glucose (supporting electrolyte = 0.10M phosphate buffer pH 7.4; scan rate = 100 mV s^{-1})

Optimisation of Meso-fluidic CL System

After the immobilisation procedure had been validated, AuNP/GOx was applied and immobilised on pencil carbon lead and was employed in the meso-fluidic CL reaction of D-glucose. Optimisation of variables affecting the sensitivity of D-glucose determination was performed, in which hydrogen peroxide was initially used as the working standard. In this part of the investigation the manifold as shown in Figure 1(C) was used; however the AuNP/GOx-immobilised pencil lead mini-column was omitted from the experiments. The different variables that affect the performance of the method were optimised via a univariate approach. Triplicate injections, each of 1.0×10^{-4}M

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H_2O_2 standard solution were evaluated with each set of variables. The range over which each variable was investigated and the optimum condition of each variable are listed in Table 1.

Table 1. Variables of meso-fluidic CL system optimised in this study

Variable	Range studied	Initial value	Optimal value
PMT applied potential (V)	500 - 900	900	880
CL reagents			
Co(II) concentration (M)	0 - 1.0×10^{-3}	0	1.0×10^{-7}
Luminol concentration (M)	1.0×10^{-5} - 6.0×10^{-4}	1.0×10^{-4}	4.0×10^{-4}
Carrier solution			
PBS concentration, pH 7.4 (M)	0.01 - 0.20	0.10	0.05
Individual flow rate of CL reagent streams and carrier stream (mL min^{-1})	0.1 - 0.5	0.2	0.4
Sample injection volume (μL)	5 - 25	10	20

The working potential of the constant power supply was optimised. The voltage ranging between 500-900 V was increased stepwise and the current representing CL intensity (in mV) was measured after an injection of 1.0×10^{-4}M H_2O_2 standard solution at each potential step. As expected, both noise and analytical signals increased as the PMT voltage increased. At the sensitivity of 880 V, the optimum working condition was achieved, which was selected for subsequent experiments.

The effect of varying the concentration of the Co(II) catalyst in a mixture with 1.0×10^{-4}M luminol solution in alkaline medium was analysed. It was observed that the sensitivity of CL detection of H_2O_2 was highest at the Co(II) concentration of 1.0×10^{-7} M. Increasing the concentration of Co(II) further resulted in a significant decrease in sensitivity due to the quenching effect of the catalyst at higher concentrations [23].

The luminol concentration (1.0×10^{-5}-6.0×10^{-4} M) was optimised next. The best sensitivity, evaluated along with the sample throughput, was determined to be at 4.0×10^{-4} M (Figure 4). This concentration was used later in optimising the remaining variables as well as in the application of the developed meso-fluidic CL system to the actual glucose analysis. At higher luminol concentrations, intensity signals further increased but the detection required longer time and the sample throughput decreased.

The carrier solution, which was a phosphate buffer, was also optimised by varying its concentration between 0.01-0.20 M. The maximum CL intensity was obtained when the concentration of the phosphate buffer was 0.05 M, which was used in all further studies.

The effects of flow rate and sample injection volume on the sensitivity and sample throughput were also optimised for the determination of 1.0×10^{-4}M H_2O_2. To simplify the flow system, the flow rates of all carrier and reagent streams were set to equal and were studied over the range of 0.1-0.5 mL min^{-1}, from which a rate of 0.4 mL min^{-1} was determined to produce an optimum CL value. Beyond 0.4 mL min^{-1}, the increment in sensitivity was indistinct and large amounts of CL reagent and carrier solution were consumed. Furthermore, the meso-fluidic device leaked at higher flow rates due to the high pressure within the meso-scale flow lines.

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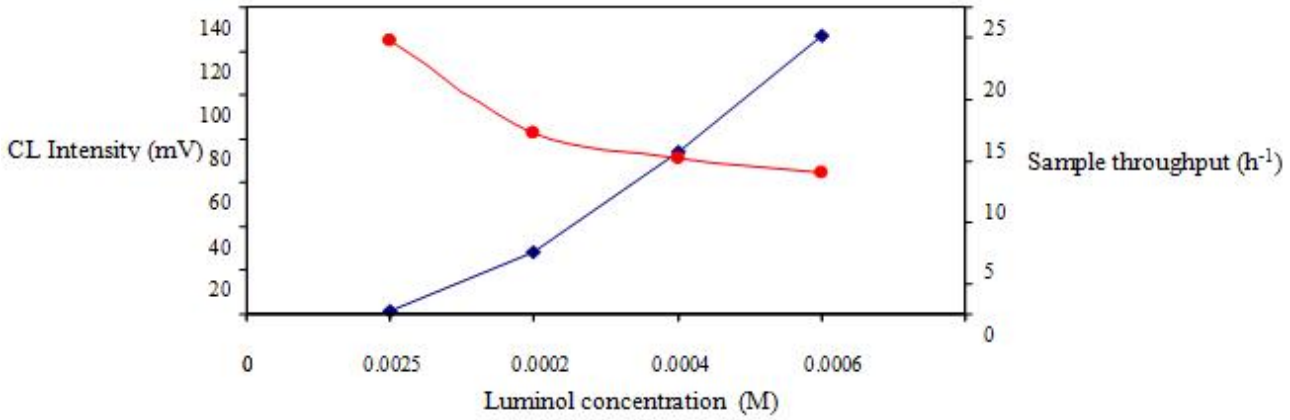

Figure 4. Effect of luminol concentration on CL intensity (-◆-) and sample throughput (-●-). Conditions: CL reagent = 1.0 × 10^{-7} M Co(II) solution in mixture with respective luminol concentrations; carrier solution = 0.10 M phosphate buffer; flow rate = 0.20 mL min^{-1}; sample standard = 1.0× 0^{-4}M H_2O_2; sample injection volume = 10 µL

On the other hand, an increase in sample volume normally leads to an increase in the emitted CL signal. The influence of the sample volume (5-25 µL) on the CL intensity was investigated and the optimum sample injection volume that gave the best compromise between good sensitivity, reproducibility and reasonable sample throughput was 20 µL.

After optimising the essential variables for the best sensitivity for the meso-fluidic CL system, they were then applied to the determination of D-glucose using the AuNP/GOx-immobilised carbon lead mini-column.

Linearity and Calibration Plot Using Meso-fluidic CL System

With hydrogen peroxide standard solutions

At the optimised conditions of the meso-fluidic CL system, the sensitivity, dynamic range and limit of detection of hydrogen peroxide were determined. By polynomial regression, the dynamic range was determined to be between a concentration of 1.0×10^{-7}-4.0×10^{-5} M H_2O_2; $CL_{intensity} = 0.084\ c^2 + 4.211c - 2.291$ (r^2 = 0.9987). Beyond 4.0×10^{-5} M of H_2O_2, the meso-fluidic CL system started to overload.

Experimentally, the detection limit, which gave an intensity signal that was three times the background noise, was found to be as low as 4.0×10^{-8} M. The sensitivity was determined to be 1.77 $\times10^6$ mV M^{-1}, which was obtained from at least 6 samples, covering the range of 1.0×10^{-7}-1.0×10^{-6} M for the linear calibration plot. The sample throughput, based on the average sample throughput of the calibration signals, was calculated to be 104 injections per hour.

With AuNP/GOx-immobilised pencil lead mini-column for the meso-fluidic CL determination of D-glucose

With the developed meso-fluidic CL method, the determination of D-glucose was performed by utilising an AuNP/GOx-immobilised pencil lead mini-column that was incorporated into the meso-fluidic conduit as shown in Figure 1(C). The calibration graph for D-glucose was constructed using the least-square regression of various amounts of the glucose standard. At least 12 samples, covering the whole range (5.0×10^{-5}-1.2×10^{-3} M) of glucose, were used based on the calibration curve method. Each point of the calibration graph corresponded to the mean value of three

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independent peak measurements. The linearity regression equation is $CL_{intensity} = 8.469C_{Glu} - 3.101$, $r^2 = 0.9946$. The limit of detection, defined as three times the background noise, was experimentally determined to be 1.0×10^{-5} M. The average sample throughput obtained from the signal intensities was 30 h^{-1}.

The AuNP/GOx enzymatic bioreactor could be used reliably within 14 days when the CL response was more than 80% at the storage temperature of 4°C in phosphate buffer or at least for four days of continuous and daily use.

Interference Study

The effects of common interferences were examined by preparing synthetic mixtures containing 3×10^{-4}M glucose standard solution in the presence of different concentrations of common interferences, namely L-ascorbic acid, 4-acetamidophenol, uric acid, salicylic acid, D-galactose, citric acid, cholesterol, oxalic acid and caffeine. Then the CL signals of these solutions were measured by the AuNP/GOx bioreactor. The effect of interference was not considered to be significant if it caused a relative error of less than 5% with 3×10^{-4}M glucose standard solution. The results showed that the glucose standard solution with high concentrations of common oxidising substances experienced significant interfering effects similar to those occurring in most GOx biosensors [22]. The tolerable concentrations of the interfering substances tested are shown in Table 2.

Table 2. Tolerable concentrations of some common interferences on CL signal of 3×10^{-4}M glucose

Tolerable concentration	Substance
1:1	L-ascorbic acid, 4-acetamidophenol, uric acid, salicylic acid
1:10	D-galactose, citric acid, cholesterol
1:100	oxalic acid, caffeine

Application of Meso-fluidic CL Method to Real Samples

The proposed method was applied to the determination of glucose in samples of energy drinks, blood serums and a commercial oral rehydration solution. In the case of the beverages, samples were diluted with the phosphate buffer (pH 7.4). An appropriate dilution of some serum samples from diabetic patients was required since their blood glucose levels normally ranged between 2.5-25 mM. The results of the determination of glucose in all samples are shown in Table 3. The spectroscopic enzyme and HPLC assay results were found to be statistically indistinguishable from those obtained from the AuNP/GOx bioreactor at 95% confidence level by the Student t-test in regard to accuracy and precision.

The average recoveries of the glucose standard solution were measured by the proposed method after spiking three different amounts of glucose standard solution in the energy drink samples. The recoveries were in the range of 96.0-100.4%.

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Table 3. Comparison of glucose analysis results obtained by meso-fluidic CL method with spectroscopic enzyme assay and HPLC method

Sample	Dilution factor	Glucose concentration (mM(			
		AuNP/ GOx method	Spectroscopic enzyme assay [a]	HPLC method [b]	t-Value [c]
Serum1	2	14.02±0.42	14.89±0.64	-	1.81
Serum2	2	12.61±0.54	13.11±0.64	-	1.09
Serum3	-	9.08±0.23	9.81±0.64	-	2.09
Serum4	-	6.62±0.45	7.54±0.64	-	2.16
Serum5	-	2.43±0.21	2.57±0.32	-	0.78
Serum6	-	7.47±0.78	8.05±0.32	-	1.46
Bev1	3	26.12±012.	-	25.35±0.26	0.43
Bev2	3	29.34±0.60	-	28.47±1.50	1.36
Bev3	3	32.90±1.65	-	31.54±0.10	1.42
ORS[d]	-	1.853±0.054[e]	-	2.025±0.065[e]	2.58

[a] Spectrophotometric enzyme assay method [24]
[b] AOAC method [25]
[c] *t*-Critical = 2.776 for $P < 0.05$
[d] Oral rehydration solution
[e] Amount in g/100mL

A comparison of the proposed method with other CL methods is shown in Table 4. An advantage of the present technique is the easy set-up and the fact that the enzyme immobilisation on the bioreactor by drop casting technique is less complicated. The AuNP facilitate a strong binding between GOx and pencil lead and this prolongs the bioreactor lifetime. Furthermore, the meso-fluidics procedure consumes small amounts of reagents and it is easy to develop further automated techniques based on this procedure.

Table 4. Comparison of different CL methods for determination of glucose

No.	Method	Bioreactor/ solid support	Linearity range (M)	Limit of detection (M)	Bioreactor lifetime	Reference
1	FI-CL	GOx/Pore glass beads (3.5cm ×2.5mm)	6.8×10^{-7}-8.2×10^{-6} (r = 0.9933)	4×10^{-8}	200 injections (2 weeks)	[15]
2	SIA-CL	GOx/Nylon tubular column (1m: i.d. 1 mm; o.d.1.78 mm)	3×10^{-5}-6×10^{-4} (r = 0.9993)	1.5×10^{-5}	-	[16]
3	FI-CL	GOx/Egg shell membrane in glass column (55mm× i.d 3mm)	1×10^{-6}-1×10^{-4} (r = 0.9965)	5×10^{-7}	200 injections (5 months)	[17]
4	FI-CL	Sol-gel immobilised with AuNP/U-shape glass (100mm, i.d.3 mm)	1×10^{-5}-1×10^{-3} (r = 0.9965)	5×10^{-6}	150 injections (14 days)	[18]
5	Meso-fluidic CL	GOx/AuNP/pencil lead (20mm×2 mm×0.5 mm)	5×10^{-5}-1.2 10^{-3} (r = 0.9946)	1×10^{-5}	200 injections (14 days)	Present method

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CONCLUSIONS

A simple meso-fluidic device with the incorporation of a mini-column of enzymatic bioreactor for the chemiluminescence determination of D-glucose has been fabricated and demonstrated to be an effective tool in obtaining low levels of detection. The AuNP/GOx-immobilised pencil lead mini-column is a practical bioreactor that allows the selective determination of glucose in liquid samples, requiring only very small amounts of sample and reagents. The sensitivity and dynamic range obtained from the device are good with a low limit of detection compared to the ordinary enzymatic spectrometric methods. The developed method provides an alternative determination of glucose in real food and biological samples with results that agree well with standard official methods.

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