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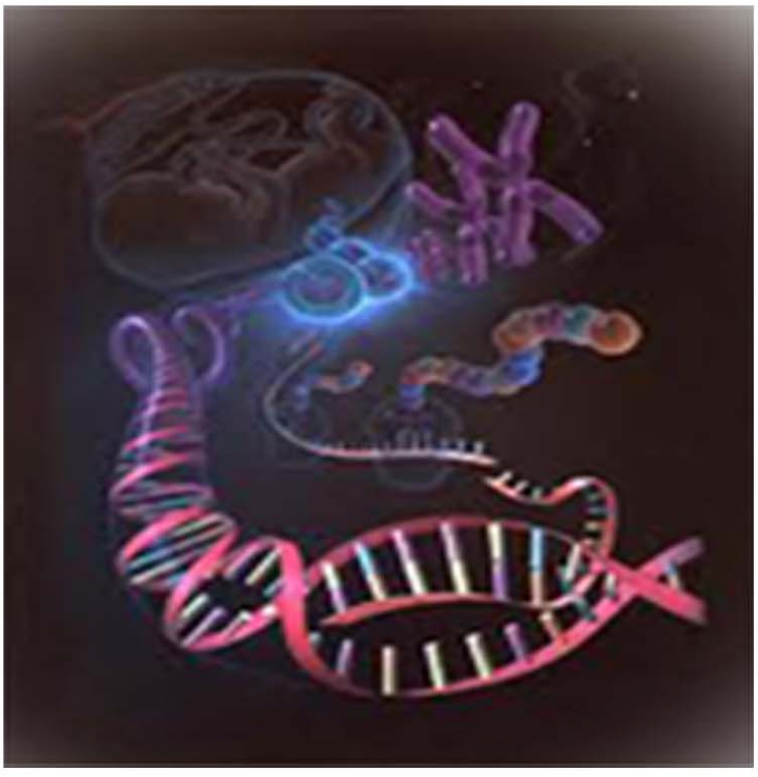

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Research Paper

FLOW-INJECTION CHEMILUMINESCENCE DETERMINATION OF GLUCOSE USING POTASSIUM PERMANGANATE-LUMINOL CL SYSTEM

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A new flow-injection chemiluminescence method was developed for the determination of glucose in physiologic liquids, which was based on the enhancement effect of glucose on the chemiluminescence signal generated by $KMnO_4$-luminol system in alkaline medium. Under optimized conditions, a linear correlation was established between the chemiluminescence intensity and the glucose concentration in the range of 7.2×10^{-5} to 2.3×10^{-8} M with a detection limit (3σ) of 1.2×10^{-9} M. The relative standard deviation for 11 measurements is 2.12%. The method was applied to determine the glucose concentration in the human serum samples and the results are consistent with those obtained by the standard spectrometric method.

Keywords: Flow-injection, Chemiluminescence, Blood serum glucose

INTRODUCTION

Glucose is the mostly used monosaccharide found in the nature. In fact, it is found to used in huge number of manufactured products such as drugs, foodstuffs, and it is used as raw material for a number of products of transformation such as alcohol, acids and so on. The glucose constitutes the main source of energy in living organism (Peng *et al*., 2011). However the presence of glucose excess in the human organism can result in the malfunction of the latter, which explains the importance of its monitoring in the biological fluids during the clinical diagnostic.

Up to day many methods are proposed for the glucose determination each of which has its own strength and weakness. Most of the reported methods are based on the enzymatic oxidation of glucose using the glucose oxidase. The enzymatic methods are generally coupled with other technique such as fluorescence (Zargoosh *et al.*, 2011; Sierra *et al.,* 1997; Sung *et al.*, 2012; Sierra *et al.*, 1998), electrochemical (Gvozdenoviæ *et al.*, 2011; Shervedani *et al.*,

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2006; Feng *et al.*, 2009), chromatographic [Oszwaldowski *et al.*, 2000; Kobayashi *et al.*, 1983; Kiba *et al.*, 1993], or flow-injection (Liu *et al.*, 1996; Trojanowicz *et al.*, 1990). The enzymatic methods have appreciable selectivity (Kobayashi *et al.*, 1983; Singh *et al.*, 2010), however they are deemed tedious (Kanchana *et al.*, 2007) and suffer of drawback such as low sensitivity or limited linear range (Zargoosh *et al.*, 2011). In addition, these methods are costly and analytically troublesome because of the instability of glucose oxidase solution. The electrochemical method using enzyme immobilization on the electrode surface appears as an accepted method (Zargoosh *et al.*, 2011). However, the loss of enzyme or its deactivation in critical pH or temperature of the medium is concern (Huang *et al.*, 2010). Therefore, developing a novel sensitive, rapid, simple and cheap method for glucose determination in biological fluids would show appealing alternative. In this objective, the flow-injection chemiluminescence known for its sensitivity, wide linear range and simplicity (Silva *et al.*, 2002) can bring an efficient solution. Some methods based on the flow injection chemiluminescence using enzymatic oxidation in the presence of luminol have been reported (Huang *et al.*, 1991; Kiba *et al.*, 1992; Worsfold *et al.*, 1984). But here the encountered problem is that this kind of oxidation must be carried out at acidic medium (about pH=5), whereas the chemiluminephore is efficient only in alkaline medium. So the realization of such method requires complicated process and equipments.

The luminol or 5-amino-2,3-dihydrophthalazine-1, 4-dione is certainly the mostly used chemiluminescence reagent. The chemiluminescent oxidation of luminol with diverse oxidizing reagent has been largely used for the determination of various organic and inorganic compounds in diverse area such as clinical, pharmaceutical, biochemical and environmental analysis (Xu *et al.*, 2012; Haghighi *et al.*, 2010; Kanwal *et al.*, 2010; Huertas-Pérez *et al.*, 2008; Adcock *et al.*, 2007). The aqueous solution of $KMnO_4$-luminol system has often been used. Although the $KMnO_4$ chemiluminescence systems have been largely developed in acidic medium (Satienperakul *et al.*, 2010; McDermott *et al.*, 2011; Percy *et al.*, 2010; Chen *et al.*, 2010), its use in alkaline medium remains very few reported. For instance Easwaramoorthy *et al.* have developed the paracetamol detection using luminol-$KMnO_4$ system and pH 12.0 (K_2HPO_4/NaOH) buffer solutions (Easwaramoorthy *et al.*, 2001). Wang *et al*. have reported the determination of terbutaline sulfate determination based on enhancement of luminol-$KMnO_4$ alkaline system (Wang *et al.*, 2004). Huertas-perez *et al.* have developed the carbamate residues detection based on luminol-$KMnO_4$ alkaline system (Huertas-Pérez *et al.,* 2004).

Herein, a new flow-injection chemiluminescence method is developed based on the enhancement effect of glucose on the chemiluminescence signal generated by $KMnO_4$-luminol system in alkaline medium. In optimized conditions the method has been successfully applied to determine the glucose in human serum samples.

MATERIALS AND METHODS

REAGENTS

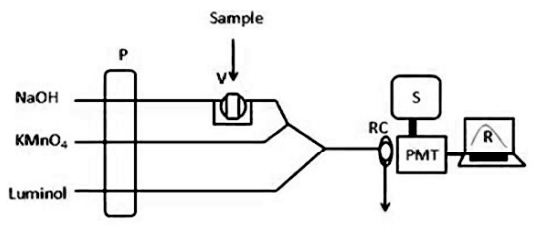
All the chemicals were of analytical grade and were used without further purification. Potassium permanganate and sodium hydroxide were purchased from Tianjin Bohai chemical reagent factory (China). Luminol was purchased from

Tianjin Fuchen Chemical Reagent Factory. Glucose was purchased from Sinopharm Chemical Reagent Co., Ltd. The trichloroacetic acid was purchased from Aldrich Chemical Co. All solutions were prepared with deionized water with resistivity higher 18 MΩ cm^{-1}. The luminol stock solution was prepared by dissolving 1.77 mg with 10^{-2} M NaOH solution in 100 ml brown calibrated flask, and stocked at 4°C. $KMnO_4$ stock solution was prepared by dissolving 79 mg with deionized water in 25 ml calibrated flask and stocked in black plastic bag in the dark. The work solutions were prepared by diluting the required amount with the deionized water before use.

Apparatus

The CL analysis was performed on the Flow Injection Analysis processor FIA-3110 (Beijing Titan instruments co. Ltd.). The schematic diagram of the system is shown on Figure 1. The injection system consists of peristaltic pumps (P), a sixteen-hole eight-ways valve (V) for reagents and samples delivering, a digital system for flow time control, the ultra-weak luminescence analyzer (type BPCL manufactured by Beijing Institute of Bio-physics, Chinese Academy of Science), the Photomultiplier Tube (PMT) operating at 1080 V and 30°C. The temperature in the CL reaction chamber is automatically readjusted by temperature control system. A FIAT monitor/data processing mechanism serves to record the CL signal. The flow cell is the homemade coil, made by coiling 30 cm of colorless glass tube (1 mm i.d. and 2 mm o.d.) into a spiral disk shape. PTFE tubing (0.5 mm i.d. Shenyang Zhaofa Institute of Automatic Analysis, China) and 150 µL loop were used as connecting material in the FI-CL system.

Figure 1: Schematic diagram of flow-injection Chemiluminescence Detection System



Note: P - peristaltic pump; V - eight-way valve; RC - reaction chamber; PMT - photomultiplier tube; S - power supplier; R - recorder

Sample Preparation

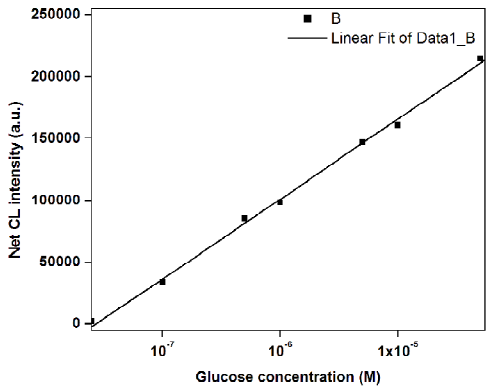
The human serum samples were provided by the local hospital. To deproteinize the sample, 5 mL of 4% trichloroacetic acid solution was added to 500 µL of each sample in graduated essay tube of 10 mL and completed by distilled water. The mixtures were kept 5 min and then centrifuged at 3500 rpm for 10 min. And then 2 mL of the supernatants were diluted to 100 ml in the graduated flasks.

Measurement Procedure

The scheme of flow injection chemiluminescence measurement is represented in Figure 1. The sample solution was injected into the carrier stream (1 ×10^{-3} M NaOH solution) using the injection valve (V) equipped with a 150 µL loop. The carrier-sample stream merge with the 5×10^{-5} M $KMnO_4$ solution and then with the 5 ×10^{-5} M luminol solution, all carried by pump P at a flow rate of 4.5 mL/min and running time of 30 s. The mixture is well mixed in the tubing prior to Reaction Chamber (RC) where the reaction takes place. The registered CL signal in the reaction chamber was compared to the one generated by Blank. The sample concentration was determined by plotting the calibration graph.

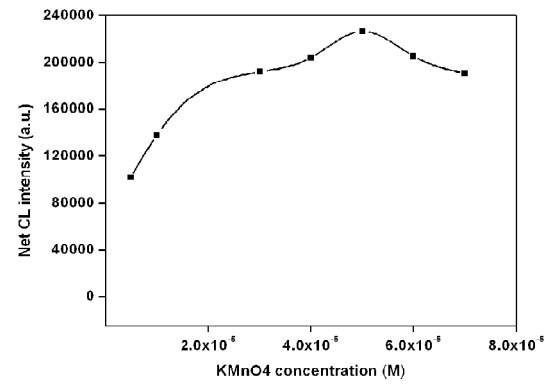
The calibration graph was plotted by measuring the CL signal of a series of glucose solutions with different concentration (Figure 2). The graph of the net CL intensity $\Delta I = I_s - I_o$ (Is, I_o are CL intensity generated by luminescence system with and without glucose, respectively) versus the glucose concentration was plotted.

Figure 2: Calibration Graphic of Glucose Determination Condition: 5 ×10^{-5} M of $KMnO_4$; 5×10^{-5} M of luminal; 1 ×10^{-3} M of NaOH



RESULTS AND DISCUSSION

Figure 3: Effect of $KMnO_4$ Concentration on the CL intensity of $KMnO_4$-NaOH-luminol-glucose system Condition: 1 ×10^{-3} M of NaOH; 5×10^{-5} M of luminol; 1 ×10^{-5} M of glucose



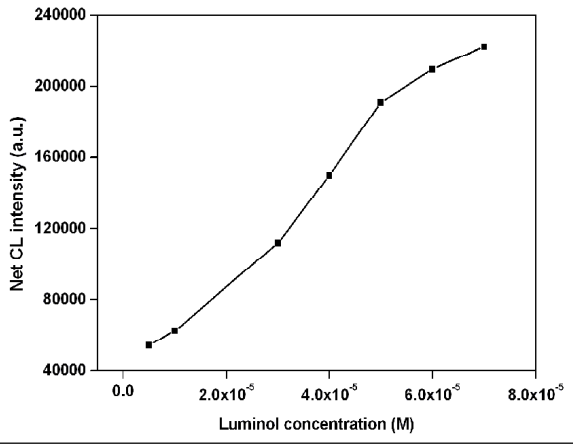
Optimization of $KMnO_4$ Concentration

The influence of $KMnO_4$ concentration on the net CL intensity generated by 1 ×10^{-5} M glucose was studied in the range of 5 ×10^{-6} M to 7 ×10^{-5} M, and the results are shown in Figure 3. It can be seen from Figure 3 that the net CL intensity increases with the increase of $KMnO_4$ concentration up to 5 ×10^{-5} M, and then decreases with the further increase of $KMnO_4$ concentration. This could be attributed to the self-absorption by $KMnO_4$ at high concentration or the formation of precipitate in alkaline medium (Wang *et al*., 2004). So 5 ×10^{-5} M $KMnO_4$ was chosen in the further experiments.

Optimization of Luminol Concentration

The effect of luminol concentration on the net CL intensity generated by 1 ×10^{-5} M glucose was studied in the range of 5×10^{-6} to 7×10^{-5} M. As shown in Figure 4, the net CL intensity gradually increases with the increase of luminol concentration. But taking into account the rational consumption of reagent and the fact that the

Figure 4: Effect of Luminol Concentration on the CL Intensity of $KMnO_4$-NaOH-luminol-glucose System Condition: 5 ×10^{-5} M of $KMnO_4$; 1 ×10^{-3} M of NaOH; 1 ×10^{-5} M of glucose

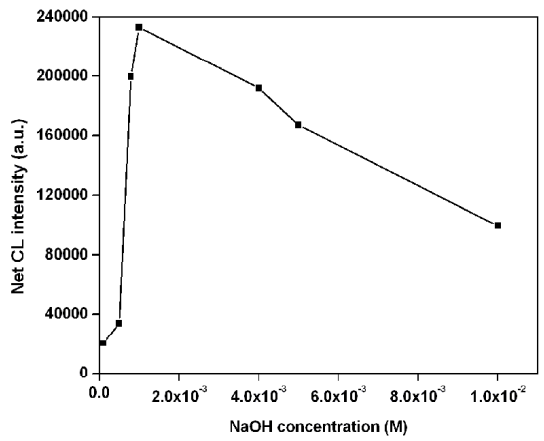


sufficient CL intensity was performed at 5×10^{-5} M, this concentration of luminol was chosen in further experiments.

Optimization of Sodium Hydroxide Concentration

The glucose stability as well as the luminol CL efficiency depends on the medium pH. Therefore the concentration of NaOH is a critical parameter. The effect of NaOH concentration was investigated in the range 1 $\times10^{-4}$ to 1 $\times10^{-2}$ M, and the results are shown in Figure 5. It can be seen that the net CL intensity increases with the increase of NaOH concentration up to 1 $\times10^{-3}$ M, and then it decreases dramatically. Therefore 1 $\times10^{-3}$ M NaOH was chosen in this study.

Figure 5: Effect of NaOH Concentration on the CL Intensity of $KMnO_4$-NaOH-luminol-Glucose System
Condition: 5 $\times10^{-5}$ M of $KMnO_4$; 5×10^{-5} M of luminal; 1 $\times10^{-5}$ M of glucose



Optimization of Flow Rates

The flow rate and sample volume are two critical parameters for flow-injection analysis in term of sensitivity, time and reagent consumption. In one hand, the high flow rate can reduce the CL intensity owing to low time of contact between reactants. In other hand, the slow flow rate provides better contact time of reagents, however reduce the sample throughput. In this case, it is appropriate to find an intermediate value of this parameter which can provide a sufficient CL intensity without an extreme sacrifice of sample throughput. The influence of the flow rate was studied simultaneously for the $KMnO_4$, the luminol and NaOH in the range of 0.5 mL min^{-1} to 5 mL min^{-1} by investigating the signal-to-noise ratio at different flow rates. The sufficient ratio with minimum reagent consumptions and time waste were observed at the rate of 4.5 mL min^{-1} for these solutions. Therefore this value was employed for further experiments.

Interference of Foreign Species

To evaluate the method selectivity, the influences of foreign species were examined by adding a certain amount of interfering species (which are supposed to coexist in the real sample with the analyte) to 1×10^{-5} M of glucose solutions. The tolerance of each coexistent substrate was taken as the highest concentration yielding a relative error less than ±5 %. The results in the Table 1 showed that these species can not affect the analysis results in their existing proportion in real sample.

Possible CL Reaction Mechanism

According to previous reports, it is well accepted that the excited manganese ion Mn (II) resulting from $KMnO_4$ reduction is likely to be able to emit the luminescence (Adcock *et al*., 2007; Tsaplev, 1991; Barnett *et al.*, 1993; Hindson *et al.*, 2001). Moreover, the oxidation of luminol result in the formation of excited 3-aminophthalate which is recognized as powerful CL emitter at around 425

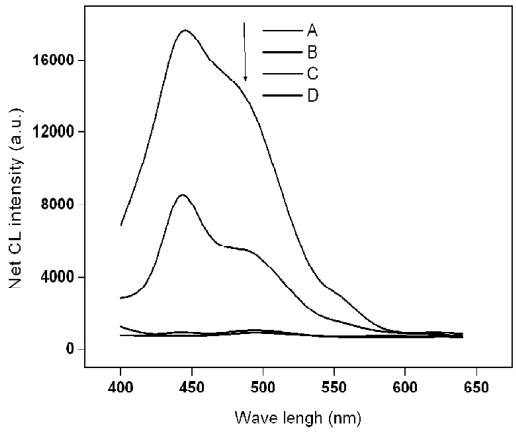
Table 1: Interference Tolerance from Some Coexisting Chemical Species on the Determination of Glucose

Foreign Species	Tolerance (fold)	Relative Deviation (%)
Na^+	1000	+3.98
K^+	500	-2.92
Mg^{2+}	150	+3.96
Ca^{2+}	100	-3.68
Fe^{2+}	250	-2.94
Urea, acid citric	25	-3.73
Cl^-	1500	+4.46
NO^{3-}	300	+3.25
SO_4^{2-}	50	-2.32
CO_3^{2-}, PO_4^{3-}	500	+3.93
$C_2O_4^{2-}$	100	-2.92
CH_3COO^-	25	-3.69
NH_4^+	500	+3.93
Lactose, sucrose	100	-2.92
Ascorbic acid	25	-3.69
L-glutamic acid	25	-3.69

nm (Li *et al.*, 2011). In addition, the possibility of emitting intermediate species is to be considered.

To elucidate the real CL emitter of the proposed luminescence system, the emission spectra of $KMnO_4$-NaOH-luminol-glucose system (A), $KMnO_4$-NaOH-luminol reaction (B), luminol-NaOH-glucose (C) and $KMnO_4$-NaOH-glucose (D) systems were investigated and the results are shown in Figure 6. Although it is well known that glucose in the strong alkaline medium can undergo the oxidation owing to its aldehyde group (Silin, 1967), it can be noticed in Figure 5 that no peak appears on the curves C and D. This indicates that the redox reaction between $KMnO_4$

Figure 6: CL Spectra of $KMnO_4$-NaOH-luminol-glucose System (A), $KMnO_4$-NaOH-luminol reaction (B), luminol-NaOH-glucose (C) and $KMnO_4$-NaOH-glucose (D) System



and glucose do not generate any emitter on one hand; on other hand there is no CL reaction between glucose and luminol. However a weak peak is noticed on the curves B; and a high peak is observed on the curve A. All these peaks are at the same wavelength of 425 nm, which corresponds to the 3-aminophthalate emission wavelength. The curve B confirms the commonly known oxidation between $KMnO_4$ and luminol (Wang *et al*., 2004). However, the enhancement noticed on curve A proves the formation of intermediate product from $KMnO_4$-glucose reaction, which has a more efficient CL yield with alkaline luminol. It is known that the oxidation of

Table 2: Comparisons of Different Methods for Glucose Determination

Method	System	Linear range (M)	Detection limit (M)	Reference
Reversed-phase liquid chromatographic	Vanadium(V)/H_2O_2/2-(5-bromo-2-pyridylazo)-5-diethylaminophenol	5×10^{-5} –2×10^{-3}	5×10^{-5}	[Oszwa³dowski *et al.*, 2000]
Spectrophotometry	Fe_3O_4/ABTS	5×10^{-5} –1×10^{-3}	3×10^{-5}	[Wei *et al.*, 2008]
Flow-injection	Hydroquinone/Fe(III)	1×10^{-6} –1×10^{-3}	5×10^{-7}	[Katsumata *et al.*, 2000]
Fluorimetry	Glucose oxidase/O2	5×10^{-4} –2×10^{-2}	4×10^{-4}	[Sierra *et al.*, 1997]
Fluoride optical sensing	GOD/HRP/Fluoride	9×10^{-4} –4×10^{-2}	8×10^{-4}	[Abd-Rabboh*et al.*, 2007]
Fluorimetry	Silica sol/gel encapsulated GOD	1×10^{-4} –2×10^{-2}	6×10^{-5}	[Chang *et al.*, 2010]
Potentiometry	Glasy electrode/ calyx[4]arene	1.6×10^{-4} –1.2×10^{-3}	2×10^{-5}	[Jin *et al.*, 2009]
Photoluminescence	Quantum dots–bienzyme hybrid	1×10^{-6} –1.5×10^{-4}	1×10^{-8}	[Yuan *et al.*, 2008]
Photoluminescent spectroscopy	GOD /MSA-CdSe/ZnS QDs	2×10^{-4} –1×10^{-2}	1×10^{-4}	[Huang *et al.*, 2008]
Chemiluminescence	KMnO4-NaOH-luminol	2.3×10^{-8} –7.2×10^{-5}	1.2×10^{-9}	This work

Table 3: Determination of Glucose Determination in the Human Blood Serum Samples

Sample Measurement			Standard Added Measurement		
Sample	Proposed Method Mean (mM) ± SD (%)[b]	Standard Method Mean (mM) ± SD (%)[b]	Added (mM)	Found (mM)	Recovery (%)
1	5.40±0.11	5.18±0.15	5.0	10.32±0.11	99.23
2	5.86±0.12	5.68±0.16	4.50	10.62±0.08	102.50
3	6.11±0.14	6.23±0.11	6.50	12.45±0.16	98.73
4	5.62±0.10	5.91±0.12	5.50	11.28±0.13	101.44
5	6.39±0.09	6.50±0.14	7.0	13.14±0.10	98.13

Note: [a] Spectrophotometric method; [b] Average from three determinations.

glucose can generate the hydrogen peroxide (Zargoosh *et al*., 2011; Yang *et al*., 1995; Zhao *et al*., 2006). So it can be deduced that the hydrogen peroxide generated from glucose oxidation by $KMnO_4$ has reacted with luminol and the excited 3-aminophthalate resulting from this oxidation emits the CL. So 3-aminophthalate is the CL emitter in the luminescence system.

Analytical Performance

Under the optimized conditions, the dynamic linear range for glucose determination was found from 7.2×10^{-5} to 2.3×10^{-8} M. The linear regression equation is following: $\Delta CL = 473083.82 + 62473.29 \times \log C$ (where C is the molar concentration of glucose), the correlation coefficient is R=0.995. The relative standard deviation for 11 measurements is 2.12%. The limit of detection (3σ) is 1.2×10^{-9}. The comparison between the proposed method and other reported methods for glucose determination in detection limit and linear range was summed up in Table *2*. It could be seen from Table 2 that the sensitivity of this proposed method was better than the other well-known methods, and is simpler.

Analysis of Serum Samples

The proposed luminescence method has been used to determine the glucose concentration in five different human serum samples and the results were compared with those obtained with the standard method, the obtained results by the two methods are shown in Table 3. It can be seen from Table 3 that there is no significant differences between the obtained results by the two methods. The recoveries of the three serum samples were found to be in the range of 98.13% to 102.5%. The results demonstrated the reliability of the present CL method for detecting glucose in practical serum samples.

CONCLUSION

A new flow-injection chemiluminescence method for glucose determination has been developed, which is based on the ability of glucose to enhance the CL signal of $KMnO_4$-luminol system. Under the optimized condition, a linear correlation was established between the chemiluminescence intensity and glucose concentration in the range of 7.2×10^{-5} to 2.3×10^{-8} M with a detection limit of 1.2×10^{-9} M. The possible reaction mechanism was discussed. The established method has been used to determine the glucose concentration in the human serum samples and the results are consistent with those found with the standard spectrophotometric method. The proposed method found its advantage in its wide linear range, simplicity, and lower detection limit.

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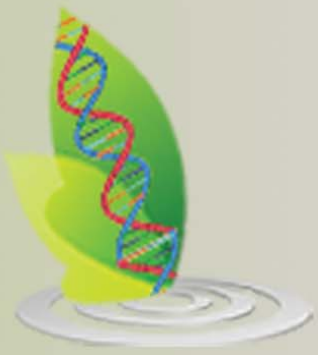

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