Flow Injection Analysis of Hydrogen Peroxide with Peroxyoxalate Chemiluminescence Detection

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Summary: A rapid and sensitive flow injection method is presented for the determination of hydrogen peroxide with chemiluminescence (CL) detection. CL is produced by the oxidation of bis(2,4,6-trichlorophenyl) oxalate by hydrogen peroxide in the presence of a fluorescent compound, perylene. The emission was monitored with a purpose-built flow injection CL detector. The responses between hydrogen peroxide concentration and CL intensity were linear over the range of (1×10^{-9} M-1×10^{-5}M) with a c.v. of 5.6% for 10^{-9}M hydrogen peroxide and 4.6 for 1×10^{-5}M hydrogen peroxide.

Introduction

Applications of CL and bioluminescence in analytical chemistry have attracted considerable interest in recent years [1,2]. The majority of work done in the field of CL is being devoted to solution phase CL system and flow injection analysis formats [3]. Peroxyoxalate CL reactions are the hydrogen peroxide oxidation of aryl oxalate esters in the presence of fluorophores. These reactions are the most efficient of non-biological CL reactions with quantum efficiency of 20-30% [4].

One of the most common oxalate ester is bis (2,4,6-trichlorophenyl) oxalate which requires hydrogen peroxide as oxidant and perylene as fluorophore [5,6]. Hydrogen peroxide can be generated by a number of oxidases. Therefore, its determination, selectively and sensitively is a pre-requisite for the field of biomedical chemistry [7]. Peroxyoxalate CL system provides a suitable detection system for the sensitive and selective determination of hydrogen peroxide [8]. A post-column peroxyoxalate-CL method in being used for the determination of hydrogen peroxide with a commercially available CL detector [9]. The linear range was 5-100 pmol with a limit of detection of 9.9×10^{-8}M.

We present a purpose built flow injection CL detector for the determination of hydrogen peroxide with peroxyoxalate CL reaction. The method is simple, rapid and sensitive and can be applied for the determination of enzymes/substrates that generate hydrogen peroxide.

Results and Discussion

Optimization of hydrogen peroxide manifold

The reaction conditions with respect to flow rate, pH and reagents concentration were optimized for hydrogen peroxide determination (Table-1). The optimum flow rate for maximum CL emission was 2.0 mL/min in both channels. The signal decreased above 2.0 mL/min which means that maximum CL occurred after the sample has passed through the glass flow cell. Therefore, a flow rate of 2.0 mL/min for both channels was used for subsequent experiments. The concentration of reagents used had significant effect on the peroxyoxalate reaction. The emission increased with increasing perylene concentration up to 1×10^{-9} M, above which perylene had little effect on CL intensity. The composition of acetonitrle/buffer (Imidazole) were investigated from 100% pure acetonitrile to 50/50 ratio of acetonitrile/buffer mixture. Maximum CL was obtained when 100% pure acetonitrile was used. The effect of pH were investigated over the range of 6.9-8.0, the optimum pH was 7.0 which agrees with previously reported results [5].

Calibration data for hydrogen peroxide

The results from triplicate injection of hydrogen peroxide over the range of 1×10^{-9} - 1×10^{-3}M are shown in Table-2. The CL intensity is linear over the range 1×10^{-9} - 1×10^{-5}M, above which the system saturated with respect to hydrogen peroxide concentration. No blank signal was obtained which shows that the method is

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Table-1: Effect of flow rate, pH and reagents on CL intensity.

Flow rate ml/min	0.5	1.0	2.0	3.0	4.0
CL intensity* (mV)	0.24	0.66	0.90	0.82	0.76
pH	6.0	6.5	7.0	7.5	8.0
CL Intensity* (mV)	0.28	0.54	0.90	0.82	0.76
Perylene (M)	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$	$1x10^{-3}$	$1x10^{-2}$
CL Intensity* (mV)	0.25	0.60	1.30	5.50	5.80
Acetonitrile%)	100	90	80	60	50
CL Intesnsity* (mV)	1.80	0.85	0.40	0.24	0.12

*Mean of three injections.

Table-2: Calibration data for hydrogen peroxide determination

Hydrogen peroxide (M)	CL Intensity* (mV)	r.s.d. (%)
1×10^{-9}	0.05	5.6
1×10^{-8}	0.08	5.4
1×10^{-7}	0.14	5.1
1×10^{-6}	0.48	4.2
1×10^{-5}	0.94	4.6
1×10^{-4}	1.35	4.0
1×10^{-3}	1.35	3.4

*Mean of three injection.

attractive for enzymes/substrates determinations in samples of clinical importance. A log -log calibration graph shows the dynamic range of peroxyoxalate CL system for hydrogen peroxide.

Experimental

Reagents

All chemicals were purchased from Sigma unless otherwise stated. A stock solution of bis(2,4,6-trichlorophenyl) oxalate (TCPO, 10 mM) was prepared by dissolving 0.45 g in 100 ml acetonitrile. Working solutions were prepared in acetonitrile by serial dilution of the stock solution. Stock perylene (1 mM) as fluorescent compound was prepared by dissolving 0.025 g of compound in 100 mL of acetonitrile. Imidazole buffer (0.05 M) was prepared by dissolving 3.4 g in 1 litre of water, pH was adjusted with 0.1 M of HCl. A stock (0.1 M) of hydrogen peroxide (Merck, 30% v/v) was prepared by dilution of 1 mL of hydrogen peroxide in 100 mL of water. Distilled and deionized water was used and all chemicals were of Analytical grade.

Instrumentation and procedure

Fig. 1 shows a purpose build flow injection CL analyzer for the determination of hydrogen peroxide using peroxyoxalate CL system. Acetonitrile and imidazole buffer (1 mM) streams were pumped at 1 mL/min using a peristaltic pump (Reglo 100) with poly (venyl chloride) pump tubing. Teflon tubing (0.5 mm i.d.) was used

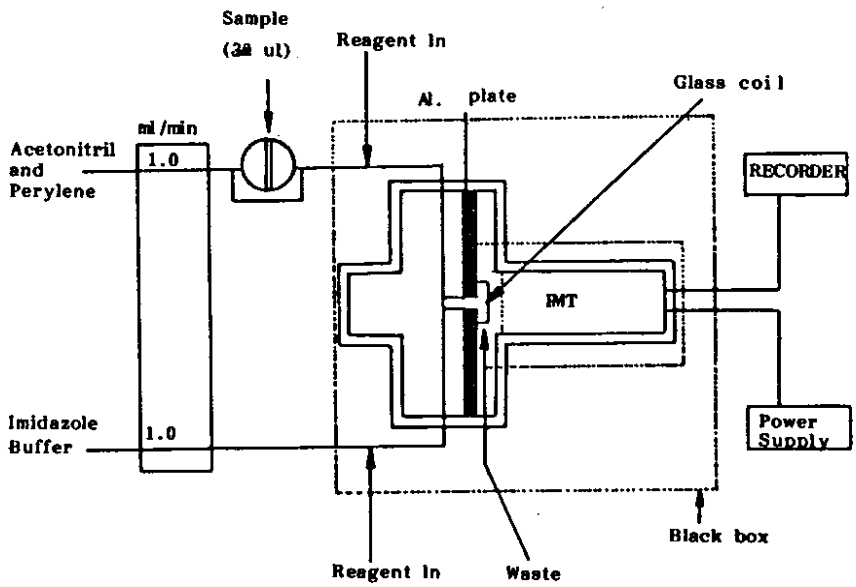

Fig. 1: A purpose - Built flow injection CL analyzer for hydrogen peroxide determi-nation.

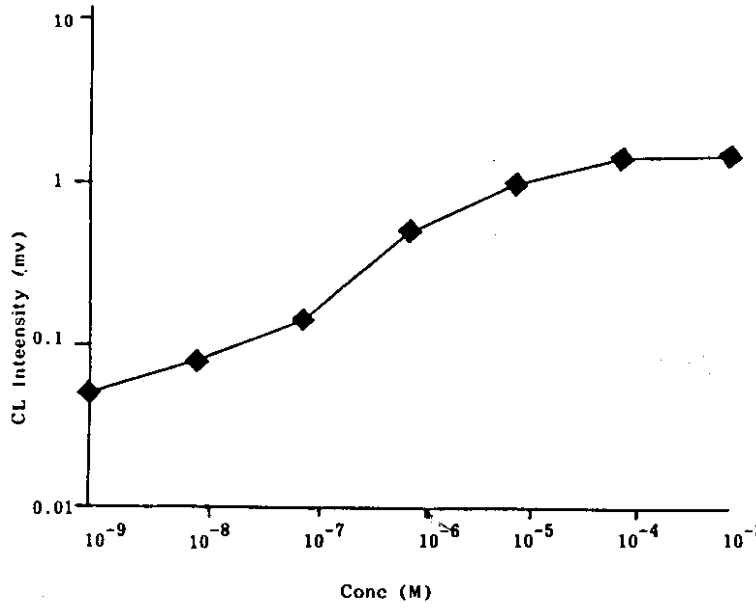

Fig. 2: Calibration graph for hydrogen peroxide determination.

throughout the manifold. Hydrogen peroxide standard (30 µl) was injected using injection valve (Rheodyne 5020) into a stream of acetonitrile containing TCPO (1mM) and perylene (0.1 mM). The injected sample zone was merged with a stream of imidazole buffer (1mM, pH 7.0). The two streams travelled 1.8 cm before passing into a glass coil (1.0 mm i.d. x 80 mm length). The coil was positioned in front of an end-window photomultiplier tube (Thorn EMI 9789QB). An aluminium foil was placed behind the coil to reflect light on the photocathode. The detector output were recorded using a strip chart recorder (Kipp and Zonen BD 40). The photomultiplier tube, glass coil and T-piece were placed in a black box to further minimize the stray light [10].

Conclusions

The results shows that flow injection is an excellent tool for studying CL reactions. The method provides simple, rapid and inexpensive means of determination of hydrogen peroxide. The peroxyoxalate CL system is suitable for enzymes/substrates analysis in various clinical samples.

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