New design and Modulating of Spectroinc 20 to the Home- Made Semi-Automated Chemiluminescence Flow Injection System and Determination of Hydrogen Peroxide in Pharmaceutical Formulations

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Abstract: ***A novel design has been constructing and modulating of Spectroinc 20 to measure the chemiluminescence by the home-made semi-automated flow injection system .The proposed method was direct, simple and rapid for determining Hydrogen peroxide in the rang 3.3-106 µgml^{-1} based on chemiluminescence by oxidation with luminol in aqueous sodium carbonate(PH 10) . The detection limit (3×noice) of 1.6 µgml^{-1}, the r.s.d % for fife determination of 26.5 µgml^{-1} Hydrogen peroxide was 0.35%.. The applicability of the method was demonstrated by the determination of Hydrogen peroxide in pharmaceutical formulations.***

Keywords: Spectrinc 20 ;Chemiluminescence determination; Flow injection; Luminol; Hydrogen peroxide; pharmaceutical formulations

1. Introduction

Chemiluminescence (CL) is generally defined as the emission of light[1] (ultraviolet, visible or infrared) during the process of a chemical reaction. CL detection has advantages including simplicity of operation, low cost of instrumentation, high selectivity, wide dynamic range, reproducibility and rapidity[2]. There are two basic measurement approaches with CL: static samples and flowing streams, flowing stream methods are almost always used with gas-phase CL and are very common with solution-phase CL. CL reagent and analyte streams continuously flow and are mixed together, with the mixture continuing on in the flowing stream[3] . Observation occurs at a fixed position after mixing. The volume between the point of mixing and the point of observation, plus the flow rate in this region, determines the time in the CL emission intensity-versus-reaction-time profile at which observation occurs, and the observed emission intensity remains constant at this value[1,4]. For maximum sensitivity, it is desirable to adjust the flow rate, observation cell volume, and transfer line volumes so that the point of observation comes at the peak in the CL intensity-versus-time profile[5]. The combination with flow-injection analysis (FIA) has made chemiluminescence detection more attractive. Flow-injection chemiluminescence (FI-CL) methods can provide a high versatility in the determination of a wide variety of species along with rapidity, simplicity, consecutive automatism, high sensitivity, wide linear range and good reproducibility, requiring only simple and low-cost-measuring devices, and have been used for determination of many substances[6-9]. Luminol (5-aminophthalylhydrazide) is so far the most frequently used CL reagent[10]. The CL emission of luminol is basedon its oxidation by hydrogen peroxide[11], hexacyanoferrate(III), permanganate, N-bromosuccinimide (or N-chlorosuccinimide), periodate, dichromate, persulphate, dichlorocyanurate ortrichlorocyanuric acid, chlorate and electrogenerated hypobromite in alkaline medium[12]. According to the well-known mechanism(fig 1), the supposed emitter is excited 3-aminophthalate anion whose maximum emission occurs at425 nm[10,13]. A lot of methods have been proposed for its determination including spectrophotometry, fluorimetry, high performance liquid chromatography, amperometry[14]and flow injection Chemiluminescence method is commonly used in the determination of hydrogen peroxide because of its low detection limit and wide dynamic range that can be achieved with relatively simple instrumentation[15]. In this laboratory at the time of this work of combining chemiluminescence with FIA was put forward, no detector existed to accomplish that, therefore, the aim of this study specially focused upon constructing and building –up a home- made semi-automated chemiluminescence flow injection system. This system can readily be assembled from simple, inexpensive, off the shelf components and evaluated the accuracy of this system.

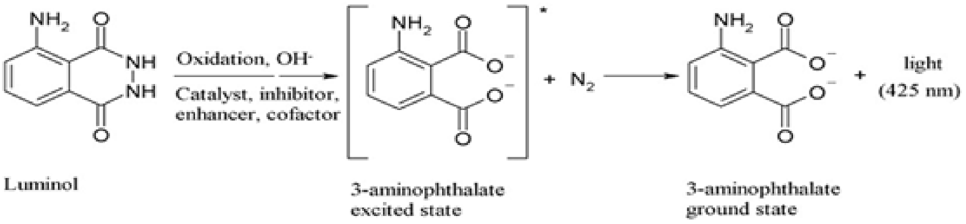

Figure 1: Luminol CL scheme.

2. Experimental

2.1 Reagents

During analytical applications with a home-made system deionized distilled water was used throughout and all reagents employed were analytical grade unless otherwise stated. All stock solutions were prepared in deionized distilled water.

- 0.1M Na_2CO_3 buffer solution: was prepared by dissolving 10.50 g of Na_2CO_3 and 0.5M NaOH to exactly 1liter[16].
- 0.01 M Luminol solution: (5-amino-2,3-dihydro-1,4_phthalazinedione) (Sigma, St. Louis, MO, USA) was prepared by dissolving 0.1772 g of luminol (97%) in 100 ml of 0.1M Na_2CO_3 solution the buffer(pH 10)[17].
- 0. 1M Hydrogen peroxide[17] was prepared by diluting2.8 ml of 30% concentered Hydrogen peroxide (Sigma, St. Louis, MO, USA) in 250 ml . The working and standard solutions were prepared by stepwise dilution by deionized water.

2.2. Instruments and flow System

To monitor the chemiluminescence with high sensitivity the flow system required a low pulsation flow to give a really stable baseline. Bidirectional pump (ISMATEC ,REGLO, ISM796,Switzerland) used to accomplish this, so it was used in the system (Fig.2a,b) and 1.00 mm (i.d.) standard pump tubing was used with this pump to propel 10^{-3} M lumenol as a carrier stream . The sample was introduced into the carrier stream through an injection valve (Rheodyne, Califorina) equipped with variable sample loop .A Spectroinc 20(fig 3) adapted to be chemiluminescence detector by removing source and the monochromator and change of sensitivity for operational amplifiers (Fig 4) system as connected directly with Gas filled photo tube (CETRON CEA95) (Fig 5) photoelectric device is the vacuum phototubes' which consists of a semi cylindrical cathode and a wire anode sealed inside an evacuated trans-parent envelope . The concave surface of the electrode supports a layer of photo emissive material that tends to emit electrons when it is irradiated. When a voltage is applied across the electrodes, the emitted electrons flow to the wire anode generating a photocurrent that is generally about one tenth as great as that associated with a photovoltaic cell for a given radiant intensity. In contrast, however, amplification is easily accomplished (Fig 6) because the phototube has a high electrical resistance [18]. The PMT single was recorder by (Siemens Kompenso graph model 7KC 1032-8BC).The PMT was attached directly(Fig 7a ,b) to the home-made glass flow coil .It was made by winding desired length (about 35 cm) 0f 0.8 mm i.d glass tubing which is design in our laboratory. The coil is fixed by mean of transparent adhesive to the surface of a black plate, the central area of which had previously. The foil enhance the collection of the emitted light for chemiluminescence only(Fig 7 c ,d), also in all experiments Teflon tubing of 0.5 mm (i.d.) was used for the rest of the flow system. The peak heights of the system were recorded with output sensitivity 500 mV and with speed control (3 cm /h) in order to eliminated the effect of the noise in the system and also to achieve smooth peaks response.

2.3 Procedure

The carrier stream was 10^{-3} M Luminol solution run into the manifold (Fig.2) at flow rate 3 ml min^{-1}. A 60 µL as a sample volume of Hydrogen peroxide was injected manually through the injection valve into carrier stream. Reaction coil of 5 cm length was inserting before the detector and the chemiluminescence of the Hydrogen peroxide sample was measured by the home –made system. The recorded peak height can be related to the concentration of injected sample.

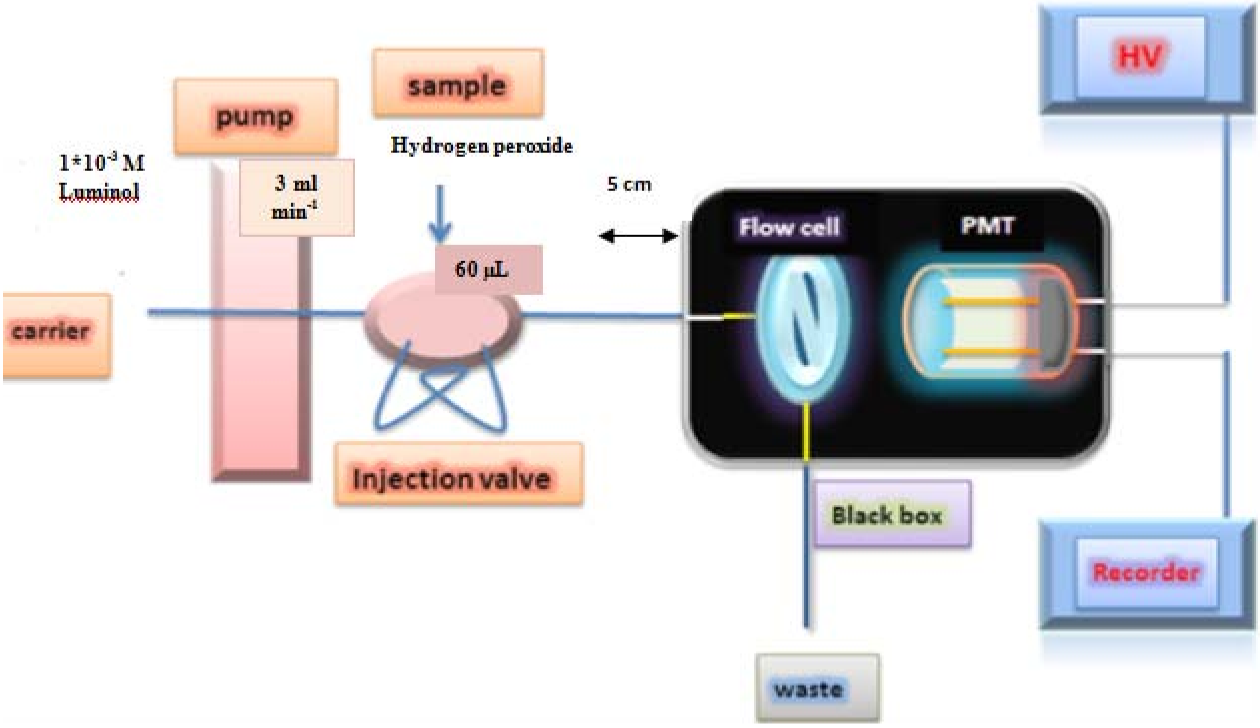

Figure 2a: scheme of Home-made semi-automated chemiluminescence flow injection system

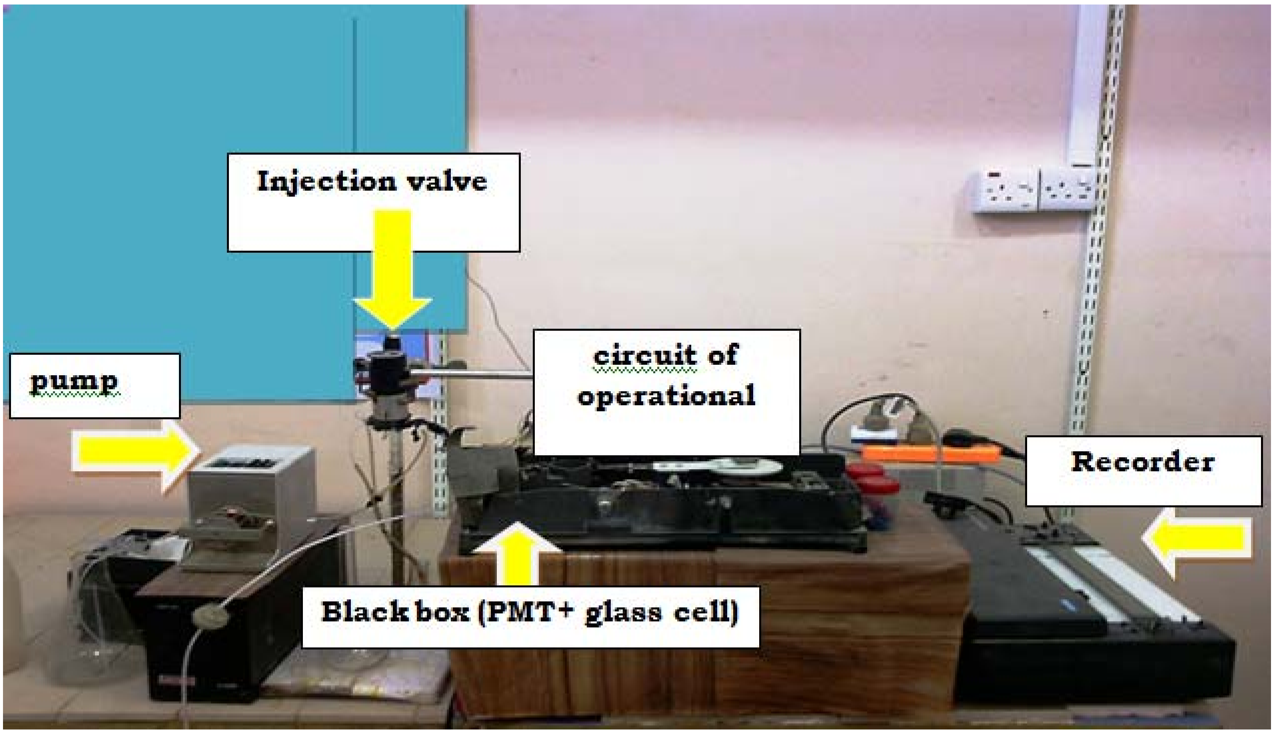

Figure 2b: Home-made of FICL system

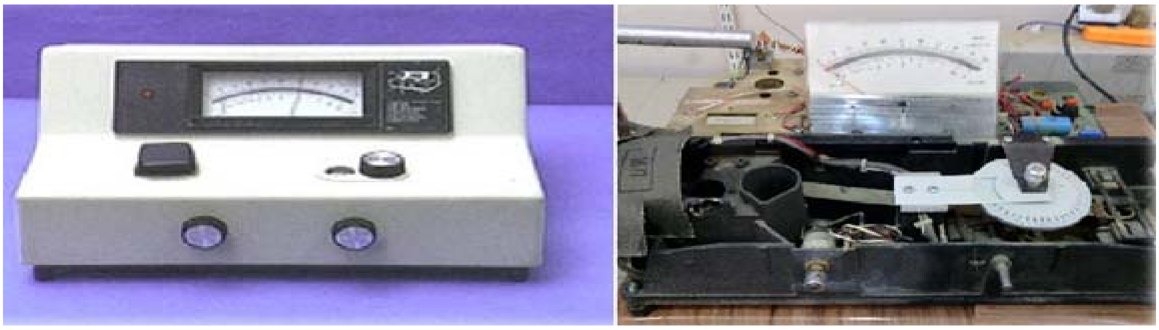

Figure 3: Spectroinc 20

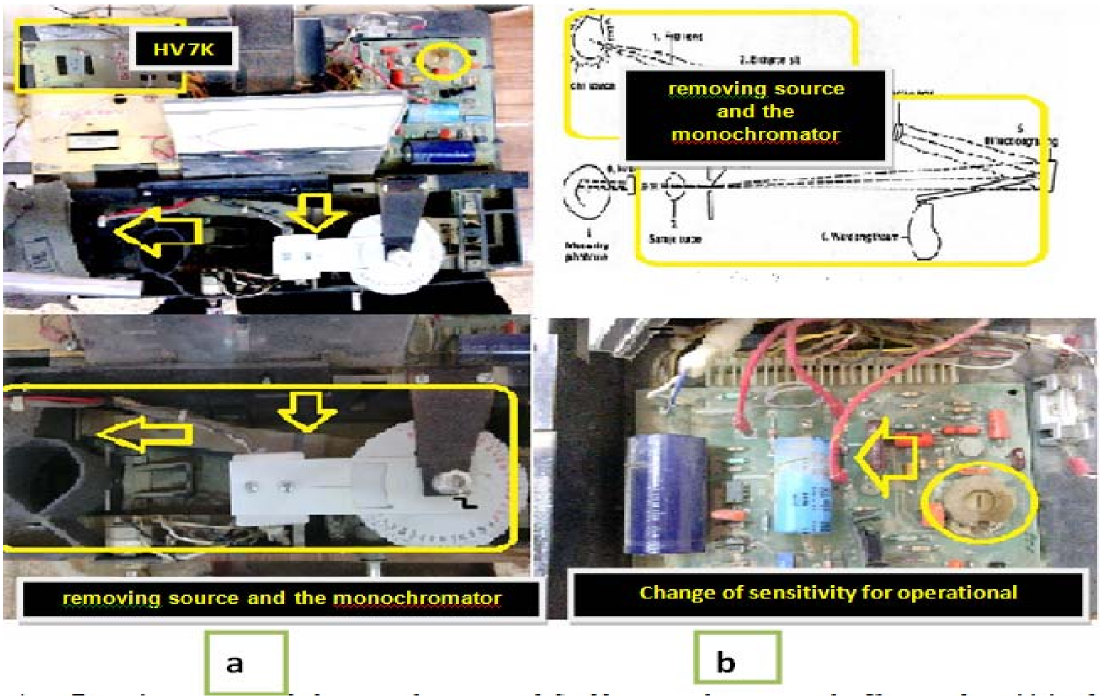

Figure 4: a- Removing source and the monochromator of Sp 20 spectrophotometer, b- Change of sensitivity for operational amplifiers

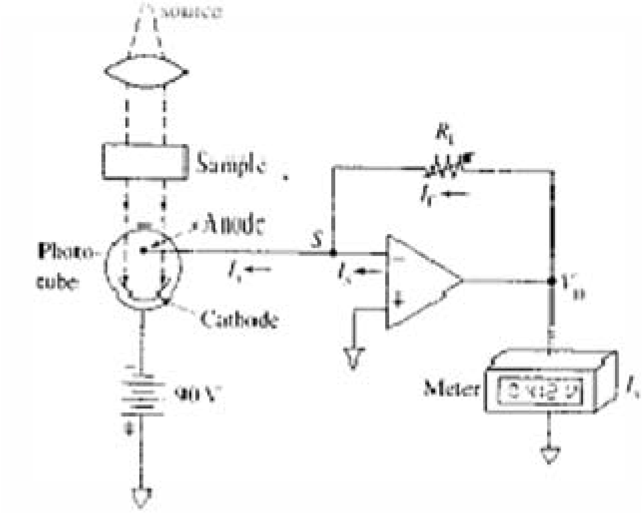

Figure 5: Gas filled photo tube Fig.6 A circuit of an operational amplifier

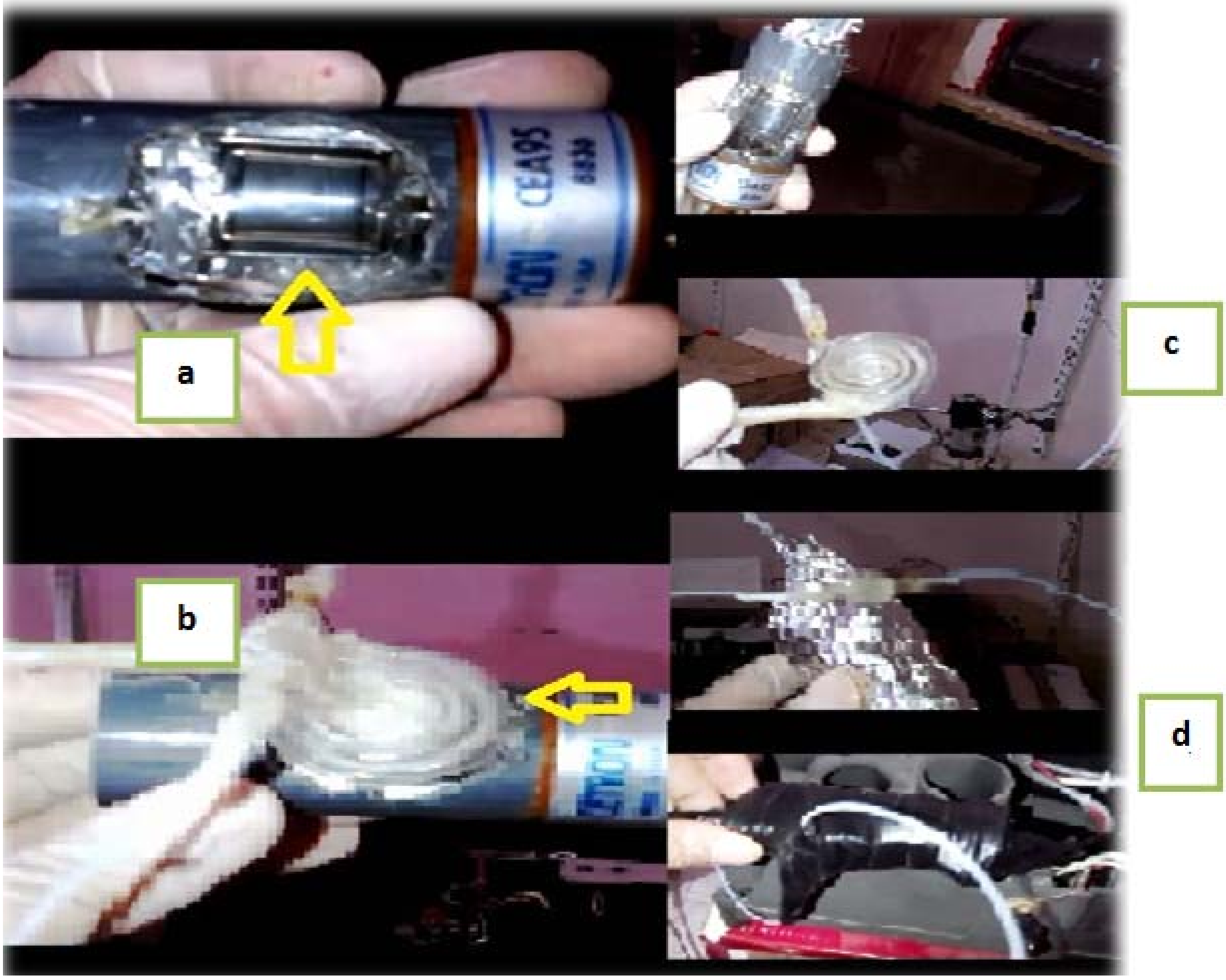

Figure 7a, b: The PMT was attached directly to the home-made glass flow coil, c glass flow, d covered with sheet of aluminum foil and black chamber of PMT

2.3 Procedure

The carrier stream was 1*10^{-3} M Luminol solution run into the manifold (Fig.2) at flow rate 3 ml min^{-1}. A 60 µL as a sample volume of hydrogen peroxide was injected manually through the injection valve into carrier stream. Reaction coil of 5 cm length was inserting before the detector and the chemiluminescence of the hydrogen peroxide sample was measured by the home –made system. The recorded peak height can be related to the concentration of injected sample

3. Result and discussion

3.1 Optimization of Manifold Parameters and Reagent Concentrations

The FI manifold is optimized for rapid determinations of hydrogen peroxide by conducting a series of experiments When the chemiluminescence FI system in(Fig 2) was used the flow rate increased almost parabolically with increasing the flow rate (Fig 8), so 3 min h^{-1} was selected for subsequent work due to smooth and good reproducibility of the obtained peaks . The peak height increased almost parabolically with increasing of the in injected volume (Fig 9) between 10 -150 µL. The maximum peak height was obtained when 120µL was injected, but the peak shape was smooth distorted, so 60 µL was injected in subsequent experiment (Fig 10) indicated that the peak height increased with increasing the luminol concentration in the range $3*10^{-4}$ -5.0* 10^{-3} M. It is well know that increasing in luminol concentration leading to more intensity of chemiluminescence .So1.0* 10^{-3} M was selected as an optimum concentration[19].

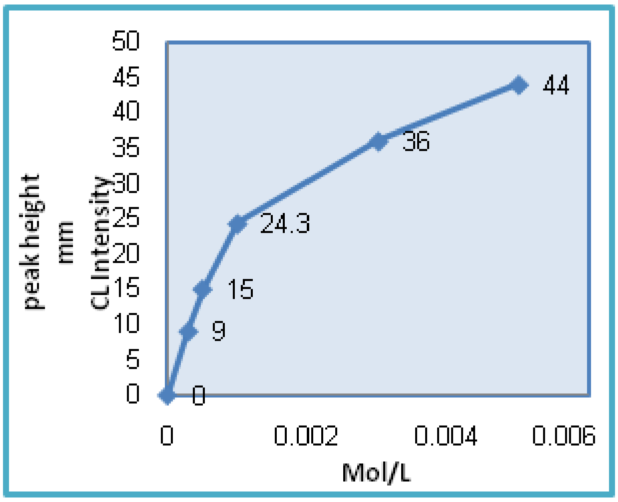

Figure 10: Effect of luminal Conc. on peak height

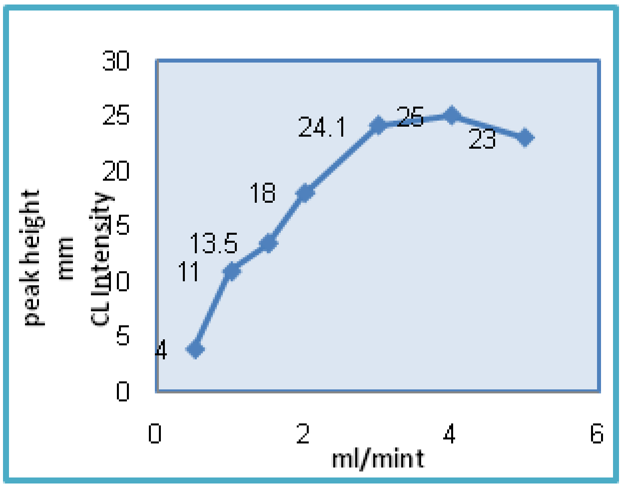

Figure 8: Effect of Flow rate on peak height of H_2O_2

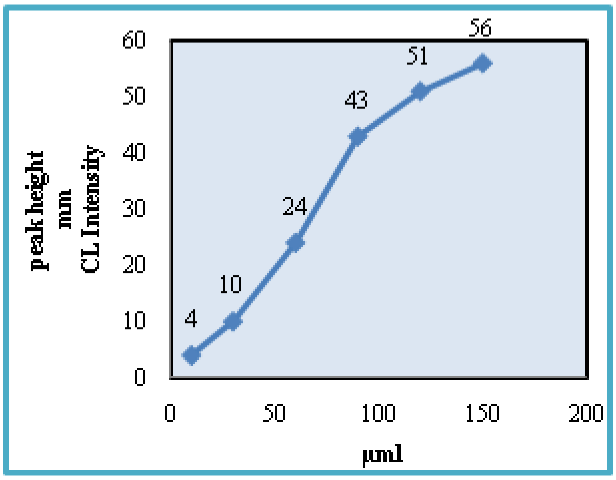

Figure 9: Effect of sample volume on peak height of H_2O_2

3.2. Calibration Characteristics

Under the optimum operating conditions, analytical characteristics[20] such as reproducibility and detection limit were investigated for hydrogen peroxide. A calibration graph of hydrogen peroxide was constructed between the CL intensity (peak height, Y, mm)and the concentration(X) range (3.3µgml^{-1}-106 µgml^{-1})in which graph was linear .This linear range allows the determination of 4 µgml^{-1} of hydrogen peroxide .The regression line of emission intensity (*I*, peak height)on hydrogen peroxide concentration (C)was I =2.0061+0.4101C (r^2=0.9943 and r=0.9987, for 6 points) with detection limit (3×noice) of1.6 µgml^{-1} .The r.s.d % for fife determination of 26.5 µgml^{-1} hydrogen peroxide was 0.35%. The sample throughput was 110 samples h^{-1} . The method was tested for several of synthetic samples of hydrogen peroxide .some of the analytical results are given in the Table(1)

Table 1: Determination 0f H_2O_2 in synthetic sample

sample	*Conc. Of H_2O_2, µgml^{-1}*		*Error%*	*RSD%*
	Taken	Found*		
1	10.0	9.85	-1.5	0.57
2	85.0	85.11	+0.13	0.57

*Average of fife replicates.

The accuracy and precision of the synthetic sample was tested by analyzing in tow different concentrations of H_2O_2 for five replicate. The values of the percentage errors (E%) and percentage relative standard deviation (RSD%) are summarized in Table (1). These values indicate the high accuracy and precision of the synthetic sample.

3.3. Application of the method

In order to demonstrate the applicability of the proposed method for the determination of H_2O_2, the method was successfully applied to the analysis of H2O2 in pharmaceutical formulation, as shown in Table(2). The assay results of proposed method were in good agreements with the declared contents. In (EAU OXYGÉNÉE, Gifrer) quantitative recoveries between98 and 98.62% were obtained ;but presenting of recovery was low obtained, because this formulation is considered combined phosphoric acid, sodium salicylate and sodium stannate as well as compounds which greatly affect the CL intensity[22] due to the formation of stable complexes or insoluble compounds,

Table 2: Application of the proposed method for determination of H_2O_2 in pharmaceutical solution

pharmaceutical Solution	Conc. Of H_2O_2, μgml^{-1}		Error %	Recovery %	RSD %
	present	Found*			
EAU OXYGÉNÉE, Gifrer (stabilisé20 volumes)6%	40	39.2	-1.93	98	0.69
	85	83.8	-1.35	98.62	0.4

***Average of fife replicates.**

3.4 Evaluation of the Proposed Method

For evaluating the competence and the success of the proposed method in the analysis of Hydrogen peroxide in pharmaceutical formulation, the results obtained were compared with those obtained by chemiluminescence flow injection method[21]. In which a quantity of solution there was good agreement between the methods**.**

4. Conclusion

In this paper , the design has been constructing and modulating of Spectroinc 20 to measure the home- made semi-automated chemiluminescence flow injection system so results were very good, high sensitivity and reproducibility when it was applied to determination some pharmaceuticals that contain hydrogen peroxide **.**

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