Monitoring Electrophilic Substitution for Determination of Amino Compounds by Flow Injection Chemiluminescence Methods

Diyar Salahuddin Ali

Department of Chemistry, College of Science, University of Salahaddin, Erbil, Iraq

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Abstract

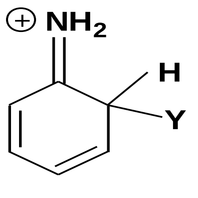
Flow injection chemiluminescence procedure is described for determination of some amino compounds, based on their reaction with bromine produced from reaction of bromate/bromide mixture in acidic medium. Some bromine is consumed and the rest catalyze the chemiluminescence intensity of luminol/H_2O_2 system. The flow injection chemiluminescence methods with merging zone principle were conducted instantly through an on-line design. Sampling rate was 95 $s.h^{-1}$. Amino compounds such as aniline, 1-naphthyl amine, o-anisidine, 2-methyl aniline and acryl amide were determined and best working conditions for their determinations were optimized. Linear calibrations of these compounds were in the range $1x10^{-7} – 1x10^{-2}$ M with correlation coefficients of (0.989), (0.985), (0.992), (0.997) and (0.980) respectively.

Keywords: Flow injection, Chemiluminescence, Bromination, Amino compounds.

Introduction:

Amino compounds have great importance in different areas, such as food, industry and fertilizers. They are present in smoke of cigarette [1], and soil [2]. Generally, nitrogen levels in different samples transformed to different nitrogenous forms exhibit potential health hazards [2]. Methods of determination of amino compounds, are thus utmost importance including fluorimetric [3], colorimetric [4], spectrophotometric [5-7], chromatographic methods [8-9] and flow injection analysis [10]. Chemiluminescence was also used for determination of amino compounds [11].

The (-NH_2) groups act as powerful activators and ortho, para directors in electrophilic aromatic substitutions. These effects were accounted for by assuming that the intermediate carbonium ion is stabilized by the structure like I and II in which nitrogen bears a positive charge and is joined to the ring by a double bond[12].



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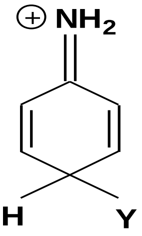


II

In the halogenation of aromatic amines the chief difficulty encountered is that the reaction proceeds too readily. Activation by the amino group is so powerful that halogen tends to enter every available ortho or para position. Thus aniline yields 2,4,6-tribromoaniline.

The bromine that produced in a flow system by mixing $KBrO_3$ and KBr in acidic medium enhances the Chemiluminescence (CL) of luminol/ H_2O_2 reaction system [13].

Thus determination of amino compounds include inhibition of the above CL reaction. The deactivation of the CL intensity is directly proportional to the amount of the amino compounds. Same principle was used for determination of phenolic compounds [14].

Experimental

Apparatus

A multichannel peristaltic pump (Desaga, 12-channel PLC), two six-way injection valves (Rheodyne U.S.A.) with 60 µl sample loop, a reaction cell (spiral 100 (l), an Ultraspoec II (LKB Biochrom) and a single beam spectrophotometer the light source of which is blocked carefully were used. Associated electronics included DC-microvoltmeter (Philips) and PE 1644 DC power supply 40V-10A (Philips). The chemiluminescence signals were recorded on a Philips PM 251 a one-line recorder.

Chemicals

All chemicals were analytical grade reagents and distilled- deionized water was used throughout. The following solutions were prepared.

Sodium carbonate solution, 0.5 M (52.99 g of Na_2CO_3) were dissolved in 1l of water. From this solution 1l of 0.1 M Na_2CO_3 was prepared.

Luminol solution, 0.01 M. (0.08858 g of luminol) were dissolved in 500 ml of 0.1 M Na_2CO_3 solution. Other solutions were prepared by suitable dilutions with 0.1 M sodium carbonate solution.

Hydrogen peroxide, 0.1 M. (9.72 ml of 35% (w/v) H_2O_2) were diluted to 1L with water.

Potassium bromate solution, 0.4 M. (33.402 g of $KBrO_3$) were dissolved in 500 ml of water.

Potassium bromide solution, 1 M. (59.5 g of KBr) were dissolved in 500 ml of water.

Stock solutions, (0.1 M each) of aniline, 1-naphthyl amine, o-anisidine, 2-methyl aniline and acryl amide were prepared by dissolving 2.325 g C_6H_7N, 3.575 g $C_{10}H_9N$, 3.20 g $C_7H_{10}NO$, 2.675 g C_7H_9N and 1.775 g C_3H_5NO each in a few milliliters of water then completed to 250 ml of distilled water.

Procedure

Fig. (1) shows a schematic diagram of the system used in which amino compound (60µl) is injected through the sample loop into a stream of distilled water which mixed with a $KBrO_3$ and KBr in 2 x 10^{-1} M HNO_3 as acidic medium which is preferable for this type of reactions [16]. A rapid bromination of the amino compounds will occur and the excess Br_2 catalyzes the chemilum-inescence of luminol/H_2O_2 reaction system. In this procedure scheme two injection valves are used to inject simultaneously both amino compounds and luminol so as to avoid continuous emission signals.

Results and Discussion

Optimizations:

Necessary optimizations include study of chemical and physical variables that affect the determination of amino compounds.

Chemical Optimizations:

Fig. (2) and (3) illustrated the results for effects of different luminol and H_2O_2 concentration respectively. $3x10^{-4}$ M luminol and $2x10^{-2}$M H_2O_2 gave the best results.

Solutions containing $2x10^{-2}$M H_2O_2, $3x10^{-4}$M luminol and $2x10^{-1}$M $KBrO_3$ and different bromide ion concentrations ($5x10^{-1}$, $3x10^{-1}$, $1x10^{-1}$, $7x10^{-2}$, $5x10^{-2}$,and $1x10^{-2}$M) were prepared in 50 ml calibrated flasks. $3x10^{-1}$M Br^- gave maximum response as shown in Fig. (4).

Physical Optimizations:

Effects of different delay coils on the CL-intensity were studied. Best emission signals recorded, as peak height (mV) was 35 cm for all determinations. Optimization of flow rate was executed depending on extent of bromination process of amino compounds. Fig. (6) illustrates the result. A flow rate of 2.5 ml/min is found to be the best as a compromise between better inhibition process and smoother emission signals.

Determination of Amino Compounds

The flow injection chemiluminescence system shown in Fig.(1) was used for the determination of amino compounds. Calibration graphs for aniline, 1-naphthyl amine, o-anisidine, 2- methyl aniline and acryl amide were plotted respectively as the logarithm of the chemiluminescence intensities expressed as peak height in mV and logarithm of concentrations. Each measurement was repeated three times.Table (2) gives the statistical data.

Conclusion

Use of electrophilic substitution and merging zone principle for detection of amino compounds by FIA-CL technique is a possible solution to many of the inherent problems. This developed method gave lower detection limits and wide linear dynamic ranges. The present method is fast, simple and sensitive allows determination of 95 $s.h^{-1}$

Amino compounds are among other organic molecules to have this fast bromination ability. Therefore, they can be determined successfully. Compared with other analytical methods (e.g., spectro-photometry, electron paramagnetic resonance, gas or liquid chromatography, and mass spectrophotometer) the present study is generally highly sensitive, and accurate for amino compounds, especially at the low concentrations [17].

Finally, the present method may successfully be used for determination of amino compounds in real samples such as industrial products. In this case design modification is necessary by introduction a suppressor unit into the FIA-CL system to remove undesired species.

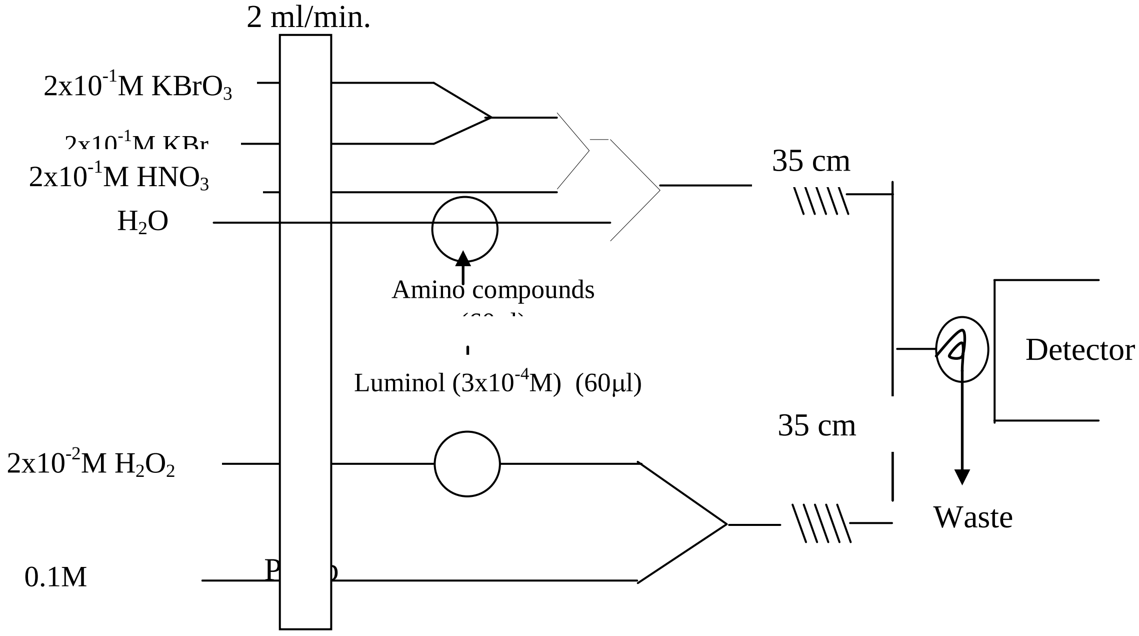


Fig. (1): Schematic diagram of the flow injection chemiluminescence system with merging zone principle for determination of amino compounds.

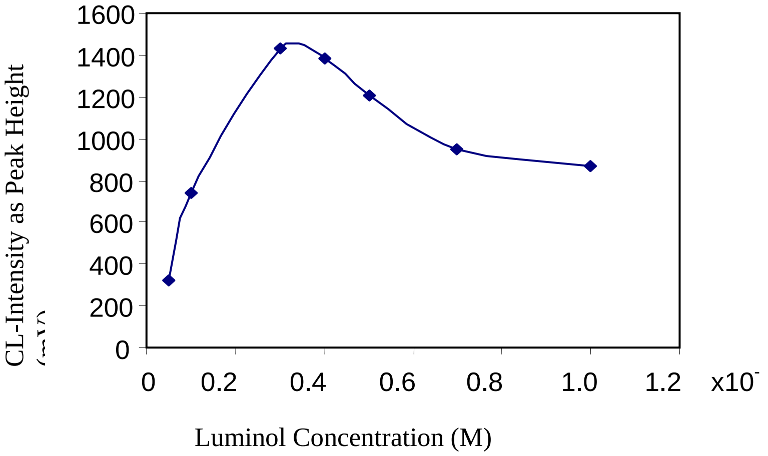


Fig. (2): Effect of luminol concentration on the CL-intensity [KBr] 1×10^{-1} [$KBrO_3$] 2×10^{-1}M,[H_2O_2] 2×10^{-2}M,and [HNO_3] 2×10^{-1}M.

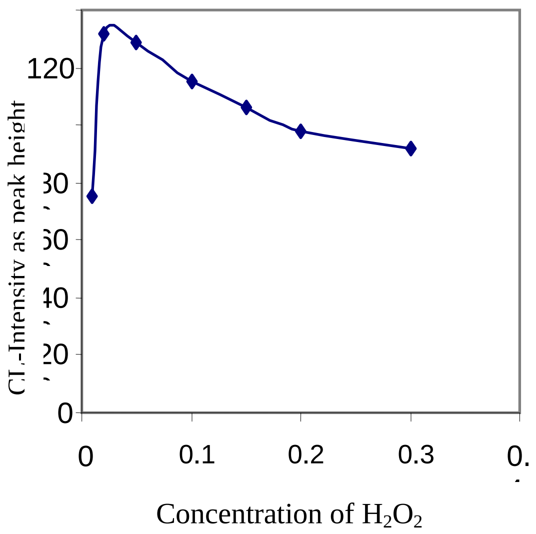


Fig. (3): Effect of hydrogen peroxide concentration o the CL- intensity [KBr]2x10^{-1}M,[KBrO$_3$] 2x10^{-4}M,[Luminol]2x10^{-4}M,and[HNO$_3$]2x10^{-}.

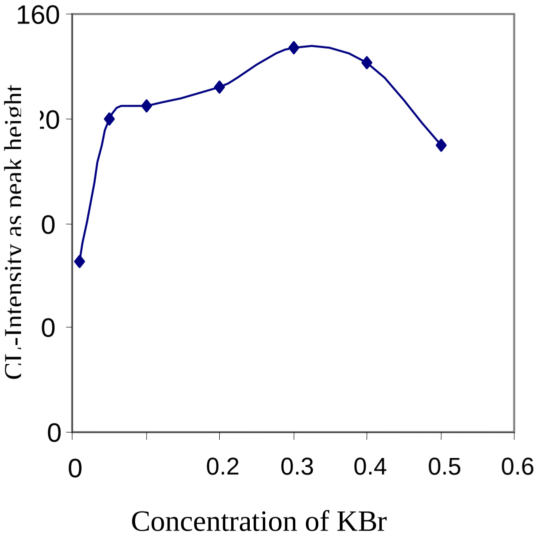


Fig. (4): Effect of bromide ion concentration on the CL- intensity [H$_2$O$_2$] 2x10^{-2}M,[KBrO$_3$]

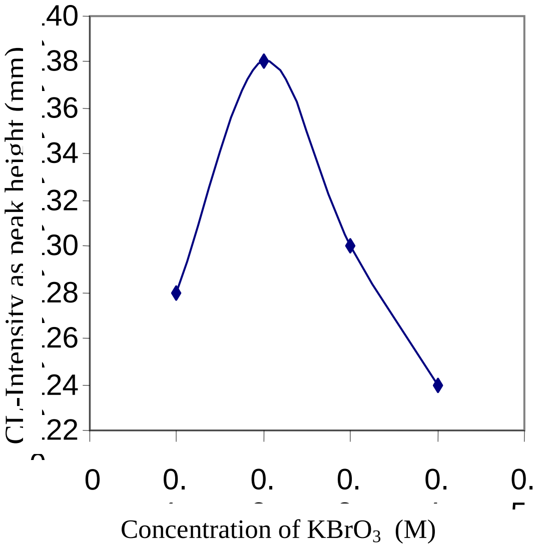


Fig. (5): Effect of bromate ion concentration on the CL- intensity [KBr]3x10^{-1}M,[KBrO$_3$] 2x10^{-4}M,[Luminol]2x10^{-4}M,and[HNO$_3$]2x10^{-1}.

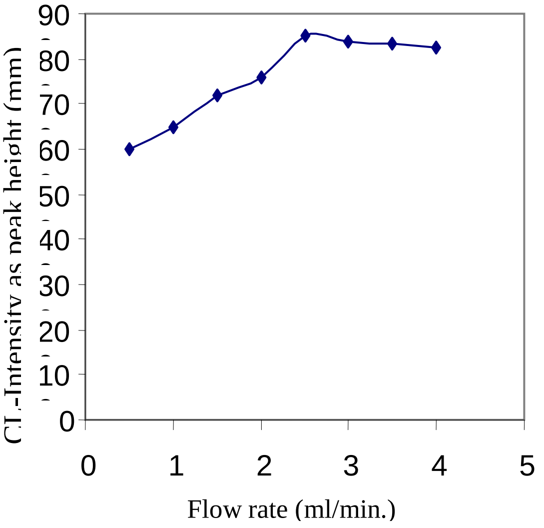


Fig. (6): Effect of flow rate on the CL- intensity KBr]3x10^{-1}M,[KBrO$_3$]2x10^{-1}M,[Luminol] 3x10^{-4}M.and[HNO$_3$]2x10^{-1}.

Table (1): Optimum working conditions for determination of amino compounds using on-line FIA-CL system

Parameter	Value
Luminol	3 x10^{-4} M
H_2O_2	2 x 10^{-2} M
HNO_3	2 x 10^{-1} M
KBr	3 x 10^{-1} M
$KBrO_3$	2 x 10^{-1} M
Flow Rate	2.5 ml/min.
Delay Coil	35 cm

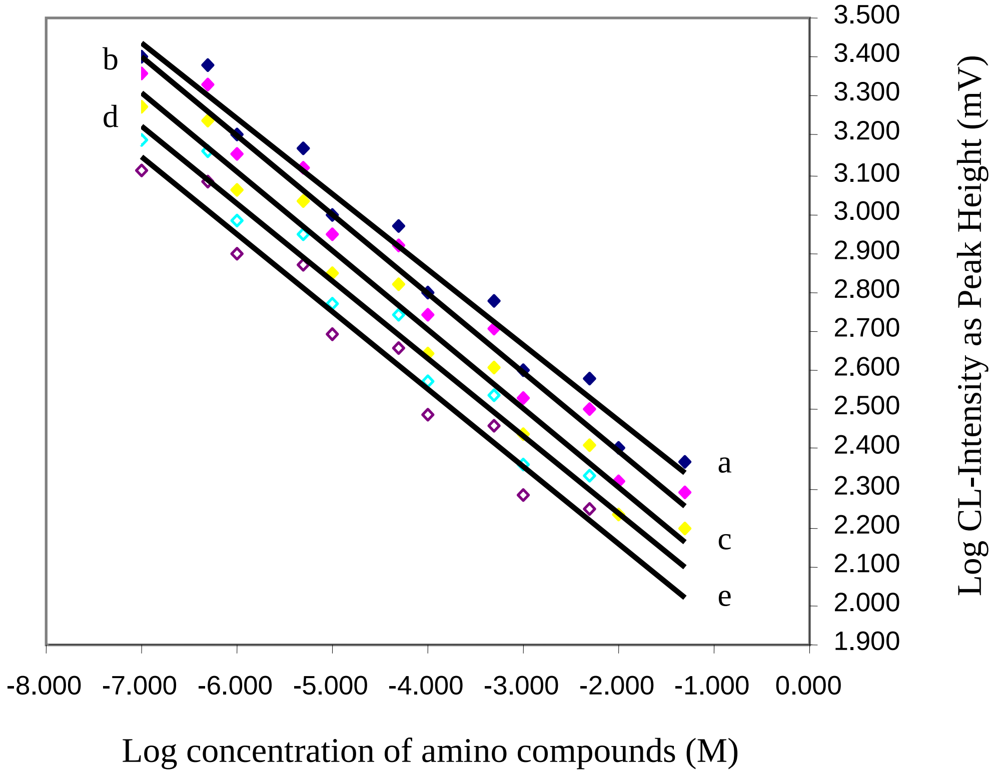
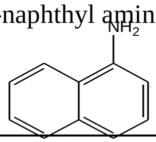
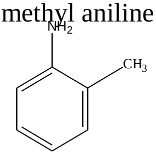
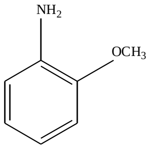
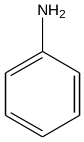


Fig.(7): Log chemiluminescence intensity expressed as average peak height (mV) versus log concentrations of amino compounds (M).

a) Acryl amide
b) 1-naphthyl amine
c) 2-methyl aniline
d) o-Anisidine
e) Aniline

Table (2): The statistical treatment of calibration results of amino compounds by FIA-CL method.

Amino compounds	Structure	Linear range M	Slope (b)	Intercept (a)	r	R.S.D.%
Acryl amide	CH_2=CH-CONH$_2$	$1x10^{-2}$ – $1x10^{-7}$	-0.18	1.65	0.989	0.025
1-naphthyl amine 		$1x10^{-2}$ – $1x10^{-7}$	-0.15	1.35	0.985	0.035
2-methyl aniline 		$1x10^{-2}$ – $1x10^{-7}$	-0.18	1.25	0.992	0.074
o-Anisidine 		$1x10^{-3}$ – $1x10^{-7}$	-0.20	1.75	0.997	0.064
Aniline 		$1x10^{-3}$ – $1x10^{-7}$	-0.20	1.71	0.980	0.075

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رصد التعويض الالكتروفيلي لتقدير المركبات الأمينية بواسطة طريقة تحليل الحقن الجرياني – البريق الكيميائي

ديار صلاح الدين علي محمد

قسم الكيماء ، كلية العلوم ، جامعة صلاح الدين ،اربيل ، العراق

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الملخص

تم وصف طريقة جديدة متطورة لتقدير مركبات الآمين بتقنية تحليل الحقن الجرياني– البريق الكيميائي باستخدام تقنية اندماج المناطق على أساس تفاعلها مع محلول البروم الناتج من تفاعل البروميد مع البرومات في الوسط الحامضي. سرعة التقنية بلغت ٩٥ أنموذج / ساعة . من المركبات الأمينية التي استخدمت في هذه الطريقة مركبات الانيلين ، أورثوأنيسدين ، ٢– ميثيل أنيلين ، ١– نفثيل أمين و أكريل أميد . تم الحصول على مديات مستقيمة لمنحنيات المعايرة بين $1x10^{-7}$ – $1x10^{-2}$ مع معاملات التغير 0.989 , 0.985 , 0.992 ،0.997 و 0.980 على التوالي.