Journal of University of Zakho, Vol.1, (A) No.2, Pp 798-813, 2013

DETEMINATION OF UREA USING FIA-CL SYSTEM

Wshyar **A**. **I**smail[1] And **A**zad **T**. **F**aizullah[2]
[1] Dept. Chemistry, College of Education, University of Salahaddin, Erbil, Iraq
[2] Dept. Chemistry, College of Science, University of Salahaddin, Erbil, Iraq
(Accepted for publication: June 9, 2013)

ABSTRACT

In this work a sensitive chemiluminescence (CL) method for the determination of urea was investigated using a reaction between hypobromite and urea in basic medium. The hypobromite (OBr-) was generated by on-line reaction of Br_2 with KOH, where Br_2 was previously generated by another on-line reaction of $KBrO_3$ with KBr in acidic medium. The proposed method was applied successfully for the determination of urea in human blood serum after elimination of interferences by several methods. The important one was the introduction of a dialysis unit into the FIA-CL system to eliminate proteins interfering effects by placing it in the sample stream with some modification in the arrangement of FIA-CL system. The linear range of the method in the absence of dialysis unit, where the interference effects were solved by dilution, was $5.0 \times 10^{-7} – 1.0 \times 10^{-2}$ mol/l urea with a correlation coefficient of 0.9958, a detection limit 8.0×10^{-8} mol/l urea and sample throughput of 180 sample/h. In the presence of dialysis unit, however, these were $1.25 \times 10^{-5} – 0.26$ mol/l urea, 0.9987, 2.5×10^{-6} mol/l urea and 35 sample/h.

***KEYWORDS:** Urea, Flow injection, Chemiluminescence, Serum.*

INTRODUCTION:

Urea was first discovered in human urine by H.M. Rouelle in 1773. It was synthesized in 1828 by Friedrich Wohler and was the first organic compound to be synthesized from inorganic starting materials (Partington, 1962). Urea is produced commercially by the dehydration of ammonium carbamate (NH_2COONH_4) at elevated temperature and pressure. Ammonium carbamate is obtained by direct reaction of ammonia with carbon dioxide. These reactions are normally carried out simultaneously in a high pressure reactor (Partington, 1964).

Urea is used as a nitrogen release fertilizer as it hydrolyses back to $2NH_2$ and CO_2 but its most common impurity (biuret, NH_2-CO-NH-CO-NH_2) must be present at less than 2% as it impairs plant growth. It is also used in many multi-component solid fertilizer formulations. Its action of nitrogen release is due to the conditions favoring the reagent side of the equilibrium's, which produce urea (Overdahl et al., 1991).

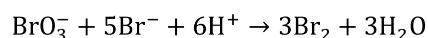
Urea or carbamide, $CO(NH_2)_2$ is the major end product of protein nitrogen metabolism in human and mammals. It is synthesized in the liver from ammonia and carbondioxide, which is produced by amino acid determination. Following synthesis, urea is transported to the kidney where the majority is excreted in the urine. Depending on renal function however, as much as 40% of the urea may be re-absorbed making urea levels a diagnostic illness, which range from congenital heart failure to dehydration to shock. Other factors, which might alter the urea concentration in blood and plasma, are protein intake, sever liver disease, and increased protein catabolism (Henery, 1974). The measurement of urea is important in clinical analyses. Urea determination in blood, urine and subcutaneous tissue samples is useful for the diagnosis of renal dysfunction. The concentration of plasma urea may range from 150 to 420 μg/ml in normal persons and from 1500 to 2500 μg/ml in persons suffering from dysfunctions. High value of urea in blood is related to a rise in the level of toxins and a diminution of the capacity of the kidneys to eliminate waste metabolites. The monitoring of this parameter helps diagnose renal dysfunction's to include the patients in a dialysis program. This clinical interest has spurred the development of numerous analytical techniques for urea (Kanagasabapathy & Sudarshan, 2000). However, the urea quantification uses conventional methods, such as some techniques use the formation of ammonia by the action of enzyme urease found in Soya and Jack beans. The ammonia formed has been determined by aeration into acid (Varley et al., 1980) and calorimetrically by nesslerisation or by the Berthelot reaction with phenol and hypochlorite. For many years the ureas - nesslerisation method was by far the most commonly used although trouble was experienced from turbidity, color instability and non – linear calibration. The application in 1960 of the reaction of ammonia with phenol and hypochlorite first noted by Berthelotas long ago as 1859 gives a more

sensitive technique with a more stable color (Frei et al., 1995). The Berthelot method was developed and modified by many workers (Varley et al., 1980, Patton .C. J. & Crouch S. R., 1977) based on hydrolyzed urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with salicylate and hypochlorite in presence of sodium nitroprusside to form a green compound (2,2–dicarboxylindophenol) whose absorbance can be measured at 600 nm. The intensity of formed color is proportional to urea nitrogen concentration in sample. The Berthelot reaction also has been adapted for use with the autoanalyzer by Wilcox et al. (1966), Wilson (1966) and Raimundo&Pasquni (1991). It is used with automated analysis of the method makes it very suitable for pediatric work. A colorimetric method the product formed when urea itself reacts with diacetyl was devised by Fearon (1939) but because of the non – linearity of the colour and the need to heat with strong acid at near 100 ^{o}C it did not a chive widespread use until adopted for the autoanalyzer (Haslam, 1966).

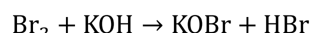
Other colorimetric analyses were used for urea. The colorimetric reagents included diacetylmonoxime in the presence of thiosemicarbazide (Rahmatullah & Boyed, 1980, Anton et al., 1990) phthaldehyde, naphthylethylen-diamine (Lequang et al., 1987, Narinesingh et al., 1992), chromotropic acid (Gindler & Daskalakis, 1982) and 4-hydroxycoumarin (Radionov et al., 1997).

A plant tissue – based CL biosensor for urea combined with FIA was employed by Wei Qin et al. (2000). The analytical reagents involved in the CL reaction, including luminol and permanganate, were both immobilized on anion exchange resin columns, while the biological material soybean tissue was packed in a mini-glass column. By the ureas–catalyzed reaction in the plant tissue column, urea was hydrolyzed to NH_4^+ and HCO_3^-. The anion produced could release luminol from the anion–exchange column with immobilized luminol, which then reacted with permanganate eluted from the anion–exchange permanganate column with sodium hydroxide, thus producing a CL signal. The CL emission intensity was linear with urea concentration in the range 4.0×10^{-6} – 4.0×10^{-4} mol/l; the detection limit was 2.0×10^{-6} mol/l. The biosensor was applied successfully to the analysis of urea in urine samples. In the last few years has been found a reaction between urea and hypobromite in alkaline solution produce CL with a maximum wavelength at 510 nm (Hu et al., 1994). A CL detection method was used for the determination of urea in human urine and natural aqueous samples, which combined this CL reaction with a FIA system. The CL intensity was linear with urea concentration in the range 5.0×10^{-7} – 5.0×10^{-5} mol/l; the detection limit was 9.0×10^{-8} mol/l. In that method sodium hypobromite solution was prepared manually by mixing saturated bromine water with sodium hydroxide solution. The method suffers from the limitation in the way that Br_2 is unstable and toxic, so inhalation its vapor causes to barrenness (sterility). The OBr^- on the other hand is highly active substance and loses its activity with time affected by light in a room temperature. Thus that method is lacks a reproducibility, and health safety, and depend on classical manual preparation.

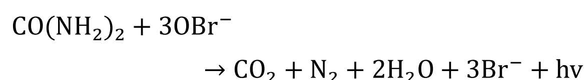
In this work the former method was developed by use of a new three-way inlet flow cell and modified to improve its reproducibility and health safety by an online preparing Br_2 from reaction between bromate, bromide in acidic medium. By an online flow, OBr^- will be formed from a reaction between generated Br_2 and potassium hydroxide in the entrance of flow cell, and reacts with urea immediately to produce CL emission as illustrated in the following equations:



$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O$$



$$Br_2 + KOH \rightarrow KOBr + HBr$$



$$CO(NH_2)_2 + 3OBr^- \rightarrow CO_2 + N_2 + 2H_2O + 3Br^- + hv$$

The CL emission is proportional to the concentration of urea with a wide linear range.

EXPERIMENTAL

1. Apparatus:

The flow system employed in this work Fig. (1) consisted of two peristaltic pumps. One (DESAGAPLG multipurpose peristaltic pump with 12-channels and variable speed) delivered a solution of $KBrO_3$ (0.35 mol/l) at a flow rate of 1.7 ml/min, solutions of KBr (0.85 mol/l) and HCl (0.15 mol/l) at a flow rate of 1.4 ml.min^{-1}. The other (HAAKEBU with 4-channels and variable speed) delivered a carrier stream solution at height flow rate of 5.8 ml/min^{-1}. The silicon rubber pump tubes with (1.5, 2.0 and 2.4 mm i.d.) were used to transport the solutions.

Polytetra-flouroethylene (PTFE) tubing (0.75 mm i.d.) was used to connect all components in the flow system. The stream of $KBrO_3$ is merged with the stream of KBr at a Y–shaped Perspex piece and mixed in a mixing coil of length 55 cm, 2 mm i.d. and the resulting stream is then merged with the HCl stream at a Y-shaped Perspex piece. The reaction proceeds in a reacting coil of 220 cm length, 2 mm i.d. to generate bromine water, which is merged and the latter reacts with the sample stream, and the stream of KOH at the inlet point of the CL flow cell. The tubing of the sample, KOH, bromine water and waste streams were covered with black insulating tape to prevent a fiber optic effect introducing stray light into the detector. The urea solution was injected by a six-way injection valve (Rheodyne U.S.A) in to the water carrier stream. The chemiluminescence reaction cell is a scroll Pyrex glass tube (1.8 mm, i.d., 4 mm o.d.) made by winding the total length of the glass tube of 28 cm, positioned at the detecting window of the light – tight box of a detector made by modifying PyeUnicam, SP8–300 uv/vis spectrophotometer. For maximum light collection by the photomultiplier tube, the coil was backed with mirror. The photomultiplier was operated at 1200 volt, provided by a stable power supply of the modified spectrophotometer. The emission was converted into current by the PMT, and the intensity of the current was recorded with x-t potentiometer recorder (Type PM 8251 Philips).

2. Reagents:

All solutions were prepared from analytical – reagent grade materials unless otherwise stated. Double distilled water was used throughout this work unless otherwise stated. During a second distillation processes 0.1 mol/l $KMnO_4$ solution is added to eliminate effects from organic compounds that may affect the CL reaction.

- A stock solution of urea, (0.1 mol/l) was prepared by dissolving 6.0062 g of urea (BDH) in sufficient water and diluting to 1.0 liter with water. The stock standard solution was stored in a refrigerator at 2 – 8 °C, a way from light. Working standards were prepared from the stock solution by appropriate dilutions with water.

- Potassium bromide stock standard solution (1.0 mol/l), was prepared by dissolving 119.0020 g of KBr (Riedel – DeHaen) in sufficient water, transferred to the solution to a volumetric flask and diluted to 1.0 liter with water. The stock standard solution was diluted as required.

- Potassium bromate stock standard solution, (0.35 mol/l) was prepared by dissolving 58.4500 g of $KBrO_3$ (Fluka) in sufficient water, transferring the solution into a calibrated flask and diluting to 1.0 liter with water. The stock standard solution was diluted as required.

- Potassium permanganate solution (0.20 mol/l) was prepared by dissolving 31.6080 g of potassium permanganate (Hopkin and Williams) in a little of water. Potassium permanganate solution was boiled for 15 minutes then filtered through glass wool and the volume was completed to 1.0 liter in a calibrated volumetric flask with water. This solution was standardized against 0.10 mol/l standard sodium oxalate solution (Vogle, 1979). This solution was kept in the dark. Other solutions were prepared by serial dilutions of the stock solution.

- Potassium hydroxide solution (1.0 mol/l) was prepared by dissolving 56.1100 g of KOH (Merk) in a little of water free from CO_2 (boiled and cooled double distilled water) the volume was completed to 1.0 liter with water, in a volumetric flask.

- Hydrochloric acid solution (1.0 mol/l) was prepared by dilution of 85.9 ml of 36% (m/m) HCl (sp. g.r. 1.1789) (Fluka) with water, in a 1.0 liter volumetric flask.

- Sulfuric acid solution (1.0 mol/l) was prepared by dilution of 111.0 ml of 96% (m/m) H_2SO_4 (sp. gr. 1.84 g/l) (BDH) with water, in a 2.0 liter volumetric flask.

- Phosphoric acid solution (1.0 mol/l) was prepared by dilution of 136.5 ml of 85% (m/m) H_3PO_4 (sp. g. 1.69 g/ml) (BDH) with water, in a 2.0 liter volumetric flask.

- Nitric acid solution (1.0 mol/l) was prepared by dilution of 53.7 ml of 70% m/m, HNO_3 (sp. gr. 1.42 g/ml) (BDH) with water, in a 1.0 liter volumetric flask.

- Hydrogen peroxide (1.0 mol/l) was prepared by dilution 11.63 ml of 35% H_2O_2 (sp. gr. 1.13 g/ml) (Fluka) with water, in a 1.0 liter volumetric flask. The solution was protected from light.

- A stock standard solution of 40.0 g/l bovine serum albumin (BSA) was prepared by dissolving 4.0 g of BSA (Fluka) in sufficient water and diluted to 100.0 ml with water.

3. General Procedure

The FIA-CL in Fig. (1) was operated. A 400 µl portion of urea was injected into the carrier stream of water then merged with KOH and bromine streams (generated from the bromate – bromide – acid) at the center of the CL flow cell

to produce a CL emission signal. The concentration of urea was quantified by CL intensity. A calibration graph was constructed from log. CL intensity (mV) versus log concentration of the urea measured, mol/l.

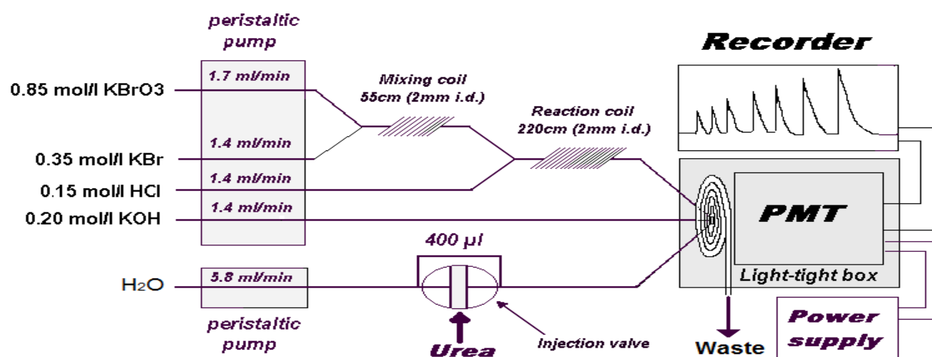


Fig. (1): Schematic diagram of the FIA-CL system used for the determination of urea.

RESULTS AND DISCUSSION

In this work , trials were made using different oxidants, such as sodium hypochlorite, potassium hypobromite, bromine water, potassium dichromate, potassium permanganate, potassium bromate, potassium persulfate, sodium periodate and hydrogen peroxide, in an acidic or basic medium. The maximum intensity was obtained upon using potassium hypobromite in a basic medium. Since it is known that potassium hypobromite is unstable, thus the potassium hypobromite on-line generated from the reaction between bromine water and potassium hydroxide was used instead of potassium hypobromite directly to oxidation of urea in basic medium. Bromine water itself was; in turn, generated on-line in the flow system between BrO_3^- and Br^- in acid medium (Shakir & Faizullah, 1989).

1. Optimization of the Experimental Variables

A series of experiments were conducted to establish optimum values of the analytical variables, The Optimized parameters included reagent concentrations and some physical variables, including the flow rate, sample volume and manifold design. These experiments are started using the following experimental chemical and physical variables, 0.25 mol/l $KBrO_3$, 0.10 mol/l H_2SO_4, 0.30 mol/l KOH, 1.1 ml/min flow rate of each line, 100 µl sample volume, 110 cm length of mixing coil (2 mm i.d.), 330 cm length of reacting coil (2 mm i.d.) and 28 cm length of CL flow cell (1.8 mm i.d.).

A. **Configuration of flow cell:**

To get maximum CL intensity the configuration of flow cell was studied as follow:

The size of flow cell was studied by using two cells, the first was made by winding glass tube of a length of 16 cm with internal diameter of 1.8 mm, and the second was made by winding glass tube of a length of 28 cm with internal diameter of 1.8 mm. The merging and entrance point of reagent lines is in rim of both cells as it shown in Fig. (2). As illustrated in Table (1) sizable flow cell given the highest response when urea was injected at concentration 1.0×10^{-4} and 1.0×10^{-3} mol/l. Thus this cell type was selected in subsequent studies.

Study of position of merging lines of chemical reactants in a flow cell by using two models in this study: In the first the point of merging reagent lines was in the rim of the flow cell, which was previously chosen as a best and in the second the merging and entrance point was in center of the flow cell. In this study both flow cells had same sizable volume. As illustrated in Table (1) the cell with a centered merging point gave highest response when 1.0×10^{-2} mol/l urea was injected. Accordingly this was selected as a best cell for use in subsequent studies.

Table (1): Effect of flow cell configaration on the CL intensity of 1X10-2mol/l urea.

Flow cell			CL intensity (mV)
Size	Length*(cm)	Entrance position	
Small	17	sidding	15
Big	28	sidding	17
Big	28	central	18

* Length of glass tube was used for making the flow cell.

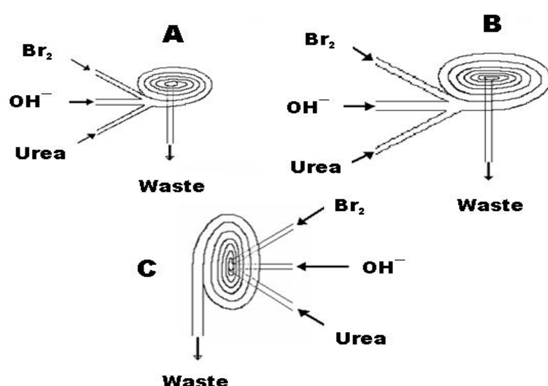


Fig.(2): Configuration of flow cell, where (A) a small flow cell, (B) a big flow cell with a siding entrance, and (C) a big flow cell with a central entrance.

B. Effect of KBr Concentration

Effect of KBr concentrations on the CL intensity (peak height, mV) were investigated in the range of 0.40 - 0.90 mol/l, while keeping all other variables constant. This study was applied for 1.0×10^{-2}mol/l urea. The results shown in Fig. (3) indicate that 0.85 mol/l KBr gives the largest CL intensity. Therefore this concentration was used for subsequent work.

C. Effect of $KBrO_3$ Concentration

The concentration of $KBrO_3$ strongly affects the concentration of the CL reaction species OBr^-, consequently affected the CL intensity. Effects of $KBrO_3$ concentrations on the CL intensity revealed were studied in the range of 0.10 - 0.35 mol/l, for 1.0×10^{-2} mol/l urea. As illustrated in Fig. (3), a CL intensity increase with an increase in bromate concentration. Since it is known that the solubility of $KBrO_3$ not permits to prepare solutions up to 0.35 mol/l. Hence 0.35 mol/l $KBrO_3$ was used in further studies.

D. Effect of different acids

In this work, characteristics of different acids including H_2SO_4, HCl, and HNO_3 were studied. The acids were used as a medium to create the environment necessary for generating bromine water from the reaction between bromate and bromide. Two urea concentrations, 1.0×10^{-4} and 5.0×10^{-4} mol/l were applied. The results are shown in Table (2). Maximum CL intensity was obtained from 0.20 mol/l hydrochloric acid for the two urea concentrations applied. Thus, hydrochloric acid was found the most suitable medium for a sensitive measurement of urea.

E. Effect of hydrochloric acid concentration

Keeping other experimental conditions at their optimized values, effects of HCl concentration on the CL intensity in the range 0.10 - 0.25 mol/l were studied for 1.0×10^{-3} mol/l urea. Fig. (3) illustrates that the CL intensity reaches a maximum for a hydrochloride acid concentration of about 0.15 mol/l for both urea concentrations .Therefore this concentration was employed in subsequent experiments.

Table (2): Effect of different acids on the CL intensity of urea.

Concentration of urea mol/l	CL intensity (mV)		
	0.1 mol/l H_2SO_4	0.2 mol/l HCl	0.2 mol/l HNO_3
$1X10^{-4}$	17.4	19.8	18.9
$5X10^{-4}$	1.71	1.87	1.79

F. Effect of Potassium Hydroxide concentration:

The concentration of KOH in the chemiluminescent flow cell not only affects strongly the concentration of CL reaction species (OBr^-), but also affects the medium necessary to create, the alkaline environment necessary for the CL reaction between urea and hypobromite. Thus, potassium hydroxide was used to react with Br_2 stream to produce on-Line OBr^-, and to create basic medium. The effect of KOH concentration was investigated in the range of 0.10 - 0.40 mol/l, for 1.0×10^{-3} mol/l urea. Fig. (3) illustrates that of KOH concentration of 0.20 mol/l gave best results. Hence, 0.20 mol/l KOH was used for the present work.

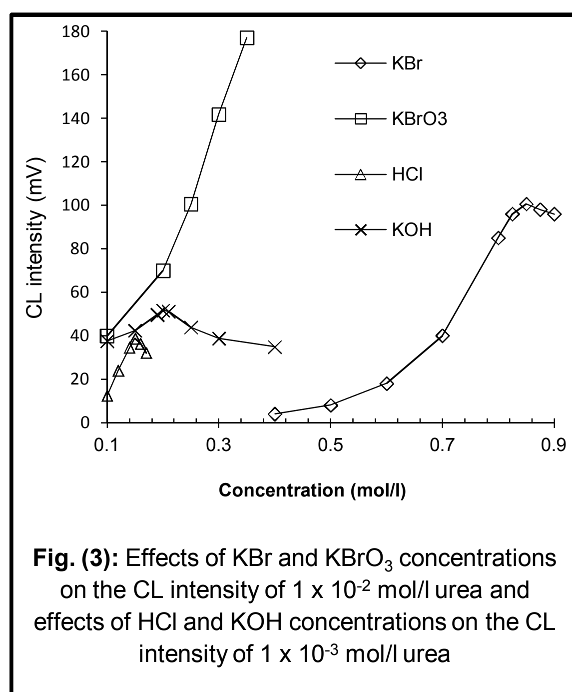


Fig. (3): Effects of KBr and $KBrO_3$ concentrations on the CL intensity of 1 x 10^{-2} mol/l urea and effects of HCl and KOH concentrations on the CL intensity of 1 x 10^{-3} mol/l urea

G. Effect of all lines flow rate

The flow rates of all lines between 0.4 – 2.0 ml/min were studied, keeping all other optimized conditions constant with equal flow rates in each

line. The results obtained show that 1.4 ml/min is the best flow rate for each line (Fig. 4), as the CL intensity depends on rapid mixing of reagents.

H. Effect of bromate stream flow rate

In this study it was used pump tubes with different diameters to producing variable flow rate of bromate stream. The flow rate of the bromate stream was studied while keeping all other conditions constant over the range 1.4 – 2.0 ml/min. The obtained results show that 1.7 ml/min is the best bromate flow rate for determination of urea by FIA-CL system (Fig. 4). This study was used for complete the previous study of effect $KBrO_3$ concentration of the CL intensity (sec.1.C), and this result emphasizes reliability of results for former study.

I. Effect of sample stream flow rate:

In this study it was used another peristaltic pump with remain previous peristaltic pump for other lines at the optimum flow rate. The influence of sample stream flow rate on the CL response was investigated in the range of 3.5 - 10.5 ml/min. It was studied for 1.0×10^{-2} mol/l urea. Fig. (4) shows continue increases in CL intensity as flow rate increases up to 5.8 ml/min. Despite the increase, no significant changes were observed in the peak shape. However, the duration for obtaining one peak was decreased as flow rate increased. In monitoring CL by FIA it is of great importance that a sufficiently high flow rate is used in order that the excited product reaches the detector in a minimum time and hence maximum collection of the emitted light can be achieved (Faizullah, 1985). The results shown in Fig. (4) confirm that the present CL reaction of urea is very fast and the excited product at the entrance of the CL reaction flow cell needs rapid transport to the coil the cell for maximum light output to be monitored. The CL intensity not only depends on reach of excited product to detector in a minimum time but also depend on mixing rapid of reagents. Finally, the flow rate of 5.8 ml/min was selected as optimum for high CL intensity and rapid response.

J. Effect of mixing coil length:

For the efficient mix between bromate and bromide, mixing coil was used in this system and its length was varied from 5.0 to 220.0 cm with constant internal diameter (2 mm). Experiments showed that a suitable length for a high CL intensity was 55.0 cm; shorter or longer mixing coil lengths caused a decrease in the CL intensity (Fig. 5), owing to an incomplete mixing or a considerable relatively reducing flow rate of bromine water by making pressure due to increase length of mixing coil.

K. Effect of reaction coil length:

Different lengths of reaction coil from 5.0 - 330.0 cm with constant internal diameter (2 mm) were tested. The results are shown in Fig. (5). It was found that 220.0 cm coil length gave the best result. Due to the instability of bromine water formed its favored to react immediately with the KOH and urea. Therefore using longer reaction coil leads to decrease in CL intensity.

L. Effect of the sample volume

The variation in CL intensity with the injected sample volume in the range 50 - 300 µl was studied. The obtained results showed that there was an increase in the CL intensity up to 400 µl, above which the CL intensity was almost constant (Fig. 6). Therefore the chosen volume was 400 µl for determination of urea by FIA-CL system.

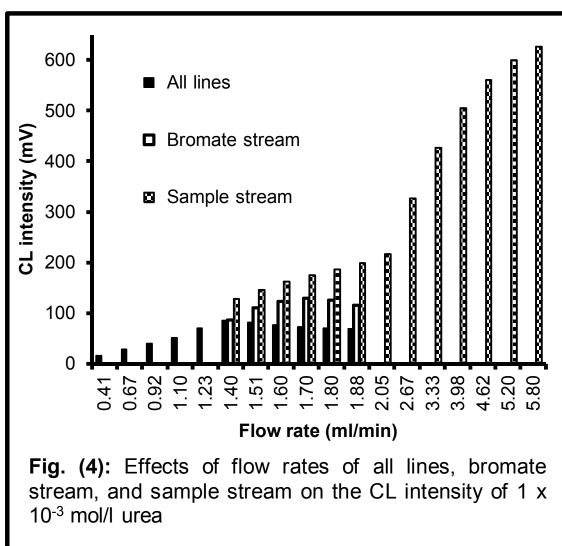


Fig. (4): Effects of flow rates of all lines, bromate stream, and sample stream on the CL intensity of 1 x 10^{-3} mol/l urea

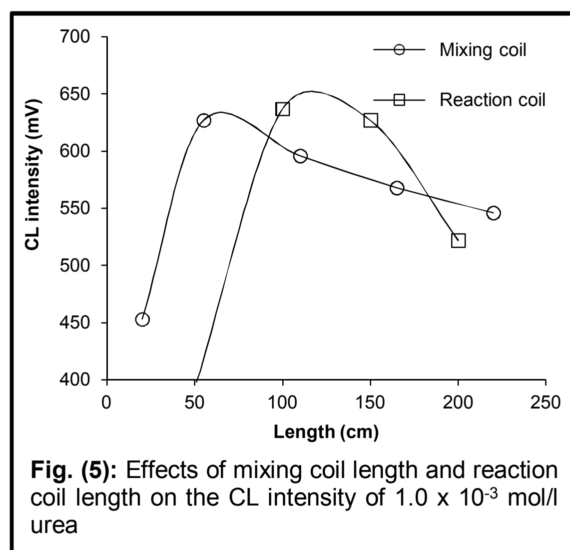


Fig. (5): Effects of mixing coil length and reaction coil length on the CL intensity of 1.0 x 10^{-3} mol/l urea

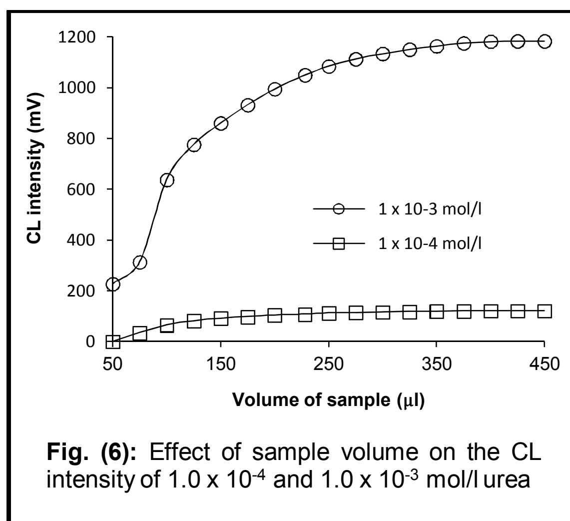


Fig. (6): Effect of sample volume on the CL intensity of 1.0 x 10^{-4} and 1.0 x 10^{-3} mol/l urea

M. Effect of glass beads reactor and depulsing column

Two packed reactor, (10.0 cm long, 2.0 mm i.d.) filled with glass beads (100 - 200 mesh) and furnished with glass wool of both ends to retain the glass beads were positioned in KOH stream line after peristaltic pump and the second one putted in sample stream between peristaltic pump and injection value in order to give stable and repeatable results. In other side two depulsing coil (2.00 m long, 1.0 mm i.d.) were used instead of two glass beads reactor in same positions and same purpose. In these studies 1.0×10^{-4} mol/1 urea was injected 25 times in the presence and absence of the glass beads reactors, and depulsing coil respectively. Table (3) illustrated that the stability and repeatability of the signals in the presence of the coils were better than in either of the other two conditions, so the two depulsing coil were chosen to improve repeatability of the FIA-CL system.

Table (3): Effect of glass bead reactor or depulsing column on response repitablilty of 1.0 X 10^{-4} mol/l urea.

States	$\overline{X}$ [a]	S.D [b]	R.S.D [c]
Absence of G.B.R [d] and R.C [e]	120.8 38	±2.1326	1.7648
Presence of the G.B.R	118.936	±1.9732	1.6590
Presence of the R.C.	115.792	±1.7424	1.5048

[a] Mean, [b] Standard deviation, [c] Relative standard deviation, [d] glass bead reactor, [e] depulsing column.

2. Calibration graph

Once the chemical and physical variables have been optimized to achieve the maximum CL intensity (CL.I), a series of standard solution over the concentration range (5.0 x 10^{-8} – 0.1 mol/l) was injected, each as three replicate, to test the linearity of the calibration graph. A plot of Log CL.I versus Log concentration of urea is shown in Fig. (7). The responses were linear for concentration between 5.0 x 10^{-7} and 1.0 x 10^{-2} mol/1 for urea. The linear regression equation was Log CL.I = 5.9502 + 0.9720 Log [urea]. The detection limits (S/N=3) of urea was 8.0 x 10^{-8} mol/l. The RSD for urea (5.0 x 10^{-4} mol/l) was (1.50) (n = 9). A complete analysis, including sampling and washing, could be performed in 14 sec.

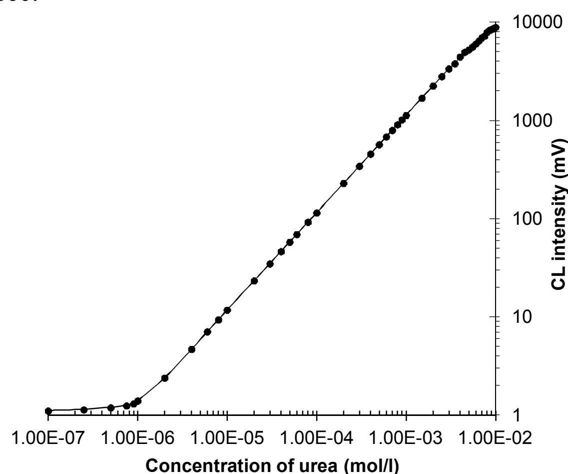


Fig. (7): Calibration curve of urea

3. Interference Study

In order to assess the possible analytical applications of the proposed method, the effect of various organic and inorganic compounds coexisting in human blood serum were investigated as follow:

Under optimum conditions for urea determination the influence of various compounds were studied by analyzing various amounts of each compound instead of urea. As illustrated in Table (4), all compounds had no significant effect on the determination of urea, but only ammonium ion and albumin were produced a weak CL relative to CL of urea.

The effect of various organic and inorganic compounds were investigated by analyzing synthetic sample solutions contain 1.0 X 10^{-5} mol/l (10 µg/ml) of urea and various amount of each compounds. The relative error results are given in Table (4). Weak negative interfering effects were observed from some cations (Cr^{3+}, Co^{2+}, Cd^{2+}, Zn^{2+}) which may be due to the formation of complexes with urea, or it may be attributed to the consumption of hypobromate solution, or due to precipitation of some of these cations by produced insoluble hydroxide compounds with alkaline medium of the CL reaction, which will effect on the CL.I

negatively, and relatively high negative interfering effects were observed from NH_4^+ and H^+ cations. As illustrated in Table (4) all anions had no significant effects and most organic compounds had weak negative interfering effects due to the consumption of OBr^- solution which will reduce the CL.I, but high concentration of creatinin had significant negative effect. Table (4) showed that the high positive effect was observed from high concentration of albumin and weak positive effects were observed from bilirubin, uric acid and high concentration of ethanol.

Table (4): Effect of interferences on the CL.I of 50 µg/ml (8.3×10^{-4} mol/l) urea.

Interference	Conc. of interference µg/ml	CL.I (mV)	E%[a]	Interference	Conc. of interference µg/ml	CL.I (mV)	E%[a]
Br^-	100	944	-0.3	Diethylamin	100	910	-3.9
SO_4^{2-}	100	947	-0.0	L – Histidin	100	920	-2.9
OH^-	100	944	-0.3	Aniline	100	930	-1.8
H^+	100	807	-14.8	Acetamide	100	924	-2.4
Cr^{3+}	100	908	-4.1	Citric acid	100	930	-1.8
PO_4^{3-}	100	947	-0.0	Hydrazin	100	903	-4.6
F^-	100	945	-0.2	Thiourea	100	912	-3.7
NO_2^-	100	947	0.0	Salcylic acid	100	927	-2.1
I^-	100	946	-0.1	Cystiene	100	915	-3.4
NO_3^-	100	947	0.0	Cystien	100	925	-2.3
Fe^{3+}	100	945	-0.2	Albumin	100	972	+2.6
Cu^{2+}	100	943	-0.4	Albumin	2000	1115	+17.6
Cd^{2+}	100	925	-2.3	Cholestrol	100	924	-2.4
Co^{2+}	100	915	-3.4	Triglesrides	100	908	-4.1
Zn^{2+}	100	923	-2.5	Creatinin	20	766	-19.1
NH_4^+	100	876	-7.5	Creatiinin	2	940	-0.7
Triethylentetramine	100	912	-3.7	P– cresol	100	944	-0.3
Formic acid	100	912	-3.7	3.5-dinitrosalicytic acid	100	940	-0.7
Triethanol-amine	100	915	-3.4	Hydroxylamin hydrochloride	100	930	-1.8
Maltose	100	912	-3.7	Uric acid	100	955	+0.8
Glucose	100	901	-4.9	Bilirubin	20	974	+2.9
Ethanol	50%	975	+3.0				

Where a is error%

4. Eliminating of interference effects

A. Eliminating of cations interference effects

Interfering effects of cations were eliminated by introducing an ion–exchange minicolumn filled with (Amberlit 120, 100 - 200 mesh) in sodium form at which all interfering cations exchanged by sodium ions from the resin; the latter has not any interfering effect on CL.I. The effect of three column length 5.0, 7.5 and 10.0 cm with 2 mm i.d. were studied on the ability of eliminating interferences. As illustrated in Table (5) the 10.0 cm column offered best ion exchange ability to eliminate all interfering effects from (NH^{4+}, H^+ and Cr^{3+}) at the concentrations 10.0 and 100.0 µg/ml, but in application this method was not used, for it is capable to use sample dilution process to remove cations in different.

B. Eliminating of bilirubin and creatinine interference effects

Interfering effects of bilirubin and creatinine can be removes by diluting the samples, because the normal range averages of urea, bilirubin and creatinine are equal to 37.8, 18, 13.6 µg/ml respectively. For example when the concentration of urea diluted ten times to 37.8 µg/ml so the concentration of bilirubin and creatinine will be 1.80 and 1.36 µg/ml respectively and their effects nearly disappeared as it shows in Table (4).

C. Eliminating of proteins interfering effect

In case of albumins, many attempts have been tried for eliminating its interfering effect such as:

Table (5): Eliminating of cations interference effects by using an ion-exchange minicolumn.

C L.I (mV) before putting ion-exchange minicolumn				C L.I (mV) after putting ion-exchange minicolumn							
*Urea	*Urea & *NH_4^+	*Urea & *H^+	*Urea & *Cr^{+3}	*Urea	*Urea & *NH_4^+	*Urea & *H^+	*Urea & *Cr^{3+}	** M.C.L (cm)	E% NH_4^+	E% H^+	E% Cr^{3+}
194	185	180	190	173	168	165	171	5.0	-2.90	-4.62	-1.16
194	185	180	190	163	160	158	162	7.5	-1.84	-3.07	-0.61
194	185	180	190	152	151	151	152	10	-0.66	-0.66	0.00

Where * is 10 µg/l, and ** is minicolumn length.

a- **Eliminating of albumins interfering effect using a suppressor minicolumn**

Use of 10.0 cm suppressor minicolumn, (2mm i.d.) packed with (Amberlite 120, 100 - 200 mesh) in Cu^{2+} and Zn^{2+} form respectively and putted in sample stream after injection valve, in order to precipitate albumins with Cu^{2+} and Zn^{2+} in suppressor minicolumn, but attempts were unsuccessful as shown in Table (6). In this study they were used a synthetic sample solutions contain 10 µg/ml of urea, various amount of albumin and 0.10 mol/l KOH. To preparing suppressor columns, the resin was stored in water for 2 days to achieve the required swelling. Cu^{2+} or Zn^{2+}– form resin was prepared by passing 50 ml of 1.0 mol/l $Cu(NO_3)_2$ or $Zn(NO_3)_2$ respectively through the Na^+ form column (10.0 cm long, 2 mm i.d.) for 50 min at 1ml/min. Water was passed until the test (with ammonium hydroxide) shows the absence of Cu^{2+} or Zn^{2+} ions respectively in the effluent.

Table (6): Attempts to eliminate proteins interfering effect by suppresser column

Synthetic sample	Added urea µg/ml	Added albumin µg/ml	In the absence of suppresser column		In the presence of suppresser column on Cu^{2+} form		In the presence of suppresser column on Zn^{2+} form	
			Found urea µg/ml	Recovery%	Found urea µg/ml	Recovery%	Found urea µg/ml	Recovery%
1	50	500	52.6	105.2	52.4	104.8	52.5	105.0
2	50	1000	54.8	109.6	54.5	109.0	54.6	109.2
3	50	1500	57.0	114.0	56.6	113.2	56.8	113.6
4	50	2000	59.2	118.4	58.5	117	58.9	117.8

b- **Eliminating of albumins interfering effect byprecipitation:**

Albumin from a synthetic samples, (consisting of an aqueous solution containing appropriate albumin found in normal serum34000 – 46000 µg/ml, and urea 330 – 420 µg/ml (Frei et al., 1995)) was precipitated,prior to analyzing the sample, by amixture of zinc sulphate (0.174 mol/l) and barium hydroxide (0.170 mol/l) (Somogyi, 1945).The filtrating solution was then neutralized with HCl (0.010 mol/l), and further diluted with distill water for the analysis. Table (7) illustrated that the interfering effect of albumin was eliminated completely by precipitating. This method is not only successful for precipitating albumin, but also for all proteins. But this method required long time, thus it was not used for eliminating proteins interfering effects in application the proposed method on human serum samples.

Table (7): Eliminating of proteins interfering effect by precipitating

Synthetic sample	Added Urea*	Added albumin*	[a]Found Urea*	Recovery%
1	330	34000	334	101.2
2	350	38000	354	101.1
3	380	42000	388	102.1
4	420	46000	428	101.9

[a]average of three determination

Table (8): Eliminating of proteins interfering effect by dilution of sample

Synthetic sample	Added Urea*	Added albumin*	[a]Found Urea*	Recovery%
1	0.100	11	0.100	100
2	0.200	53	0.504	100.8
3	0.750	80	0.758	101.1
4	1.000	106	1.012	101.2

[a]average of three determination

c- **Eliminating of albumins interfering effect by dilution:**

Most of proteins as sort of globulins precipitate in distill water, so globulin kind can be separate in human serum sample by diluted with distill water through precipitate. While albumin remained because it is soluble in distill water, and the interfering effect of albumin can be decrease by dilution. As it known normal level average of urea and albumin in human serum are equal to 378 and 40000 µg/ml respectively and the linear range of the proposed method was between 5.0 x 10^{-7}– 1.0 x 10^{-2} mol/l urea or 600.6 – 0.030 µg/ml urea. Therefore urea can be determined by the proposed method in the range 1.0 – 0.030 µg/ml without serious interfering effect of albumin, because when concentration of urea in human serum is in this range, albumins concentration falls within the range 3.2 – 105.8 µg/l, which had not significant interfering effect as illustrated in Table (4). Under optimum condition the elimination albumin interfering effect was investigated by determined urea in a synthetic sample solutions contain appropriate urea found in the range 1.0 – 0.030 µg/ml, and various amount of albumin found in the range 3.2 – 105.8 µg/l. As observed in Table (8) there are not significant interfering effects of albumin. Hence this method is suitable for elimination of interfering effect of proteins for application the propose method in human serum.

d- **Eliminating interferences using a dialyser unit:**

Adialyser unit is introduced to the system to eliminate proteins interfering effects by placing it in the sample stream with some modification in the configuration of FIA-CL as shown in Fig. (8). Functioning of the continuous dialysis depends on the membrane area, residence time of the sample and mixing, and therefore on pumping rates, temperature, membrane thickness, concentration of solutions, and their viscosity (Ruzicka & Hansen, 1976).Therefore it was decided to optimize some new physical parameters such as; membrane area, thermostated delay coil, recipient stream flow rate and internal pressure of carrier stream and reoptimization of sample volume and carrier stream flow rate.

i. Configuration of the dialyser unit

The Cenco UNMF MKII dialyser module (agents: Techmation Ltd.) with dialysis membrane (Cat no. PJC – 400 – 150H, M. Wt. Cat-off 12000-14000 dalton, Gallenkamp) was used in this study. The dialyser consists of two rectangular blocks of varying length (7.5 and 60.0 cm) depending on the degree of dialysis needed. The U shaped semicircular grooves are again cut in opposing faces and are separated by the dialysis membrane. The grooved surface of the plates are exact mirror images and can be located one over the other by metal spigots and holes as shown in Fig. (9). A sheet of semi permeable membrane is clamped between the plates to produce a long U shaped of circular cross-section with a diameter of 1.5 mm, divided longitudinally by the dialysis membrane. Nipples on the periphery of the blocks allow the sample an recipient streams to enter and leave, each stream traversing one side of the dialyser. It is important to ensure that the two streams flow in the same direction. Operation in the counter – current fashion gives rise to very poor separation of one sample is greatly reduced. Care must also be taken to ensure that the output from the recipient stream is the one which enters the remainder of the analytical system (Varleyet al., 1980). This module achieves the separation of small and large molecules by allowing the former to pass through a semi-permeable membrane from the donor (sample) stream to a recipient stream.

ii. **Pretreatment of the dialyser unit**

Commercial dialysis membranes are not always pure and should be cleaned for exacting work. Common contamination is glycerol added as humectants, sulfides used to plasticize the membranes, and traces of heavy metals. A cleaning procedure recommended by Boyer R. F. (Boyer, 1986). The dialyserunit was cleaned by passing 1.0% acetic acid solution through donor and recipient paths of the dialyser unit for 1.0 hour at 1.0 ml/min, then distilled water passed until the test (with phenol red (Skoog & West, 1976) shows the absence of acetic acid. To remove metal ions, boiling basic EDTA solution (1.0% Na_2CO_3, 1.0 X 10^{-3} mol/l EDTA) was passed through both paths for 1.0 hour at 1.0 ml/min , then hot distilled water passed for 10 min at 1.0 ml/min . The dialyser unit was stored until using time by filling their paths with distilled water at 4 °C containing a few drops of 1.0% NaN_3 as a preservative , then ends of both paths (entrance and exist) closed off with stoppers.

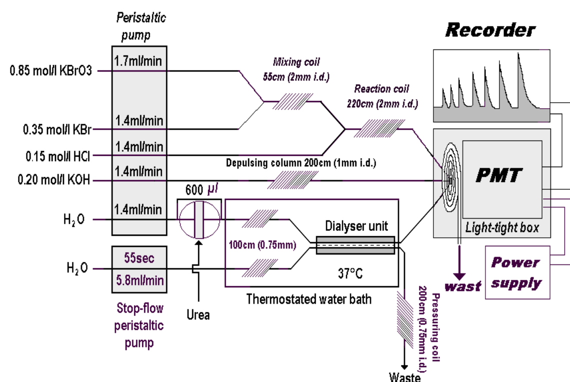


Fig. (8): Schematic diagram of FIA-CL system combined with dialyser unit for determination of urea in human blood serum

iii. Effect of membrane area

Using the FIA-CL system in Fig. (8), with 1.0 ml/min flow rate of both carrier (donor) and recipient streams, while keeping other parameters under optimum conditions. It was used a synthetic sample solution contain normal level average of urea, proteins and sodium chloride i.e. 378, 61500 and 9000 µg/ml respectively. The effect of membrane area was investigated on the efficiency of the dialyses process represented by CL intensity (peak height, mV). Dialyser units with different lengths vary from 7.5 to 60.0 cm were employed. These dialyser units were connected with each other with total length vary from 7.5 – 202.5 cm.

The results are shown in Fig. (10), which indicate that the CL intensity increase (efficiency of the dialyses) with an increase in length of the dialyser (area of dialyser membrane). However, the increasing is not much (more) due to dilution of sample during passes the long path of the dialyser and due to slow dialyses process in general. As a compromise between the longest length required for an effective dialyses and the minimum length for limitation of sample dilution, 180 cm length was selected as the suitable length of dialyser unit in subsequent experiments.

iv. Effect of thermostated delay coil

For the efficient dialyses process, stainless steel coil for carrier (donor) and recipient streams were immersed in the thermostatically controlled water bath at 37 °C on the input side of the dialyser units, ensure temperature equilibrium before dialysis starts, they are length were varid from 50 to 200 cm, while other experimental conditions were maintained at the optimum values. The results are shown in Fig. (10). Initially, increasing the coil length up to 100 cm was accompanied by increasing efficiency, i.e. the CL.I. At longer coil length the CL.I was decreased due to dilution, indicating that the temperature of both stream reached to equilibrium at 37 °C.

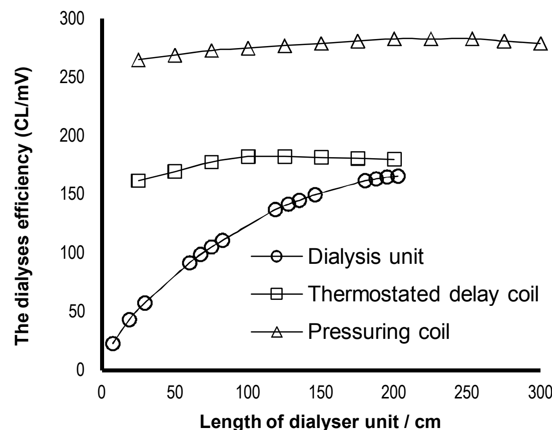


Fig. (10): Effects of dialyser unit length, thermostated delay coil length and pressue drop in the carrier stream (length of pressuring coil) on the dialysis effeciency of synthetic sample containing 378, 61500 and 9000 µg/ml urea, albumin and sodium chloride respectively

v. Effect of sample volume

As the efficiency of dialysis of continuously moving stream is only about few percentage , the dialyser serve not only as a separator of substances to be determined from interfering components, but also as an effective dilutor of the substances, i.e. increase the dispersion. An efficient way of decreasing dispersion is to increase the sample volume (Ruzicka & Hansen, 1980).Under the optimum the effect of sample volume on the CL.I was investigated over the range 400 to 1000 µl. The obtained results showed that there was an increase in the CL.I up to 600 µl, above which the intensity was almost constant. Therefore, the chosen volume was 600 µl in subsequent experiments.

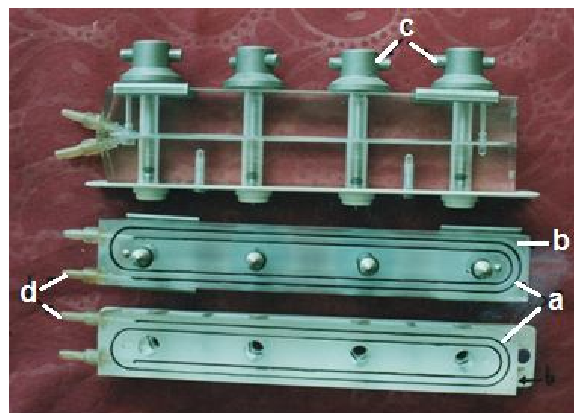


Fig. (9): Photograph of the dialyser unit. (a) U shaped semicircular grooves, (b) Rectangular blocks, (c) metal spigots, (d) Nipples.

vi. Effect of carrier stream flow rate

Fixing the flow rate of recipient stream at 1.0 ml/min, the effect of carrier stream flow rate on the efficiency of the synthetic sample dialyses as well as the CL.I was studied over the range 0.35 – 3.0 ml/min, while other parameters were kept constant. As shown in Fig. (11) that the flow rates of the carrier stream produced only a slight decrease in the CL.I as it increase, but as a compromise between the efficiency of the dialyser process and the time required for a signal measurement (rate of sampling), 1.4 ml/min was selected as the optimum flow rate of carrier stream. Flow rates less than 1.4 ml/min may be used if sampling rate is not considered.

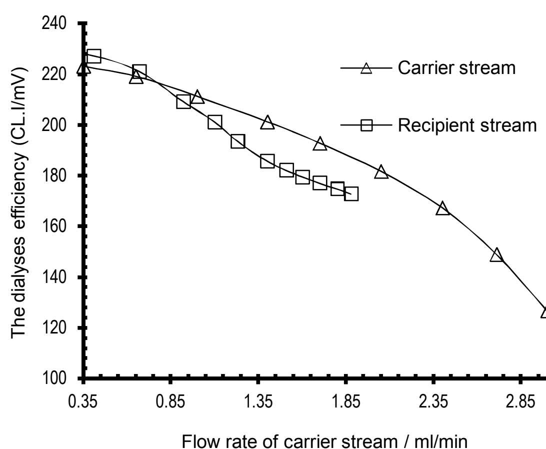


Fig. (11): Effects of carrier and recipient stream flow rate on the dialysis efficiency of synthetic sample containing 378, 61500 and 9000 µg/ml urea, albumin and sodium chloride respectively

vii. Effect of recipient stream flow rate

The effect of flow rate of recipient stream (distilled water) on the efficiency of the synthetic sample dialyses was studied over the range 0.17 – 2.0 ml/min, while keeping all other conditions constant at optimum values. Fig. (11) shows a continuous decrease in peak height, i.e. the efficiency of the dialyses as flow rate increases. As a compromise between best flow rates required for maximum efficiency of the dialyses and best flow rate (5.8 ml/min) required for the very fast CL reaction (sec.1.I), it was decided to stop the recipient stream during passing the synthetic sample through the donor path of the dialyser unit, then pumping it at the optimum flow rate (5.8 ml/min) in subsequent experiments.

viii. Effect of recipient stream stopped time

There are different purposes for operating FIA system in the stopped–flow–mode, provide easier way to optimize the analyte to the reagent ratio to obtain linear response with higher sensitivity, back ground signals can be eliminated as they readily unchanged during the stopped flow interval, reaction time can be increased without increasing dispersion or the length of coil or decreasing the flow rate , while consuming reagent solution only when needed rather than continuously (Ruzicka & Hansen, 1988). In this study, the peristaltic pump of the recipient stream was halted at the moment of sample injected to the carrier stream, for different times over the range (10 – 90 sec).The stream was then pumped at the 5.8 ml/min, (sec.1.I). The effect of different recipient stream stopped time was investigated on the efficiency of the synthetic sample dialyses, while flow rate of the carrier stream keeping at 1.4 ml/min and all other conditions at optimum values. The results indicate that 55 sec is suitable time to stopping recipient stream with best yield of the dialysis as well as the CL.I, due to best concentration gradient between the sides of the membrane and best resistance time of the synthetic sample in the dialyser unit.

ix. Effect of pressure drop in the carrier stream (internal pressure of carrier stream)

The effect of pressure drop in the carrier stream was represented by influence of the carrier stream tube length (pressuring coil) after the dialyser unit (Basson & Van Staden, 1979). Under optimum conditions the influence of the pressuring coil length on the efficiency of the synthetic sample dialyses was studied over the range 20 to 300 cm (0.75 mm i.d.) as shown in Fig. (10). Maximum efficiency is attained with a coil length of 200 cm. Therefore the coil with length 200 cm (0.75 mm i.d.) was putted on the carrier stream path after the dialyser unit to produce suitable pressures, which increase the efficiency of the dialysis.

Once the physical variables have been optimized to achieve the maximum efficiency of the dialyses process, the newer FIA-CL system combined with dialyser unit was used to eliminating the proteins interfering effects. In this study it was used synthetic sample solutions consisting of an aqueous solution containing appropriate urea and albumin (instate total proteins) found in normal serum, i.e. albumin (50000 – 73000 µg/ml) , and urea (330 – 420 µg/ml) respectively . The results in Table (9) indicate that the interfering effect of albumin was eliminated completely by the dialyses process. This method is not only successful for

eliminating proteins interfering effect, but also for all other interferences by it is dilution effect as was pointed out before (Section 4.C.c).

Table (9): Eliminating of albumin interfering effect by dialyser

Synthetic sample	Added urea μg/ml	Added Albumin μg/ml	Found[a] urea μg/ml	Recovery%
1	330	50000	332	100.9
2	350	55750	352	100.6
3	380	61500	382	100.5
4	400	97250	403	100.8
5	420	73000	423	100.7

[a] average of three determination.

5. Calibration graph for determination of urea in the presence of dialyser unit

Under the optimum conditions, and operating FIA-CL-dialysis system in Fig. (8) calibration graphs for determination of urea constructed.The graph is linear for concentrations between (1.25×10^{-5} – 0.26 mol/l) urea. The linear graph has a regression coefficient of 0.9987 (n = 26) and the graph satisfies the least square equation Log CL intensity (av. (n=3) mV) = 4.5777 + 0.9642 [concentration of urea in mol/l]. The detection limit (3 times blank noise) was 2.5×10^{-6} mol/l and the mean RSD was 0.89%. The sampling rate was 35 s/h.

APPLICATION

In order to study the validity of the proposed method, it was applied to the determination of urea in human serum in the presence dialyser unit and in the absence it.

1. In the absence of dialyser unit (in state of eliminating of interfering effect by sample dilution)

A 0.1 ml of serum was diluted to 50.0 ml with distill water. To remove precipitating globulins during the diluting process a filter paper (685^3) was introduced into the entrance of the injection valve of the FIA-CL system, then a 400 µl of the diluted samples were injected in to the carrier stream of the sample. The results are given in Table (10). It is shown that the concentrations obtained by this method are in good agreement with those given by modified urea – Berthelot reaction, with a mean relative error of 2.6%.

2. In the presence of dialyser unit.

A 600 µl of human blood serum was injected directly in to in to the carrier stream of the FIA-CL system combined with dialyser unit without any dilution or any pretreatment. The results are given in Table (10). It is shown that the concentrations obtained by this method are in good agreement with those given by modified urea-Berthelot method (Frei et al., 1995) with a mean relative error of 0.67%.

DISCUSSION

The new FIA-CL system which involves on-line generating of hypobromite ion, a highly active CL reaction reagent, which used for the determination of urea. Compared with other methods for the determination of urea, the proposed method is sensitive, rapid, accurate and simple. The dynamic range of the method in the absence of dialyser unit is very wide $7.0X10^{-7}$–$1.0X10^{-2}$ mol/l with a through put of 180 samples/hour. The dynamic range of the method in the presence of dialyser unit is very wide 1.25×10^{-5} – 0.26 mol/l with a through put of 35 samples/hour. The method is useful for determining urea in human blood serum with on-

Table (10): Results of analysis of urea in human serum

Sample	Concentration of urea μg/ml [a]			E% of proposed method with dilution	E% of proposed method with dialyser
	by modified Berthelot method	proposed method with dilution	proposed method with dialyser		
1	350	358	352	+2.3	+0.6
2	940	968	932	+3.0	-0.9
3	350	358	348	+2.3	-0.6
4	158	160	159	+1.3	+0.6
5	300	305	299	+1.7	-0.3
6	4500	4715	4545	+4.8	+1.0
7	347	354	346	+2.0	-0.6
8	2020	2095	2008	+3.7	-0.6
9	869	894	876	+2.9	+0.8
10	413	423	411	+2.4	-0.5
11	739	759	746	+2.7	+0.9
				2.6[b]	0.67[b]

[a] average of three determination.

line eliminating of interfering effects by dialyser unit or with minimizes interfering effects by dilution of sample.

REFERENCES

Anton S., Gutierrez M. C., Gomez- Henz A. & Perez Bendito D. (1990). Kinetic determination of urea in serum by stopped-flow spectrophotometry. *Anal.Chim.Acta.* 230, 145-150.

Basson W.D. and Van Staden J. F. (1979).Direct determination of Calcium in Milk on a Non-segmented Continuos Flow System. *Analyst,* 104, 419 – 424.

Boyer, R. F. (1986). Modern experimental biochemistry(4th ed.). Addison-Wesley Pub. Co. in Reading, Mass.

Faizullah A. T. (1985). Continuous flow injection analysis.Ph.D. Thesis, University of Hull.pp. 45.

Fearon W. R. (1939). The carbamidodiacetyl reaction: a test for citrulline. *Biochem. J.*, 33, 902-910.

Frei J., Hill P. G., Heach C. C., El-Nageh M. M., Riesen W. & Poller L. (1995). *Production of Basic Diagnostic Laboratory Reagents*. WHO reginal publications, eastern Mediterran series 11, Egypt.

Gindler E. M. & Daskalakis O. (1982). **Colorimetric urea determination in presence of long hydrocarbon chain amidobetaine.United States Patent, US4357144 A**.

Ruzicka J and Hansen E. H. (1976). Flow injection analysis, Part VI. The determination of phosphate and chloride in blood serum by dialysis and sample dilution. *Anal.Chim.Acta.,* 87, 353-363.

Haslam R. M. (1966). Determination of urea by autoanalyser.*Technical Bulletin, 5*(9), 220-226.

Henery J. B. &Davidsohn T. S. (1974). *Clinical Diagnosis and Management by Laboratory Methods*. Sunders and Philadelphia, PA.

Hu X., Takenaka N., Kitano M., Bandow H. & Maeda Y. (1994). Determination of trace amounts of urea by using flow injection with chemiluminescence detection. *Analyst, 119,* 1829-1833.

Kanagasabapathy A. S. & Sudarshan Kumari (2000). *Guidelines on Standard Operating Procedures for Clinical Chemistry*. World Health Organization, Regional Office for South-East Asia, New Delhi.

Lequang N. T. Migneres G., Roche D., Pelladeau M. L. &Labrousse F. (1987). Improved dye procedure for determining urea concentration by using o-phthalaldehyde and naphthylethylene-diamine. *Clin. Chem*. 33, 192-196.

Narinesingh D., Pope A. & Ngo T. T. (1992). Flow-injection analysis of serum urea using o-phthalaldehyde and naphthyl-ethylenediamine. *Talanta, 39*(10):1233-1238.

Overdahl C. J., Rehm G. W. and Meredith H. L. (1991). Fertilizer Urea. (Online), (2004). University of Minnesota/ extension. http://www.extension.umn.edu/distribution/cropsystems/DC0636.html

Partington, J. R. (Eds). (1962). *A History of Chemistry*. Macmillan and Co., Ltd.: London.

Partington, J. R. (Eds). (1964). *A History of Chemistry*. Macmillan and Co., Ltd.: London.

Patuch C. J. & Crouch S. R. (1977). Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem. 49*, 464–469.

Qin W., Zhang Z. &Peng Y. (2000). Plant tissue-based chemiluminescence flow biosensor for urea.*Anal.Chim.Acta.*, *407*, 81-86.

Radionov I., Iazovskaia S. Khatipov E. Piatigorskaia M. &Mikhllov I. (1997). A new method for urea detection in biological fluids. *Klin Lab. Diagn.*, *11*, 17-20.

Rahmatullah M. & Boyde T. R. (1980). Improvements in the determination of urea using diacetylmonoxime; methods with and without deproteinisation. *Clin.Chim.Acta., 107* (1-2) 3-9.

Raimundo Jr. I. M. &Pasquni C. (1997). Automated monosegmented flow analyser. Determination of glucose, creatinine and urea. *Analyst 122*(10), 1039-1044.

Ruzicka J. and Hansen E.H. (1980). Flow Injection Analysis. Principles, Applications and trends. *Anal.Chim.Acta.*, *114*, 19-44.

Ruzicka J. and Hansen E.H. (1988). Flow Injection Analysis (2nd ed.). Wiely Inter science, New York.

Shakir I. M. A. & Faizullah A. T. (1989). Determination of bromide using flow injection and chemiluminescence detection. *Analyst, 114*(8) 951-954.

Skoog D. A. & West D. M. (1976). Fundamental of Analytical Chemistry (3rd ed.). Holt, Reinhort and Winston, New York.

Somogy M. (1945). Determination of Blood Sugar. *J. Biol. Chem.*, *160*, 69-73.

Varley H., Gownlock A. H. & Bell M. (Eds) (1980). *Practical Clinical Biochemistry*. William Heinemann Medical books LTD, London.

Vogel A. (1979). *Text book of Macro and Semimicro Qualtitative Inorganic Analysis*. Longman Inc., New York, USA.

Wilcox A. A., Carrol W. E., Sterling R. E., Davids H. A. & Ware A. G. (1966). Use of the Berthelot reaction in the automated analysis of serum urea nitrogen. *Clin. Chem.*, *12*(3) 151-157.

Wilson B. W. (1966). Automatic estimation of urea using urease and alkaline phenol. *Clin. Chem., 12*(6), 160-168.

پوخته

لەم توێژینەوەیەدا ڕێگایەکی هەستیاری بریسکەي کیمیاوي ئەنجامدراو بە تەکنیکی شیکاریکردن بە دەرزی لێدانی ڕۆشتوو ڕوون دەکاتەوە کە بەکارهاتووە بۆ خەملاندنی یۆریا بەهۆی کارلێکی نێوان یۆریا و هایپۆبرۆمات لە ناوەندی تفتدا، دوای ئەوەی پێشتر هایپۆبرمات بە شێوەیەکی ڕۆیشتوو بە بەرهەم هاتبوو لە ئەنجامی کارلێکی نێوان Br_2 و KOH دا. هەروەها Br_2 لە کارلێکی ڕۆیشتووی پێشووتردا لە نێوان ئایۆنی برۆمات و برۆمید لە ناوەندی ترشدا بە بەرهەم هاتووە. ئەم ڕێگایە ڕاهێنراوە بۆ خەملآندنی یۆریای زەرداوی خوێنی مرۆڤ بە سەرکەوتوویی، دوای لابردنی چوونەناویەکەکان بە چەند ڕێگایەك کە گرنگترینیان بەکارهێنانی یەکەی جیاکەرەوەی پەردەییە لە ناو دەزگای شیکاریکردن بە دەرزی ڕۆیشتوو و بەستراو بە بریسکەی کیمیاوی بۆ لابردنی کاریگەری چوونەناویەکی پرۆتینەکان کە لە ناو زەرداوی خوێنی مرۆڤدا هەن، دوای ئەوەی هەندێك گۆڕانکاری پێویست لە پێکهاتەی دەزگاکە کرا. لەم ڕێگایەدا مەودای ڕاستی هێلی خەملآندنی یۆریا بەبێ بەکارهێنانی یەکەی جیاکەرەوەی پەردەیی (لێرەدا کاریگەری چوونەناویەکەکان لابرا یان زۆر کەم کرایەوە بە ڕوونکردنەوەی پەیتی نموونەکە بە ئاو) لە نێوان ($5 \times 10^{-7} - 1 \times 10^{-2}$) مۆل/لیتر بوو، نرخی هاوکۆلگەی بەستنەوەی ڕاستەهێڵەکە یەکسان بوو بە (0,9958) و سنووری ناسینەوەکەی یەکسان بوو بە (8×10^{-8})مۆل/لیتر یۆریا و توانرا بەم ڕێگایە 180 نمونە لە کاتژمێکدا بخەملێندرێت. بەلام لە کاتی بەکارهێنانی یەکەی جیاکردنەوەی پەردەیی بۆ لابردنی ماددە چوونەناویەکەکان مەودای ڕاستی هێلی خەملاندنی یۆریا بەم ڕێگایە بریتی بوو ($1,25 \times 10^{-5}$) مۆل/لیتر یۆریا بوو و هاوکۆلگەی بەستنەوەی ڕاستەهێڵەکە یەکسان بوو بە (0,9987) و سنووری ناسینەوەکەی یەکسان بوو بە ($2,5 \times 10^{-6}$) مۆل/لیتر یۆریا و توانرا بەم ڕێگایە 35 نمونە لە کاتژمێرێکدا بخەملێندرێت.

الخلاصة

في هذا البحث وصف طريقة حساسة للبريق الكيمياوي لتقدير اليوريا باستخدام التفاعل بين الهايبوبرومات و اليوريا في وسط القاعدي. تم توليد OBr^-من تفاعل مستمر بين Br_2 و KOH في وسط قاعدي، حيث تم توليد Br_2 مسبقا بطريقة مستمرة من تفاعل $KBrO_3$ و KBr في وسط حامضي. طبقت هذه الطريقة في تقدير اليوريا في مصل دم الانسان بنجاح بعد ازالة المتداخلات بعدة طرق ومن اهمها ادخال وحدة اللفصل الغشائي في نظام FIA-CL لازالة التاثير التداخلي للبروتينات الموجودة في المصل بوضعه في تيار النموذج مع بعض التحويرات في التصميم FIA-CL . كان مدى الخطية لهذه الطريقة عند عدم استخدام وحدة الفصل اللغشائي (في هذه الحالة تم ازالة تاثير المتداخلات او تقليله بواسطة تخفيف نموذج باماء المقطر) تساوي ($5 \times 10^{-7} - 1 \times 10^{-2}$) مول/لتر يوريا، و قيمة معامل ارتباطه تساوي (0,9958) و قيمة حد الكشف تساوي (8×10^{-8}) مول/لتر و بمعدل سرعة التحليل بحدود 180 نموذج/ساعة في حين كان مدى الخطية لهذه الطريقة مع استخدام وحدة الفصل الغشائي ($1,25 \times 10^{-5} - 0,26$) مول/لتر يوريا، و قيمة معامل ارتباطه تساوي (0,9987)، و حد الكشف تساوي ($2,5 \times 10^{-6}$) مول/لتر يوريا و بمعدل سرع تحليل تساوي 35 نموذج/ساعة.