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Monitoring Urea Levels During Haemodialysis with a Pulsed-Flow Chemiluminescence Analyser

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Abstract

We have developed a rapid and robust method for the determination of urea in spent haemodialysis fluid, as a measure of the efficiency of haemodialysis treatments. A novel flow analysis instrument (which generates a pulsed solution flow) was coupled with a chemiluminescence detection system, based on the oxidation of urea with the hypobromite anion. The ‘Pulsed-Flow Chemiluminescence Analyser’ exhibited high precision (1.6% RSD for a $10^{-5}$ M urea standard) and detectability ($9 \times 10^{-7}$ M, S/N = 3) as a result of the rapid and reproducible mixing of small volumes of reagent and sample at the point of detection. The proposed chemiluminescence technique and an established urease-based laboratory procedure were compared, and showed a very similar trend for the change in urea concentration during a typical haemodialysis treatment. The relative chemiluminescence response from the oxidation of species with similar structure has revealed the inherent selectivity of the light producing pathway, but a positive interference was obtained from protein when this technique was applied to the determination of urea in serum samples. The $\alpha$-amino acid arginine was identified as the predominant source of this interference.

Keywords: Chemiluminescence; Pulsed Flow; Urea; Haemodialysis
**Introduction**

Urea is used as a marker for the low-molecular-mass toxins removed during the haemodialysis treatments received by patients with chronic renal failure. It has been demonstrated that a high urea clearance correlates with long-term patient survival [1]. Patients with stable end-stage renal failure may require haemodialysis lasting 3 to 6 hours on 2 or 3 days per week [2]. The efficiency of this treatment has traditionally been determined by subjective assessments of the patient’s well-being, and an evaluation of pre- and post-dialysis blood biochemistry, with samples taken on a monthly basis. In 1995, it was recommended a target blood urea reduction of at least 55% for patients dialysing three times per week, and 85% for patients receiving biweekly dialysis treatments [1]. Whilst the modelling of patients’ urea levels based on the determination of this species in plasma or serum samples remains popular, the direct measurement of the urea transferred to the electrolyte solution (dialysate) pumped along the opposing side of the semipermeable membrane is a convenient alternative that can also provide feedback during the course of the dialysis treatment [1].

The dominant methodology for the determination of urea in clinical laboratories involves the selective urease-catalysed hydrolysis of urea, followed by the determination of ammonia with colorimetry or electrochemistry, or after further enzyme catalysed reactions [3]. Other techniques, such as the colorimetric reaction between urea and diacetyl monoxime, remain in use, particularly for matrices that contain urease inhibitors [4]. In relation to monitoring haemodialysis, Koncki and co-workers recently discussed a variety of methods proposed for the determination of urea in spent dialysate [5]. Each of the discussed methods incorporated immobilised urease, and most used conductometric or potentiometric detection of the ammonia
produced by the hydrolysis of urea. Koncki et al. noted that ammonium sensors and ammonium-based biosensors exhibit cross-sensitivity to alkali ions (particularly potassium) removed from the blood during dialysis, which they addressed with the use of a reference cell detector [5]. However, it has been reported that there is a poor uptake of proposed spent-dialysate urea sensors for clinical diagnosis [6]. Della Ciana and Caputo ascribed this lack of acceptance to problems with sample handling, instrument calibration, the cost of manufacture and quality control. In addition, there have been issues of instrumental reliability related to the “bedside” environment found in the practice of haemodialysis [6].

Pulsed flow chemistry, first reported by Wang and co-workers [7] in 1998, has been shown to be a viable alternative for automated analyses, combining the high sensitivity and precision of existing flow-based techniques with robust, small-scale instrumentation [7,8]. Solutions are propelled by the generation of short pressure pulses to produce precisely-timed bursts of liquid flow, interspersed with relatively longer periods when the solution remains static. Analysis modes are interchangeable using software settings, without physical alteration of the instrument manifold [7,8]. In a previous study we demonstrated the suitability of this instrumentation for chemiluminescence detection and applied this approach to the determination of molecules of clinical and forensic importance [8]. The appeal of chemiluminescence as a method of detection stems from its high sensitivity, wide detection ranges and, in some cases, excellent selectivity observed for the desired analytes [9].

In our recent review of methodology for the determination of urea, particular attention was given to approaches that employed chemiluminescence detection [4]. With one exception [10], the chemiluminescence-based techniques have incorporated the use of
urease and conversion of the hydrolysis products by further enzymatic reactions [11-14] or ion-exchange [15], to form, release or remove, the limiting species in a chemiluminescent reaction. The feasibility of each technique was demonstrated with preliminary testing of plasma [12,14], serum [11-13], urine [12,15] and dialysate [14] samples.

Whilst the above procedures exhibited adequate sensitivity, analytical methodology incorporating a direct chemiluminescence reaction would greatly reduce instrument complexity and reagent cost, which are particularly important factors for point-of-care urea monitoring. The only direct reaction with urea reported in the literature to exhibit significant chemiluminescence is the oxidation by hypohalites, first described by Stauff and co-workers in 1962 [16,17]. Hu et al. independently examined this chemistry and, in 1994, published a flow-injection technique for the determination of urea in urine based on the reaction between urea and hypobromite [10]. The only significant interferent present in the matrix was ammonia, although this species produced an emission at 200-fold lower intensity than that of urea, and was removed with on-line cation exchange [10]. Nevertheless, the determination of urea in urine is no longer considered important in clinical diagnosis and monitoring, other than for a crude estimation of overall nitrogen balance [18,19].

In this paper we present simple, robust and selective methodology for the determination of urea, using novel pulsed-flow instrumentation coupled with chemiluminescence detection based on the reaction with alkaline hypobromite. The application of this methodology to the rapid determination of urea during the course of a haemodialysis treatment is demonstrated, and the potential for on-line monitoring is discussed.
Experimental

Flow Injection Instrumentation

A conventional flow injection analysis manifold was assembled (Figure 1(i)) using a syringe pump (Model sp210iw, World Precision Instruments, Glen Waverly, Victoria, Australia) or peristaltic pump (Gilson Minipuls 3; John Morris Scientific, Balwyn, Victoria, Australia) with bridged PVC tubing (Protech Group, Coolum Beach, Queensland, Australia) to propel solutions through 0.8 mm i.d. PTFE tubing (Protech). Solution lines were combined with a Y-piece, samples were injected into the carrier stream via an automated 6-port valve (Valco Instruments, Schenkon, Switzerland), and detection was achieved using a custom built flow-through luminometer. The luminometer consisted of a coiled flow cell (PTFE tubing, Protech) mounted flush against the window of a photomultiplier tube (PMT; Thorn-EMI Model 9924SB, Ruislip, Middlesex, UK) which was operated at 900 V provided by a stable power supply (Model PM28BN, Thorn-EMI) via a voltage divider (C611, Thorn-EMI). The flow cell, PMT and voltage divider were encased in a padded light-tight housing, and the output from the photomultiplier was documented with a chart recorder (YEW Type 3066, Yokogawa Hokushin Electric, Tokyo, Japan).

Pulsed-Flow Instrumentation

The two-line Pulsed-Flow Chemiluminescence Analyser (Figure 1(ii)) consisted of a prototype pulsed flow chemistry module (Model C, Precision Devices, Shoreham, Victoria, Australia) and PMT (Thorn-EMI, Model 9924SB) with custom built housing. Improvement in the design of this instrument compared to that used in our previous study [8] included a detection cell constructed by machining a coiled flow pathway from a transparent acrylic block and fixing a thin film of the same material to enclose the coil. This modification reduced the pulse dampening by the flexible
tubing used to join instrument components to the previously used glass flow cell. To eliminate light piping through the solution lines, the plastic casing was altered to include sealable compartments for the sample, reagent and waste containers. A number of pulse sequences were examined. The most suitable sequence contained pulses of the sample solution to flush the sample line and detection cell, a single pulse consisting entirely of reagent solution, followed by a final pulse of sample solution. The output from the PMT was documented with a chart recorder (YEW Type 3066).

**Chemiluminescence Emission Spectra**

A Cary Eclipse spectrofluorimeter (Varian Australia, Mulgrave, Victoria, Australia), fitted with a R928 photomultiplier tube (Hamamatsu, Iwata-gun, Shizuoka-ken, Japan) was adapted for the measurement of chemiluminescence spectra with a two-line continuous flow manifold incorporating an integrated glass Y-piece and spiral flow cell (0.5 mm i.d., 90 μL volume, Embell Scientific, Murwillumbah, NSW, Australia) placed in front of the emission window of the spectrofluorimeter. A peristaltic pump with silicone pump tubing was used to continuously pump reagent and analyte solution through 0.5 mm i.d. PTFE tubing to the flow cell where the chemiluminescence reaction occurred (Figure 1(iii)). The excitation source of the spectrofluorimeter was turned off (Bio/Chemiluminescence data mode) and the final data recorded was an average of ten scans (1000 ms gate time, 1 nm data interval) with a slit width of 20 nm. Emission spectra were corrected for the wavelength dependence of the detector response and monochromator transmission by multiplication with a correction factor that was established using a 45 W quartz-halogen tungsten coiled lamp of standard spectral irradiance (OL245M, Optronics Laboratories, Orlando, FL, USA). The lamp was operated at 6.5 A d.c. supplied by a programmable constant current source (OL65A, Optronics Laboratories).
Reagents and Clinical Samples

Solutions of hypobromite were prepared by disproportionation of bromine (Hopkin & Williams, Chadwell Heath, Essex, UK) in cold aqueous sodium hydroxide (AR grade; Ajax (APS), Auburn, NSW, Australia) in a capped Schott bottle with stirring. After preparation, solutions were stored in Schott bottles covered with aluminium foil to prevent photodegradation of the hypobromite reagent [20]. For an examination of the relationship between the chemical structure of the reactant and chemiluminescence intensity, ammonium chloride (AR; BDH, Poole, Dorset, UK), urea (AR; Ajax), ammonium carbamate (LR; Pfaltz & Bauer, Waterbury, CT, USA), ammonium hydrogen carbonate (98%; Riedel-de Haën, Seelze, Germany), uric acid (98%; BDH), hydrazinium sulfate (LR; Ajax), methylurea (97%; Sigma-Aldrich, Castle Hill, NSW, Australia), guanidine hydrochloride (AR; Sigma-Aldrich), thiourea (LR; BDH), sulphamid (99%; Sigma-Aldrich), creatine (LR; Ajax), methylammonium chloride (LR; Ajax), hydroxylamine hydrochloride (LR; Ajax), and acetamide (LR; Ajax) solutions were each prepared by dissolution of the solid in deionised water followed by the appropriate dilutions, with the exception of ammonium carbamate which was dissolved in a sodium hydroxide (0.1 M) solution to improve stability. Formamide (99.5%; BDH) was purchased as a liquid and diluted with water as required. Solutions of the L-amino acids; alanine, aspartic acid, glycine, histidine, isoleucine, lysine, phenylalanine, serine, threonine, valine (each purchased from Sigma), asparagine monohydrate, glutamine, leucine, methionine (Calbiochem, La Jolla, CA, USA), glutamic acid (sodium salt), proline (BDH), tryptophan, tyrosine (Aldrich), arginine (Ajax) and cysteine (Hopkin & Williams) were prepared by dissolution of the solid in deionised water.
Spent dialysate was periodically collected from the waste line of a Fresenius Polysulfone Capillary Dialyser (Hemoflow F-series High Performance Steam, Fresenius Medical Care, Bad Homburg, Germany) during a single haemodialysis treatment performed in the Dialysis Unit of the Geelong Hospital.

Serum samples were prepared by centrifugation of whole blood (obtained from the Geelong Hospital) in a Vacutainer tube (L10259-00, Becton Dickinson, Franklin Lakes, NJ, USA) containing a clot activator. The removal of protein from the serum samples was achieved by filtering 5 mL of the sample solution through the polyethersulfone membrane contained in a Vivaspin Concentrator (Product numbers VS2001 and VS2021, with a relative-molecular-mass cut-off of 10000 and 30000 g mol$^{-1}$; VivaScience, Westford, MA, USA) by centrifugation until a constant volume was reached.
Results and Discussion

Preliminary Studies with Conventional Flow Injection Instrumentation

Using a conventional two-line flow injection manifold (Figure 1(i)) with the reagent conditions established by Hu and co-workers, we observed a detection limit (S/N = 3) of $2 \times 10^{-7}$ M urea and an optimum flow rate of 5 mL min$^{-1}$. The previous group reported a detection limit (3σ) of $9 \times 10^{-8}$ M at an optimum flow rate of 10 mL min$^{-1}$. The differences are most likely due to the variation in instrumental design, particularly as the previous researchers used a purpose-built double concentric flow cell [10]. As the concentration of urea in spent dialysate or blood is over four orders of magnitude greater than our detection limit, the difference in reported detectability for the two studies is insignificant in the context of these sample matrices.

The decomposition of hypobromite, to form bromide, bromite and bromate ions and molecular oxygen, occurs at a rate which is greatly influenced by the hypobromite concentration, the pH and temperature of the solution, and the intensity of light to which the solution is exposed [20,21]. Concerns have been raised about the use of alkaline hypobromite solutions as analytical reagents [20,22]. We examined the stability of a hypobromite solution (0.08 M) that was prepared by adding bromine (200 μL) to a sodium hydroxide solution (50 mL, 0.4 M). Syringe pumps were used to propel the solutions through the two-line FIA manifold (Figure 1(i), line 1: hypobromite solution (0.08 M), line 2: H$_2$O carrier, 5 mL min$^{-1}$ each line) to eliminate the flow-rate variation associated with peristaltic pump tubing elasticity, which leads to variations in signal intensity, particularly over long periods of time [23]. Five replicate injections of a urea standard ($1 \times 10^{-5}$ M) were performed hourly for eight hours, and at three times the following day. No significant decrease in the chemiluminescence intensity (peak height) was observed, although some minor
variation in the signal and dark current response resulted in a RSD of around 3% for all injections. It was concluded that the hypobromite solution was stable for at least one day, and was prepared daily for all the following experiments.

**Pulsed-Flow Instrumentation**

The determination of urea with the Pulsed-Flow Chemiluminescence Analyser (Figure 1(ii)) involved the placement of reagent (0.08 M hypobromite) and sample solution vials into compartments incorporated into the device and initiation of a programmed pulse sequence to transport the solutions to a mixing point and present the reacting mixture to a detection cell. Only one pulse (50 μL) of reagent solution was required for each analysis cycle (after initial priming of the reagent line) and, consequently, the reagent vial did not require refilling during the testing period. After completion of the pulse sequence, the reacting mixture remained in the detection cell until the maximum emission was reached (approximately 1.5 s), which was documented with a chart recorder. Using the Pulsed-Flow Chemiluminescence Analyser with this procedure, the limit of detection (S/N = 3) was $9 \times 10^{-7}$ M urea, and a relative standard deviation of 1.6% was calculated using 10 replicate analyses of a single urea standard solution ($1 \times 10^{-5}$ M).

**Analytical Selectivity**

In a study of the chemiluminescent oxidation of urea with alkaline hypobromite, Hu and co-workers examined the emission intensity for a variety of other species [10]. No chemiluminescence was detected when mixing hypobromite with alkylamines (1 mM), and a very weak emission was measured for a variety of carboxylic acids and amino acids (ca. 10 mM). Significant chemiluminescence was observed from the oxidation of humic acid, pyrogallol and ammonia. However, to achieve comparable
emission intensity with urea, the concentration of ammonia was over two orders of magnitude greater.

At this stage the relationship between chemiluminescence intensity and the structure of the reactants remains unresolved. The emission intensity for the oxidation of urea with hypobromite was compared to that of species with similar structure (Table 1). Unlike procedures for the determination of urea based on the reaction with diacetyl monoxime [3], similar species with ureide functionality (as demonstrated by methylurea) do not strongly interfere. The solutions containing ammonia each produced a similar emission, which was over forty times lower in intensity than that observed for a 10 mM solution of urea. The spectral distribution of the chemiluminescence accompanying the oxidation of urea and ammonia with hypobromite was found (by the previous workers) to be significantly different [24], suggesting that the reaction pathways lead to two distinct emitting species. Therefore, the inherent features of each reaction, such as the spectral distribution and reaction kinetics, can be exploited to provide additional selectivity for one of these two species. By inserting a glass filter between the chemiluminescence reaction cell and the photomultiplier tube, Hu and co-workers [25] reduced the interference from both urea and humic acids for the determination of ammonia in rainwater and fogwater using flow injection analysis methodology. However, for the determination of urea in urine, cation exchange was used to remove the interference from ammonia [10]. In our study, the relative difference in chemiluminescence intensity for urea and ammonia was found to be greater at lower analyte concentrations and dependent on both the instrument manifold and the wavelength response of the detector. Compared to the analysis of urine, the determination of urea in spent dialysate is not complicated by a high endogenous ammonia concentration [3] and therefore the cation exchange...
adopted by Hu and co-workers for the analysis of urine [10], was not necessary for the analysis of these samples.

**Determination of Urea as a Marker for Dialysis Efficiency**

The characteristics of the Pulsed-Flow Chemiluminescence Analyser – compact, robust and simple instrumentation – are ideal for on-site analysis. To demonstrate the potential of this methodology for bed-side monitoring of haemodialysis, urea was determined in spent dialysate samples, collected every 10 minutes from the waste line of a Fresenius Polysulfone Capillary Dialyser, over a 100 minute segment of a typical haemodialysis treatment performed at a local hospital. Each spent dialysate sample was analysed with the chemiluminescence method within two hours of collection and then stored in a refrigerator for analysis with an existing urease based technique the following day. High stability is observed for urea in clinical samples, if bacterial contamination is avoided [3].

Chemiluminescence assays were performed with the instrumental conditions discussed in the previous sections and with each sample diluted 100 fold with water. Nine urea standards (2.5 \times 10^{-5} \text{ M} to 2.0 \times 10^{-4} \text{ M}) were used to prepare a calibration, which closely approximated linearity (R^2 = 0.9997, log-log gradient of 1.01). Enzymatic assays were performed at a pathology laboratory (Pathcare Consulting Pathologists, Geelong, Victoria, Australia) with the Dimension Clinical Chemistry System, based on reaction with urease and glutamate dehydrogenase, and monitoring NADH concentration with the dichromic (340, 383 nm) rate technique.

No significant difference in chemiluminescence intensity was observed for urea standards prepared in water or the pre-dialysis electrolyte fluid prior to the 100-fold dilution in water performed on all standards and samples. The two techniques showed
a very similar trend for the change in analyte concentration (Figure 2). However, the results of the enzymatic analysis were consistently higher than those from the Pulsed-Flow Chemiluminescence Analyser. Overestimation when using techniques based on the enzymatic breakdown of urea may result from endogenous ammonia, particularly in patients with impaired liver function [5], but it would be premature to conclude that this was the cause for the observed difference without more extensive investigation. A deliberate interruption in the dialysate flow resulted in a temporarily raised urea concentration in the spent dialysate sample collected at 80 minutes after the initiation of the haemodialysis treatment. It was therefore concluded that the pulsed-flow chemiluminescence analyser has the potential to provide a near-continuous measure of dialysis efficiency during treatment. The technique that we have presented has a number of distinct advantages including: a simple process consisting of a single chemical reaction performed with robust instrumentation; high analytical frequency (120 to 300 samples per hour); low reagent consumption (50 μL per cycle); an inherent low response from endogenous ammonia; and the potential for on-line electrochemical generation of the reagent from a potassium bromide solution [26].

**Determination of Urea in Serum**

The Pulsed-Flow Chemiluminescence Analyser was also applied to the determination of urea in human serum samples. The results from this technique were outside the normal reference levels for urea in serum (2.1 to 7.1 mM) [27], and significantly higher than those produced with a conventional enzymatic procedure. An albumin sample, prepared at a concentration of 60 g L⁻¹ to simulate the total protein concentration in adult serum samples (60 to 80 g L⁻¹, with around 30 to 50 g L⁻¹ as albumin) [27], produced a signal comparable to the difference between the response from the chemiluminescence and enzymatic procedures. Centrifuge tubes containing a
polyethersulfone membrane with a molecular weight cut off (MWCO) of either 10000 or 30000 g mol$^{-1}$ were employed to remove the majority of the proteins contained in the serum sample, prior to analysis by both methods. After removal of the proteins, the results obtained with the proposed chemiluminescence method were in good agreement with those from the conventional enzymatic procedure (Table 2).

The obsolete manometric determination of urea, based on the reaction of urea with hypobromite and measurement of the evolved nitrogen gas, was also subject to interference from protein in serum and plasma samples [28]. It was established that the arginine content of the protein was chiefly responsible for the additional production of nitrogen [29]. Interestingly, an examination of the chemiluminescence response from the reaction of hypobromite with twenty amino acids using the pulsed-flow instrumentation revealed that arginine was also primarily responsible for the positive interference in the chemiluminescence method under investigation. The chemiluminescence responses from the amino acids, tyrosine, histidine, serine, threonine, methionine and glycine were between 0.2% and 0.02% of the arginine response, and all others tested were below 0.02%. Calibration graphs for urea and arginine, prepared using the Pulsed-Flow Chemiluminescence Analyser, revealed a slightly lower detection limit ($6 \times 10^{-7}$ M, S/N = 3) for arginine. Raj and co-workers recently examined the pre- and post-dialysis concentration of various species in the blood of patients with end-stage renal disease [30]. It was reported that arginine levels in the blood fell from around 0.11 to 0.09 mM during the 4 hr dialysis treatments. This chemiluminescence signal from arginine at these levels is insignificant compared to the response recorded for the levels of urea encountered in spent dialysate. Future patient trials will include an examination of cases where there is an increased protein metabolism or increased arginine intake due to dietary supplementation.
**Emission Spectra**

The spectral distribution of the chemiluminescence accompanying the reaction of urea with hypobromite shown in Figure 3(ii) is in agreement with the data of Stauff and Rümmler [17] and Hu and co-workers [24]. Both groups reported a broad emission, from 350 nm to at least 700 nm, with a maximum emission around 510-520 nm. Hu *et al.* [24] noted that the distribution of this broad emission was significantly effected by the relative quantum efficiency of their photomultiplier tube. The maximum emission in the corrected spectrum occurred at a wavelength of around 710 nm. We observed similar shift in the emission spectrum after multiplication with a correction factor (Figures 3(ii) and 3(iii)), but it was difficult to precisely estimate the wavelength at which the maximum intensity occurred due to the broad nature of the emission, the accompanying amplification of noise with the correction, and the dependence of the corrected spectrum on an estimation made to account for the baseline drift over the collection time.

The luminescence accompanying the reaction between L-arginine and hypobromite has a similar spectral distribution to that detected for the oxidation of urea (Figures 3(i) and 3(ii)). Although this is the first report of the emission accompanying this reaction, Stauff and Rümmler [17] had described the emission detected during the reaction of gelatine or guanidine with hypochlorite, which had a similar spectral distribution than that for the reaction of urea with either hypochlorite or hypobromite. In the case of gelatine, we assume that this was due to the presence of L-arginine in the polypeptide chain.
**Reaction Mechanism**

The mechanism proposed by Stauff with Schmid kunz [16] and discussed by Stauff and Rümmler [17] involved excited species formed by the recombination of OH or O$_2$H radicals, which are formed during the halogenation and oxidation of compounds containing –CO-NH$_2$, -C(NH)-NH$_2$ and -CONH- groups, as represented by urea, guanidine and gelatine. In light of the new evidence, including the superior emission from arginine in comparison with all other amino acids tested, and the relative chemiluminescence intensities from the reaction of hypobromite with urea, methylurea, thiourea, creatine, guanidine and arginine, the structural requirements for an intense emission could be refined to RC(NH$_2$)$_2$, where R is an oxo (O=) or a secondary amine (–NH–) group. Hu and associates [24] examined the oxidation of urea with hypobromite and tentatively attributed the emission to molecular nitrogen, a known product of the reaction. Molecular nitrogen has been proposed as the emitter or excited-state intermediate in a number of chemiluminescence reactions [24,31-33], but in each case the suggestion has not been supported by conclusive evidence, and the spectral distributions recorded for some of these chemiluminescence reactions do not coincide [24]. We are currently investigating the light-producing pathway of the chemiluminescent reaction between urea and hypobromite, and a number of related reactions.

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nursing staff and patients of the Dialysis Unit for their assistance in obtaining samples for comparative studies. Funding for this project was provided by the Australian Research Council and an Australian Postgraduate Award.
References


Figure 1.

(i) 
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reagent
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Cary Eclipse
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waste
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(ii) 
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Table 2.

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Figure 2.
Figure 3.
Figure 1. Schematic diagram of the (i) 2-line FIA, (ii) pulsed-flow manifold, and (iii) 2-line continuous reaction manifold for the collection of emission spectra. The instrumentation comprised of (a) solutions propelled with peristaltic or syringe pumps, (b) sample introduction via automated injection valve, (c) solutions combined at a Y-piece immediately prior to (d) a flow-through detector, and (e) the pulsed-flow module supplying both solution propulsion and sample introduction.

Figure 2. The concentration of urea in spent dialysate during a typical dialysis treatment, as determined by the urease (open circles) and direct chemiluminescence (solid circles) techniques.

Figure 3. Uncorrected chemiluminescence emission spectra for the reaction of hypobromite with (i) arginine and (ii) urea, and (iii) the corrected spectra for the oxidation of urea with hypobromite.

Table 1. Relative chemiluminescence intensities on reaction with hypobromite using the Pulsed-Flow Chemiluminescence Analyser.

Table 2. Determination of urea in human blood samples.