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On-line monitoring of urea in effluent liquid during haemodialysis

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Abstract: An analytical system specially built for on-line urea monitoring is reported. Measurements are carried out in the effluent of a haemodialysis machine. The measuring system employs the dialyser inflow stream as a carrier solution channel in a continuous fashion. The analyser periodically samples the outflow stream of the dialyser by means of an automatic injection valve. The analyser features a bioreactor consisting of immobilized urease and a gas-diffusion module. It is through this module that the urea is converted to ammonia gas which is transferred to another carrier channel, this transports the ammonium ion to a tubular, all-solid-state, ion-sensitive electrode. A timer controls the transport, injection, the measuring and the recording subsystems. The analyser has been used during actual haemodialysis sessions. Urea clearances were also measured in batch, using conventional spectrophotometric clinical equipment. The correlation between both methodologies was sufficient to confirm the usefulness of the developed on-line analyser to monitor the optimal length of haemodialysis sessions.

Keywords: *Urea; on-line monitoring; haemodialysis.*

Introduction

The need for analytical techniques to monitor clinically relevant variables in patients is growing. This is especially true for patients in the intensive care unit or in the surgical theatre [1]. Under these conditions, on-line information allows for a speedier diagnosis and for the adoption of adequate therapeutic decisions.

The monitoring of one or more metabolites during a haemodialysis session facilitates individualized treatment, thus enhancing the quality of life of the patient. Continuous clinical control such as the one described is not practised at present and the duration of a dialysis session is only determined as a function of renal degradation. Unfortunately, renal degradation is evaluated over a long period of time (usually several months) and singular events in the clinical evolution of a given patient are averaged out. Patients undergo haemodialysis for fixed intervals, typically for 3–4 h. This interval might not be the ideal one [2] and may entail unsuitable features: inadequate use of available equipment can ensue, resulting in haemodialysis sessions being shorter or longer than necessary and undesirable secondary effects can appear, damaging the health of the patient. This paper describes the study and application of a flow injection (FI) potentiometric biosensing system for on-line monitoring of urea. Urea is a metabolite commonly used as an indicator of renal deficiency. Urea measurements are made in dialysis effluent liquid leaving a haemodialysis machine. The resulting system permits the continuous monitoring of urea without interfering with the dialysis process since samples are taken continuously from waste effluent liquid. Urea concentration in blood can be inferred from its concentration in effluents using well-established relationships between urea concentrations in both fluids [3].

The flow injection system developed is based on an ammonium sensitive flow-through ion-selective electrode (ISE). This electrode was designed in our laboratory and features a tubular design, ideal for flow injection use. The analytical system also uses a microcolumn with immobilized urease. The electrode and the microcolumn are separated by a gas diffusion module [4]. The selectivity shown by the urease bioreactor makes sample pre-treatment unnecessary. By using a gas diffusion module, the sample matrix is prevented from coming into contact with the sensor, thus reducing

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interference effects arising from alkaline ions. In this manner greater reproducibility and stability are achieved.

Experimental

Apparatus

The flow injection system is similar to one previously used in ammonium-ion measurements in freshwater streams [4] with the addition of a column featuring an immobilized biomaterial. The flow system is represented in Fig. 1. The peristaltic pump used was a Gilson Minipuls 2. The manifold was made of Teflon tubing (0.7 mm i.d.) and an automatic, six-way Teflon valve was employed (Sirtek, Barcelona, Spain). The gas diffusion unit was built using two Perspex blocks, each of which had an excavated canal (7.2 cm long, 2.5 mm wide and 0.25 mm deep) with an entry and an exit point. A water-tight, gas permeable, membrane (GVHP 09050, Millipore) was inserted between both channels. The urease bioreactor (Appiano Bolzone, Milano, Italy) was placed immediately downstream from the injection valve. A timer periodically started the flow system (peristaltic pump, potentiometer, injection valve and recorder) and switched the injection valve as required during the operational cycle.

The detector used was an ammonium-ion selective all-solid-state (without inner reference solution) electrode, featuring a mobile-carrier PVC matrix membrane electrode in a flow-through configuration. This electrode was built following a procedure developed in our laboratory [4]. A PVC membrane is deposited on the inner surface of a hollow cylinder made of a conductive composite material. The composite used was graphite–epoxy. The reference electrode system was a double-junction Ag–AgCl electrode (Orion 90-02-00) with a 0.01 M solution of Tris (hydroxymethyl)aminomethane (Tris) of pH 7.5 as the external solutions. Potentiometric readings were made with a pH meter (Crison Digilab 517) connected to a recorder (Metrohm E 586). The flow-injection system was connected to the haemodialysis machine through two valves coupled to the input and the exit points of the dialysis liquid. Samples are taken with a three-way flow valve connected to the exit channel of the dialysis machine. The flow valve has two positions that allow sample taking or calibration without disconnection. The carrier solution is taken continuously from the entry channel of the haemodialysis machine.

Conventional urea biosensors were used for preliminary studies. The potentiometric urea biosensors were built following an all-solid-

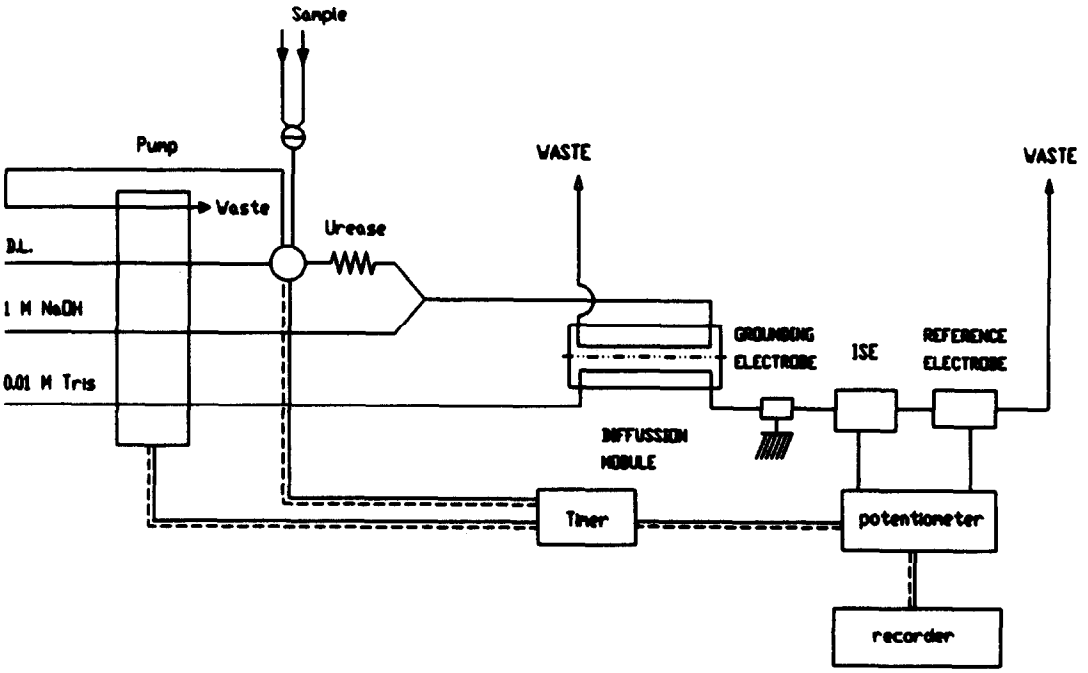

Figure 1
Schematic diagram of flow injection (FI) system for on-line monitoring of urea. DL, dialysis liquid; ISE, tubular flow-through ammonium-ion selective electrode.

state procedure previously reported [5, 6]. This procedure involves the direct deposition of the enzyme membrane on an ammonium-ion sensor. Urease is immobilized on a Nylon mesh that had been previously activated using dimethylsulphate, lysine and glutaraldehyde following a standard procedure [7, 8].

Reagents

All reagents used were pro-analysis grade. The standard ammonium chloride solutions used were made by the dilution of a stock solution.

The carrier solutions had the following composition: 0.01 M Tris(hydroxymethyl)aminomethane hydrochloride (pH 7.5) (Tris-HCl) and 1 M sodium hydroxide.

The dialysis solutions employed were Renofundina 543, Renofundina 908 and Nefrofundin (Industrias Palex, Barcelona, Spain).

The sensing membrane consisted of 0.5% nonactin (Fluka), 66.8% bis(1-butylpentyl)-adipate (Fluka) and 32.7% high relative molecular mass PVC (Fluka), all dissolved in tetrahydrofuran (Merck).

Urease (EC 3.5.15) used came from Jack beans, Type VIII, purchased from Sigma. Jack bean urease immobilized in a Nylon coil was purchased from Appiano Bolzone (Milan, Italy).

Characteristics of the haemodialysis liquid

The composition and osmolarity of the haemodialysis solution are very similar to those of plasma. It does not contain any of the solutes to be disposed of, like urea, urates, phosphate or creatinine and its electrolytic make-up is such as to correct the imbalances shown by the patient.

Two different types of dialysis liquids can be found, according to the buffer employed: acetate solutions and carbonate solutions. From point of view of blood chemistry, a carbonate haemodialysis liquid is deemed more adequate. However, problems arising from the precipitation of magnesium and calcium salts can occur. For this reason acetate solutions are more widely used. Carbonate-based solutions are reserved for patients showing liver dysfunction since acetate is metabolized in the liver. Dialysis liquids are found commercially as concentrated solutions.

The acetate-based liquids used in the present study were Renofundina 543 and Renofundina 908. Both contain potassium ions at a concentration of 1 mmol l^{-1} and 3.5 mmol l^{-1}, respectively, according to the dialysis requirements of an individual patient. The concentrate is diluted in water in a 1:34 proportion. Nefrofundin, a carbonate-based dialysis liquid, has also been studied. It has a potassium-ion concentration of 1.5 mmol l^{-1}. The liquid is commercially available in the form of two separate concentrates: an acid concentrate and a bicarbonate concentrate. To be used, one has to mix 33.2 parts water and 1 part acid concentrate or 1.8 parts bicarbonate.

Results and Discussion

Experimental characteristics under batch conditions

The response of the ammonium ISE (conventional configuration) in the various dialysis liquids was carried out following the known constant interference method. The linear range for diluted Nefrofundin and Renofundina 543 went from 2×10^{-3} to 10^{-2} M and from 4×10^{-3} M to 10^{-2} M for Renofundina 908. The lower limit of linear response is higher than has been found in previous work [5] because there is a larger interference arising from the saline nature of the dialysis liquid. The presence of K^+ and Na^+ raises the baseline. This interference is more acute in liquids containing Renofundina 908 because of its higher K^+ content (Fig. 2).

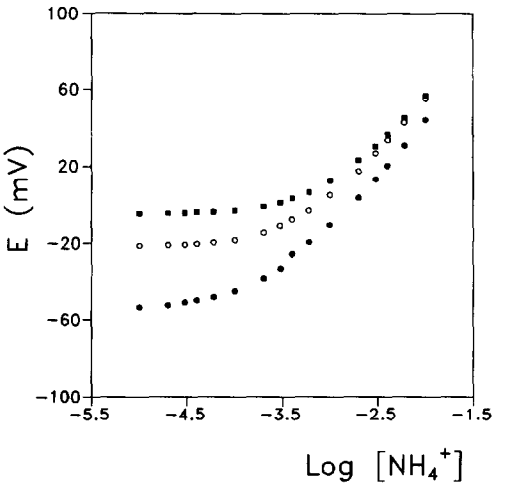

Figure 2
Calibration curves of ammonium response of the all-solid-state potentiometric sensor in presence of different haemodialysis liquids. (○) Renofundina 543; (■) Renofundina 908; (●) Nefrofundin.

The interference of the dialysis liquid on the urea biosensor was studied in the same way as with the ammonium ISE. Under normal working conditions (Tris-HCl 0.1 M, pH 7.5) the urea biosensor shows a 54 mV decade^{-1} sensitivity and a linear range from 6×10^{-5} M to 2×10^{-2} M urea. When either of the three dialysis liquids are present, a decrease of sensitivity and an increase of the lower and the upper limits of the linear response (LLLR and ULLR) are noted. The increase shown by the upper limit is possibly caused by the higher buffering capacity of the dialysis liquid when compared to the buffering capacity of the buffer solutions normally used (Fig. 3). The response reproducibility of the urea biosensor was studied performing successive calibrations in the dialysis liquid (Nefrofundin) during a single working session. Calibration parameters (regression coefficient, slope and standard potential) show negligible variations. The reproducibility of the potential reading of a single sample was also studied (4×10^{-3} M urea in a Nefrofundin solution). Previously, a calibration was performed to ensure proper response of the biosensor. The relative standard deviation for the potential measurements was 3% ($n = 10$). Samples of the dialysis effluent (Renofundina 543) were taken during a haemodialysis session for subsequent analysis using the potentiometric method presented here and the analytical method normally employed in the hospital: a standard spectrophotometric method using a Technicon RA 100 spectrometer. The results obtained using both methods were statistically compared and no significant differences were found (95% confidence level).

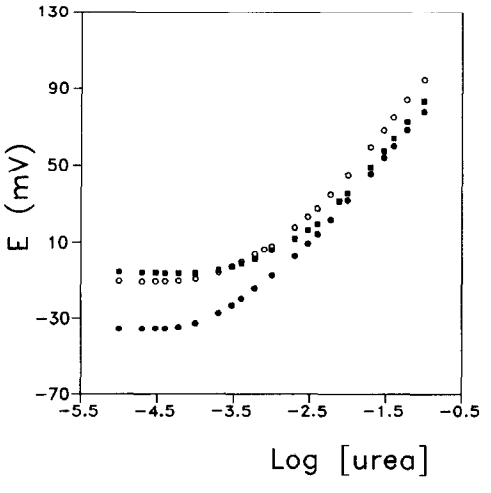

Figure 3
Calibration curves of the all-solid-state potentiometric urea biosensor in presence of different haemodialysis liquids. (○) Renofundina 543; (■) Renofundina 908; (●) Nefrofundin.

Evaluation of the flow injection system

A single channel flow injection system was used initially. In this system, the urease coil is placed immediately downstream from the injection valve. To evaluate the response of the flow system both to the ammonium ion and to urea, ammonium chloride and urea solutions were injected at different concentrations using the same carrier solution (Tris-HCl 0.01 M, pH 7.5). When the carrier solution was changed from Tris 0.01 M (pH 7.5) to dialysis liquid (Nefrofundin or Renofundina 543) it was observed that the baseline did not recover for urea concentrations equal or greater than 3×10^{-3} M.

This feature led to the modification of the flow system to accommodate a gas diffusion module (Fig. 1). Urea solutions were injected in a distilled water channel so that they were carried to the urease coil, where urea was enzymatically hydrolysed forming NH_4^+. Ammonium was rendered alkaline using a 1 M NaOH solution which merged with the water channel through a T-connector. Flow rate of the distilled water and the NaOH were 1.6 and 0.2 ml min^{-1}, respectively. The resulting NH_3 diffused through the gas permeable membrane and was picked up by a 0.01 M Tris-HCl solution (pH 7.5) counter-flowing at a rate of 0.6 ml min^{-1}. Following this method, the matrix of the sample is prevented from coming into contact with the detector, thus enhancing its lifetime and yielding a higher quality of response. Under these conditions, the injection of pure urea dilutions (from 10^{-5} to 10^{-1} M) yielded a 50 mV decade^{-1} slope and a linear range from 10^{-4} to 10^{-2} M. In order to enhance the bioreactor durability, a buffered carrier solution is preferred. Subsequently the system was evaluated changing the carrier solution from distilled water to dialysis liquid (Renofundina 543) and trying different sample injection volumes (100, 200 and 500 µl). Optimal results were obtained with a sample injection volume of 200 µl (51 mV decade^{-1} sensitivity and a linear range from 10^{-4} to 3×10^{-2} M urea). The relative standard deviation of the potential (peak height) after 30 measurements from the same sample (10^{-3} M urea in Renofundina 543) was found to be 0.8% (Fig.

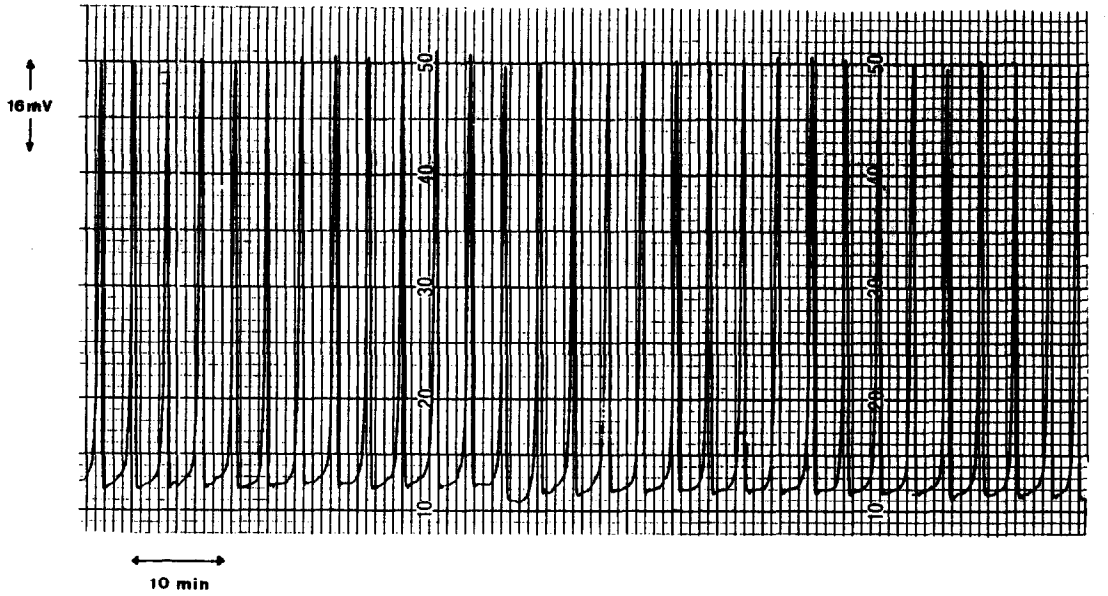

Figure 4
Strip-chart recording showing the response reproducibility of the flow-injection biosensor system with dialysis liquid as carrier. Each peak corresponds to measurements of the same sample (10^{-3} M urea in Renofundina 543).

4). Baseline recovery (time between two consecutive baselines) under the same conditions, was 4 min.

Application of the prototype

The prototype system was connected to the entry and exit channels of a haemodialysis machine (Gambro AK 10) without interfering with the dialysis process. While the dialyser was being primed with the blood of the patient, the system was calibrated by injecting standard solutions with concentrations ranging from 10^{-5} to 10^{-1} M urea employing a three-way valve. Dialysis liquid was taken from the input channel of the dialysis machine and used as the carrier solution. Under these conditions a sensitivity of 53 mV decade^{-1} and a linear range from 3×10^{-4} M to 3×10^{-2} M urea was obtained. The effluent coming out of the dialysis machine was monitored for urea content continuously. Samples were injected every 5 min under timer control.

Discrete samples from the effluent dialysis liquid were also taken at different times. Urea measurements were done in the hospital laboratory using a standard spectrophotometric method outlined above. Both set of results were compared and no significant difference was found at the 95% confidence level. Even though the laboratory data were fewer than the results yielded by the prototype system, good agreement was found between the two sets of data. Figure 5 shows urea levels measured during a haemodialysis session using the biosensing analytical system and the spectrometer method.

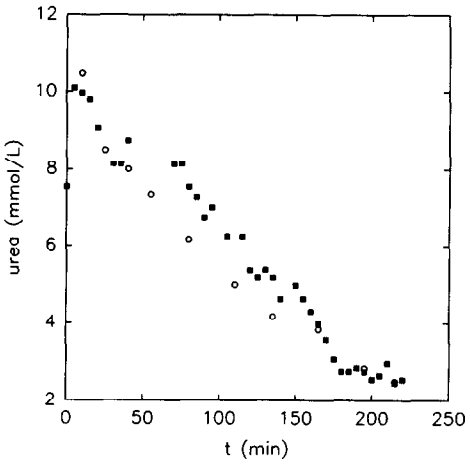

Figure 5
Monitoring of urea levels in effluent dialysis liquid with the proposed FI biosensor system during a haemodialysis session. Results are compared with spectrometric determinations performed with discrete samples. (■) Biosensor system; (○) spectrometric method.

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