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Optoelectronic detectors and flow analysis systems for determination of dialysate urea nitrogen

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ABSTRACT

Three compact flow analysis systems based on flow-through optoelectronic devices and microsolenoid pumps and valves dedicated for determination of urea in effluent liquid produced by artificial kidney in the course of hemodialysis treatments have been developed. The developed photometric devices operate according to paired detector diode principle. For the first flow analysis system an optoelectronic urea biosensor based on pH-sensitive film enzymatically modified with urease has been applied. In the second system open-tubular urease biosensor and optical detector of ammonia by Berthelot reaction have been used. The third non-enzymatic analytical system is based on optoelectronic detector of the product formed in reaction of urea with modified Ehrlich reagent. The analytical utility of developed flow analysis systems has been tested with real samples of spent dialysate. The results of dialysate urea nitrogen determination are comparable with those obtained using reference off-line method recommended for clinical analysis. Advantages and drawbacks of developed prototypes have been compared and discussed.

1. Introduction

End-stage renal disease (uremia) occurs when nephrons are degenerated and unable to remove metabolic waste products, salts and water excess from blood. Uremia leads to death unless renal replacement therapy is started. Uremic patients can survive with kidney transplantation or extracorporeal blood purification. The blood purification by dialysis performed using artificial kidney is named hemodialysis (HD). Urea, although itself is not toxic, is recognized as a main marker of uremic toxins in monitoring and modeling of hemodialysis processes [1]. Urea has several advantages as the uremic marker. Nearly 95% of protein nitrogen is metabolized into urea nitrogen so the levels of this metabolite are relatively high. As a small, polar, water-soluble and neutral molecule, urea is not bound to proteins and cells, thus easily diffuses among body-water compartments as well as across dialyzer membranes. Consequently, the changes of urea levels caused by hemodialysis treatment provide a sensitive indicator of dialyzer efficiency. On the other hand, urea generation in patient body originated exclusively from protein catabolism, so it is accepted for estimation of quantitative index of nutrition. Finally, urea, as the main final product of metabolism, provides information about other uremic toxins which also are predominantly generated from proteins. Summarizing, urea levels well reflect both, the exposition of patient to uremic toxins (from metabolism) and their removal (by hemodialysis). Urea kinetic modeling is widely recognized as the most efficient way for quantitative control, description and assessment of hemodialysis therapy.

A current trend in urea kinetic modeling is a bloodless control of artificial kidney treatment based on determination of so-called dialysate urea nitrogen (DUN) in the course of successive hemodialysis sessions [1]. A prevailing number of analytical systems dedicated for urea kinetic modeling is based on potentiometric biosensors. These are ammonium ion selective electrodes [2–4], array of electrodes (electronic tongue) [5] as well as thick-film metal oxide electrodes [6,7] enzymatically sensitized for urea. A great attention is paid for urease-based enzymatic field effect transistors dedicated for DUN determination [8–13].

There are a few literature reports devoted to optical flow analysis systems [14] and biosensors [15] applied for DUN detection. It is rather surprising taking into account a recent progress in optoelectronics and a great number of reports on application of light-emitting diodes in several fields of analytical chemistry [16,17]. Non-conventional measurement option is offered by paired

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light-emitting diodes in the form of complete optical devices in which one of them is used in conventional way as a light source, whereas the second plays the role of light detector. These, so-called paired emitter detector diodes (PEDDs) made of the cheapest optoelectronics, powered with low-voltage sources and generating easy to measure analytical signal are extremely economic optical detectors useful for photometric [18,19] as well as fluorometric [20,21], turbidimetric [19,22] and nephelometric [22,23] measurements.

PEDD detectors are miniature devices operating according to principle described by Shockley equation and the Lambert–Beer law [18]. PEDD detectors, generate voltage as an analytical signal, proportional to the absorbance of sample. It has been confirmed that the reading of such analytical signal using low input impedance voltmeter results in the significant enhancement of sensitivity caused by partial discharging of illuminated LED-detector by voltmeter [24]. Such approach causes a narrower range of linearity but the appropriate choice of power supplying LED-emitter allows to adjust the range of maximal sensitivity of device to the required range of detection. Obviously, the measurement setup based on low-budget voltmeter is more economic than this one based on pH-meter.

Moreover, they are naturally designed for miniaturization and easy to develop in the form of compact, fibreless, flow-through detectors. Optical detectors based on light-emitting diodes are recommended as compatible with multicommutation flow analysis (MCFA) [25,26]. MCFA systems based on microsolenoid valves and pumps are recommended [27] and widely used [28,29] for development of modern analytical systems.

In this contribution three different prototypes of flow analysis systems based on microsolenoid devices developed for determination of urea in spent dialysate produced by artificial kidney are presented. These MCFA systems are based on newly developed optoelectronic devices (detectors and biosensors) operating according to PEDD principle. In each case another chemical scheme for optical determination of urea has been applied.

2. Materials and methods

2.1. Reagents

Urease from *Canavalia ensiformis* (Type IX, powder, 59,400 units/G), 4-(1H-pyrrol-1-yl)benzoic acid, 99%, reagent grade (Pyr-Bac), N-Ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) of commercial grade, sodium hypochloride solution (available chlorine 10–15%), salicylic acid and p-dimethylaminebenzaldehyde (DMABA, 98%, reagent grade) were obtained from Sigma–Aldrich (Germany). All other reagents of analytical grade and solvents were obtained from POCh (Poland). For all experiments doubly distilled water was used throughout. The concentrate of electrolytes for hemodialysis (product no. 1190612, MTN) was obtained from Neubrandenburg GmbH (Germany). Sodium bicarbonate for dialysis (product no. B10789) was obtained from Gambro Lundia AB (Sweden).

2.2. Samples

Samples of spent dialysate as well as “pure” dialysate fluid were obtained from Dialysis Station at the Warsaw Medical University. They were sampled from spent fluid stream produced by artificial kidneys AK96 from Gambro Lumbia AB (Sweden) equipped with capillary dialyzers Hemoflow F of different effective surface areas (from 1.6 to 2.2 m^2) obtained from Fresenius Medical Care (Germany). The postdialysis fluid was sampled every 10 min in first hour of dialysis than every 15 min from the beginning to the finish of monitored hemodialysis treatment. The fluid produced by artificial kidneys for hemodialysis process commonly contained Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, HCO_3^-, CH_3COO^- ions and glucose at concentrations 138 mM, 3.0 mM, 0.50 mM, 1.25 mM, 109.5 mM, 32.0 mM, 3.0 mM and 8.3 mM respectively. “Pure” dialysate also was sampled from spent dialysate line before starting the blood pump of artificial kidney. The collected samples were analyzed using the developed flow systems and the reference method recommended for clinical analysis (the kinetic method with urease and glutamate dehydrogenase coupled enzymatic method) [30] using clinical analyzer Roche Hitachi Cobas 6000 model C501 (Switzerland).

2.3. Biosensing membrane

Optically pH-sensitive Prussian Blue (PB) film with immobilized urease forms bioreceptor part of the PEDD based biosensor. The non-electrolytic deposition of PB over a non-conductive surface has been described elsewhere [31,32]. Polyester roller (1 mm thick, 9.5 mm in diameter made by lathing of transparent polystyrene plate (Plastics Group, Poland) was placed on Schotch® transparent tape (from 3M, Poland) and was soaked in 0.1 M $K_3Fe(CN)_6$ in 1.0 M HCl solution saturated with Pyr-BAc (a few milligrams per liter) subjected to UV-irradiation (Phillips TLD 18W/08 lamp) for two days. To immobilize the monomolecular layer of enzyme on the Prussian Blue surface, a simple one-step carbodiimide method was applied [33]. A freshly prepared solution containing EDAC (10 mg mL^{-1}) and urease (20 mg mL^{-1}) was dropped on the film surface and left for 12 h. To remove the access of unbounded enzyme a biosensing membrane was left for 2 h in vigorously stirred 0.1 M phosphate buffer pH of 7.0.

2.4. Bioreactor

Bioreactor was made of 8 mm Ertacetal® stick by drilling and milling. The inner diameter and length of this open-tubular reactor were 2.3 mm and 3.0 cm, respectively. As proposed elsewhere [34], the internal surface of reactor was coated with carboxylated PVC by flowing through its solution in THF (60 mg mL^{-1} for 30 s) and evaporation of solvent residues at room temperature. For enzyme immobilization, the reactor was filled with a water solution of urease (20 mg mL^{-1}) containing EDAC (10 mg mL^{-1}) and left at room temperature overnight. Before the first use, the bioreactor was washed for 2 h by working buffer passing through.

2.5. Optoelectronic devices

All light-emitting diodes used in these investigations have common shape, 5.0 mm diameter and transparent lens and were obtained from Optosupply (Hong Kong). LEDs used as emitters were powered using homemade low-voltage circuit based on L272 chip. All electronic components used for the fabrication of supply unit were obtained from TME (Poland). The voltage signal generated by illuminated LED used as a detector was measured and recorded as analytical signal [24] by multimeter (model U1231A from Keysight Technologies, USA) without any additional amplification. This multimeter operating as voltmeter was connected with data storage computer using IR-to-Bluetooth® interface (model U1177A from Keysight Technologies, USA).

2.6. MCFA manifolds

The flow analysis systems were constructed using microsolenoid pumps (Product no. 120SP1210-4TE 12VDC) and three-way microsolenoid valves (product no. 100T3MP12-62 30PSI 12VDC) purchased from Bio-Chem Fluidics (Boonton, USA). The microsolenoid devices were microprocessor-controlled by