On-line monitoring of urea using enzymatic field effect transistors

William Sant, Pierre Temple-Boyer, Eric Chanié, Jérôme Launay, Augustin Martinez

ON-LINE MONITORING OF UREA USING ENZYMATIC FIELD EFFECT TRANSISTORS

W. Sant[1], P. Temple-Boyer[2,3], E.Chanié[1], J. Launay[2,3], A. Martinez[2,3]

1) HEMODIA SA, Rue du chêne vert, BP88205, F-31682 Labège, France

2) CNRS, LAAS, 7 avenue du colonel Roche, F-31077 Toulouse, France

3) Université de Toulouse; UPS, INSA, INP, ISAE; LAAS; F-31077 Toulouse, France

Abstract

Silicon and polymer-based technologies were developed in order to reach the industrial fabrication of disposable, mass-fabricated microsensors for the urea detection in liquid phase. The detection principle is based on pH-sensitive chemical field effect transistor (pH-ChemFET) microdevices. In order to realize an urea-sensitive enzymatic field effect transistor (urea-EnFET), studies involve the integration of an urease-based enzymatic layer by ink jet printing techniques, the packaging into a specific flow cell compatible with the liquid phase measurement, and the development of urea-EnFET/pH-ChemFET differential analysis technique. Thus, the urea detection properties were studied in EDTA-based and dialysate solutions, evidencing good sensitivity in concentration ranges appropriate to human kidney failure. Finally, the whole analysis system was successfully tested for the continuous monitoring of urea in artificial dialysis conditions.

Keywords: ChemFET sensor, enzymatic reaction, urea detection, flow cell analysis, haemodialysis

Introduction

Chronic end-stage kidney failure is a widely spread health disorder and is a priority for the development of health-care systems. Thus, dialysis techniques, and haemodialysis in particular, were in constant development in order to improve their efficiency. This concerns mainly the detection of the two final metabolites of the protein metabolism, i.e. urea and creatinine [1,2]. Up to now, the dialysis monitoring is still performed through standard blood analysis before and after the treatment. Thus, it is possible to define the dialysis efficiency through the determination of the Kt/V ratio where K, t and V are respectively the clearance, the dialysis duration and the patient urea distribution volume [3]. However, this measurement is usually done monthly, gives results subsequently and do not allow an evaluation of each dialysis treatment, where signs of deviations and reduced dialysis efficiency is discovered late. This could ultimately lead to increased morbidity and mortality. In order to reach further improvement in terms of kidney failure diagnosis and patient life expectancy, the haemodialysis treatment must be monitored on-line and the Kt/V ratio must be determined just next.

The concept of ionic dialysance was therefore proposed [3-5]. This technique is based on the equivalence between the sodium dialysance and the urea clearance. Thus, the dialysis efficiency is monitored using conventional electrodes by measuring the conductivity difference between dialysate inlet and outlet of the dialysis machine. Nevertheless, even if this technique gives rapidly the Kt/V ratio, there is no direct measurement of the urea concentration and therefore no way of defining the extracted urea mass during dialysis. Furthermore, even if some devices were marketed under the name "urea monitor", the high cost and difficulties of implementation stopped their development, emphasizing the medicine requirements for easiness, disposability and low cost.

Optical detection principles were also developed for the monitoring of haemodialysis sessions. Thus, the urea concentration was monitored on-line using near-infrared (NIR) absorbance measurements but the proposed solution was quite complicated [6]. In a more convenient way, the dialysis clearance was monitored continuously through the dialysate ultraviolet (UV) absorbance measurements but such method for the Kt/V ratio determination was indirect and therefore non-specific to the urea concentration [7,8].

In order to reach the direct urea measurement in dialysis conditions, the development of electrochemical detection principles was finally proposed, either based on ion sensitive electrodes (ISE) [5,9-12] or on chemical field effect transistors (ChemFET) [13-15]. From a general point of view, studies concerned the integration of the urease-based enzymatic reaction into specific flow injection systems. Indeed and at neutral pH, urease is known to be responsible for the urea hydrolysis and finally for the production of ammonia and carbonic acid deviated species: $CO(NH_2)_2 + 2H_2O$ ----------> $NH_3 + NH_4^+ + HCO_3^-$.

As a consequence, two research axis were developed. Some works concerned the monitoring of the ammonium ions NH_4^+ in solution while requiring the integration of specific ion-sensitive layers [9,11,12], some others dealt with the monitoring of $NH_4^+/NH_3/HCO_3^-$-deviated pH variations in solution while requiring pH-related differential analysis [10,13-15]. Nevertheless, in all cases, these developments and realizations based on electrochemistry provide further advantages: they can be extended to the detection of creatinine in solution [9-12,15,16], are fully compatible with the "Silicon & Polymer" microtechnologies, and allow their integration into specific flow injection systems for dialysis applications.

This paper presents the industrial development of pH-based urea-EnFET (enzymatic field effect transistor) microsensors for the urea concentration measurement. Its major goal is to build a smart analysis system based on "Silicon & Polymers" microtechnologies and "Smart cards" techniques for simple, fast and reliable diagnosis during the course of

haemodialysis sessions. Therefore, it deals with the pH-ChemFET chips mass-fabrication, the enzymatic layers integration, the packaging into specific flowcell, and the development of measurement interfaces adapted to differential analysis techniques. Finally, validation of the urea-EnFET-based analysis system is demonstrated for the on-line monitoring of urea in dialysis conditions.

Experimental

N-channel, SiO_2/Si_3N_4-gate, pH-sensitive chemical field effect transistors (pH-ChemFET) were fabricated on 6-inch, (100)-oriented, N-type (500 Ω.cm) silicon wafers using standard P-well technology. A technological process was developed in LAAS-CNRS cleanroom facilities, in collaboration with the HEMODIA company [17]. These works showed the feasibility of a ChemFET-based microsensor for detecting urea in biological solutions [16,18]. Then, this fabrication process was optimised and transferred by the HEMODIA company in an industrial foundry in order to reach fully mass fabrication at low cost, reproducibility, and high fabrication yield (figure 1).

The whole realisation of the urea-EnFET flow injection microsystems was performed using "Smart cards" techniques in order to reach automated, mass fabrication packaging processes. Two pH-ChemFET chips (size: 2.1 x 1.6 mm^2) were packaged and connected on a specific printed circuit board (called "module" hereafter) with an integrated gold pseudo-reference gate electrode used to bias the analyzed solution. The first pH-ChemFET chip was adapted to the urea detection thanks to a specific enzymatic layer [19]. A photosensitive polyvinyl alcohol-based (PVA-SbQ SPP-H-13 purchased from Toyo Gosei) liquid matrix was modified by adding a specific urease (not described for confidential reasons). The so-obtained enzymatic matrix was deposited by ink jet techniques and reticulated under UV exposure

(figure 2), leading to the realisation of the urea-sensitive enzymatic field effect transistor (urea-EnFET). The second chip was used as a pH-sensitive reference field effect transistor (pH-ReFET) to overcome any pH variations, any temporal drift and/or any shortcomings of the integrated gold pseudo-reference gate electrode, and to limit temporal drift [20,21]. Finally, the urea-EnFET/pH-ReFET modules were integrated into a specific, opaque flowcell compatible with standard fluidic and electrical connections (figure 3). All in all, starting from the pH-ChemFET chips fabrication in silicon foundry, all the packaging process was automated in order to reach the industrial realisation of urea-EnFET-based flow injection systems for dialysis applications (figure 4).

Electrical measurements were performed while biasing the gold pseudo-reference gate electrode and therefore the studied solution to the mass ($V_G = 0$) and working with constant drain-source voltage V_{DS} and drain-source current I_{DS} (typically $V_{DS} = 1$ V and $I_{DS} = 0.1$ mA) in order to work in saturation mode. A specific ChemFET-meter electronic interface was used to monitor continuously the gate-source voltage V_{GS} of both urea-EnFET and pH-ReFET microsensors. In fact, some data treatments were introduced to improve the signal to noise ratio, leading finally to one urea concentration measurement every ten seconds. So, the pH and urea measurements were possible by direct and differential analysis respectively with a sampling frequency of 0.1 Hz.

First, urea-EnFET/pH-ReFET modules were studied in order to measure by differential analysis the urea concentration in phosphate buffer saline solution ($pH_0 = 7.4$) with ethylene-diamine-tetra-acetic acid (EDTA: 1 mmol/L, purchased from Sigma) in order to stabilize the urease enzymatic activity, and in standard, freshly prepared, dialysate solutions (composition: glucose: 5.55 mmol/L, NaCl: 103 mmol/L, KCl: 1.5 mmol/L, $CaCl_2$: 1.5 mmol/L, $MgCl_2$: 0.5 mmol/L, CH_3COOH: 3 mmol/L, HCO_3Na: 36 mmol/L - $pH_0 = 7.4$). Then, the on-line monitoring of urea was tested in artificial dialysis conditions according to the following

procedure. Freshly prepared, urea-rich (urea concentration: 20 mmol/L) dialysate (pH_0 = 7.4) was purified for two hours in a GAMBRO AK200 dialysis generator (dialysate flow: 500 mL/min, filter: Smartflux L-16, UF 500 mL). The urea EnFET/pH-ReFET detection microsystem was located in a disposable external bypass loop in dialysis machine drain line in order to analyze continuously the spent dialysate (figure 5). This was done for three reasons: (i) measuring the urea dose effectively removed during the dialysis session, (ii) taking the urea microsensor away from the blood circuit for biocompatibility aspects, and (iii) preventing any degradations related to the dialysis machine cleaning and/or disinfection procedures. Finally, in order to compare urea concentration results, some dialysate samples were also analyzed by optical absorbance measurements using a COBAS Integra 800 system from ROCHE Diagnostics.

Results and discussion

A. pH measurement

SiO_2/Si_3N_4-pH-ChemFET microdevices were tested for pH measurements using a commercial Ag/AgCl wire as reference electrode. A quasi-Nernstian pH response was thus evidenced on a wide pH range (figure 6). Such pH-ChemFET technology was already studied in previous works, evidencing maximal drift lower than 4 mV/day and lifetime higher than 4 four months in the frame of water analysis applications [22]. In the frame of medical applications like haemodialysis, such phenomena were not studied since further short durations use and disposability are required. Nevertheless and according to the electrical characterization of more than two thousands pH-ChemFET microdevices (fabrication yield around 94%), the others technological specifications are given in table 1 [17].

B. Urea detection

Since the chosen urea detection principle is based on pH-ChemFET-metry, a ChemFET/ReFET differential analysis technique was used in order to cope with any pH interferences, hysteresis phenomena and/or electrochemical instabilities of the biasing gate electrode [20,21]. Thus, in the following, all the results presented for the urea detection were obtained by urea-EnFET/pH-ReFET differential measurement while using the integrated gold pseudo-reference gate electrode. Furthermore, in order to tackle off the pH-ChemFET threshold voltage discrepancy (cf. table 1), the potential origin was chosen by fixing to zero the urea-EnFET/pH-ReFET output voltage obtained for the no-urea solution (urea concentration: 0 mmol/L).

First, the urea-EnFET/pH-ReFET modules were tested in phosphate buffer saline solutions with ethylene-diamine-tetra-acetic acid (EDTA) while focusing on the [1 – 25 mmol/L] human pathological concentration range for urea in blood (figure 7). Thus, urea was detected with high voltage variations, i.e. 70 mV between 1 and 10 mmol/L, and very fast response. Such phenomenon should be related to the use of pH-metry technique for the urea detection. Indeed, the modelling of the pH-based urea-EnFET detection principles shows that the urea sensitivity depends on the initial pH value and on the different acid/base chemical systems in solution: NH_4^+/NH_3 and $H_2CO_3/HCO_3^-/CO_3^{2}$ of course (cf. equation 1) but also any others specific to the solution buffer properties [23]. Consequently, whereas the pH-ChemFET microsensor is characterized by a quasi-nernstian response (see below), the influence of the urea variation on the pH one can be amplified or attenuated according to these specific acid/base chemical systems, leading finally to the urea sensitivity increase or decrease accordingly. Here, for EDTA-rich phosphate buffer solution, an amplification

phenomenon is clearly evidenced, responsible for voltage variations higher than the Nernst sensitivity.

On the contrary, in dialysate solutions whose buffer properties are based on carbonic and acetic acids, lower voltage variations, i.e. around 20 mV per concentration decade, were obtained in the [1 – 25 mmol/L] urea concentration range, evidencing clearly an attenuation of the pH-ChemFET sensitivity (figure 8). This phenomenon should be related to the higher buffer capacity of dialysate compared to the EDTA-rich phosphate buffer solution. Nevertheless, results demonstrate that the urea-EnFET/pH-ReFET modules can be used successfully in dialysate solutions.

Furthermore, whatever the solutions, i.e. EDTA-based or dialysate, and thanks to the use of the urea-EnFET/pH-ReFET differential analysis (see below), no significant drift (<< 0.1 mV/hour) was evidenced for the urea detection (figures 7 and 8). In fact, this drift was estimated at 0.04 mV/hour during four hours (result not shown). Since the dialysis session duration should be lower than five hours and since the urea microsensors have to be disposable, such low drift is efficient for the urea dose monitoring and no further study of this phenomenon was performed.

Finally, reproducibility experiments were performed for a batch of almost fifty urea-EnFET microsensors. Thus, the fabrication yield was estimated around 83% and an output voltage V_{out} of 75.5 ± 5.5 mV was found for an EDTA-based solution with a 5 mmol/L urea concentration.

Since urea detection was shown in dialysate solutions, the urea-EnFET/pH-ReFET microsensor was finally tested for the urea on-line monitoring in dialysis conditions, using an industrial dialysis generator. As expected, the output voltage decrease, i.e. the urea concentration decrease, was evidenced during the dialysis session (figure 9). The detection of measurement artefacts was expected. Indeed, this phenomenon is related to the self-

calibration procedure of commercial dialysis generators: every thirsty minutes and during two minutes, the dialyzer is shunt, pure dialysate flows directly to the drain and the urea concentration goes to zero. Thus, the urea microsensor response shows negative measurement jumps accordingly.

In parallel with the urea-EnFET/pH-ReFET test in dialysis conditions, some spent dialysate samples were analyzed by optical absorbance measurement in order to determine their urea concentration. Thus, thanks to a linear conversion model (see hereafter), the urea concentration decrease during the dialysis session was finally predicted (figure 10). A maximal value around 6 mmol/L was found in the spent dialysate (rather than 20 mmol/L in the initial one). Such discrepancy is related to the dialysis generator procedure that introduces a ratio around three between the dialyser flow and the spent dialysate flow, so dividing roughly the urea concentration by three. Nevertheless, urea was successfully monitored in dialysis conditions. Such experimental curves will enable to optimise the dialysis duration, to calculate the extracted urea mass and therefore the dialysis efficiency. Thus, the urea on-line monitoring was successfully demonstrated and the urea-EnFET/pH-ReFET detection microsystem was shown to be fully operational.

Finally, the different urea responses of the urea-EnFET/pH-ReFET microsensor were studied according to the analyzed EDTA-based and/or dialysate-based solutions, as well as to the optical spectroscopic measurements in dialysis conditions (figure 11). For each case and taking into account the ChemFET potentiometric detection principle, empirical laws were defined between the output voltage V_{out} and the logarithm of the urea concentration (log c) with an error lower than 1%:

- EDTA-based solution: V_{out} (mV) ≈ $-35.7(\log c)^2 - 106.4(\log c) + 13.1$ (2)

- dialysate-based solution: V_{out} (mV) ≈ $-10.2(\log c)^2 - 31.2(\log c) + 20.2$ (3)

- dialysis conditions: V_{out} (mV) ≈ 34.7(log c) + 122.1 (4)

For the EDTA-based phosphate buffer solution, a parabolic law is evidenced (equation 2), leading to an amplified potentiometric variation around 92 mV in the [0.8 - 30 mmol/L] urea concentration range. Such parabolic law should be related to the pH-based saturation phenomena of the NH_4^+/NH_3 [18]. For a solution pH_0 value around 7.4 and a pH-ChemFET sensitivity around 53 mV/pH (see table 1), knowing than the urea hydrolysis cannot lead to pH higher than 9.2 [18], the maximal potentiometric variation can be estimated to 95 mV. Therefore, equation (2) is representative of the maximal detection response for the urea-EnFET/pH-ReFET microsensor.

For the dialysate solution, a parabolic law is still evidenced (equation 3), but lower potentiometric variations (around 45 mV) are found on the [0.3 - 30 mmol/L] urea concentration range. This decrease was already related to the attenuation of the pH-ChemFET sensitivity due to the dialysate higher buffer capacity (see below). Moreover, dialysate solutions are characterized by ageing phenomena in airy atmosphere [13]. These oxidation phenomena are responsible for some pH increase with time and participate also to the potentiometric variations decrease [23], even (or especially…) if freshly-prepared dialysate was initially used. This is confirmed by the empirical equation obtained in dialysis conditions (equation 4) and used previously for determining the urea concentration from the output voltage (see below). In this case, the dialysate solution used is not in contact with the atmosphere and is therefore kept chemically unchanged during the dialysis session. Thus, intermediate linear potentiometric variations (urea sensitivity around 35 mV per decade) are evidenced on the urea concentration range [0.5 – 6 mmol/L], enabling the development of simple calibration procedure for future haemodialysis applications.

4. Conclusion

Urea-EnFET microsensors were developed using "Silicon & Polymers" microtechnologies and "Smart card" techniques. The industrial process involved the mass fabrication of pH-ChemFET transistors in silicon foundry, the deposition of urease-based enzymatic layer by ink-jet printing and the mass packaging into a specific flow cell. Since good fabrication yield (between 90 and 95% for the pH-ChemFET chips, between 80 and 85% for the urea-EnFET microsensors) was obtained, differential analysis was used in order to detect urea in biological solutions. Finally, the urea-EnFET/pH-ReFET analysis system showed good urea detection properties, enabling finally the on-line monitoring of urea in artificial dialysis conditions as well as the analysis of the different urea detection responses.

This technology is ready-to-use in the frame of dialysis, but few research works have still to be continued. First, the urea concentration must be predicted from the urea-EnFET/pH-ReFET output voltage using a self-calibration technique. Actually, such automated calibration procedure involving a low and a high urea concentration samples (typically 0 and 5 mmol/L) before the dialysis session as well as optimised conversion models are being tested clinically in dialysis centre in order to improve definitively the urea measurement. Second, the pH-ChemFET-based technology has to be extended to the on-line monitoring of creatinine as well as of potassium K^+, phosphate PO_4^{3-} or calcium Ca^{2+} ions while using adapted enzymatic or ion-sensitive layers. Thus, it will be possible to detect the different (bio)chemical species of clinical interest, to monitor the blood ionic equilibrium, to identify any deviations of the dialysis treatment, to measure the dialysis efficiency, and, finally, to improve the diagnosis of chronic end-stage kidney failure.

References

[1] S. Stiller, A. Al-Bashir and H. Mann: "On-line urea monitoring during hemodialysis: a review", Saudi Journal of Kidney Dis. Transplantation, 12 (2001) 364-374

[2] R. Koncki: "Recent developments in potentiometric biosensors for medical analysis", Analytica Chemica Acta, 599 (2007) 7-15

[3] L.J. Garred, B. Canaud, J.Y. Bosc and C. Tetta: "Urea rebound and delivered Kt/V determination with a continuous urea sensor", Nephrol. Dial. Transplantation 12 (1997) 535-542

[4] F. Locatelli, U. Buoncristiani, B. Canaud, H. Köhler, T. Petitclerc and P. Zucchelli: "Haemodialysis with on-line monitoring equipment: tools or toys ?", Nephrol. Dial. Transplantation, 20 (2005) 22–33

[5] R.M. Linday and J. Sternby: "Future Direction in Dialysis Quantification", Seminars in Dialysis, 14 (2001) 300–307

[6] D.S. Cho, J.T. Olesberg, M.J. Flanigan and M.A. Arnold: "On-line near-infrared spectrometer to monitor urea removal in real time during haemodialysis", Applied Spectroscopy, 62 (2008) 866-872

[7] F. Uhlin, I. Fridolin, M. Magnusson and L. Lindberg: "Dialysis dose (Kt/V) and clearance variation sensitivity using measurement of ultraviolet-absorbance (on-line), blood urea, dialysate urea and ionic dialysance", Nephrol. Dial. Transplantation, 21 (2006) 2225-2231

[8] F. Uhlin, J. Pettersson, A. Fernstrom and L. Lindberg: "Complementary parameter for dialysis monitoring based on UV absorbance", Haemodialysis international, 13 (2009) 492-497

[9] A. Radomska, R. Koncki, K. Pyrynska and S. Glab: "Bioanalytical system for control oh haemodialysis treatment based on potentiometric biosensors for urea and creatinine", Analytica Chemica Acta, 523 (2004) 193-200

[10] H. Suzuki and Y. Matsugi: "Integrated microfluidic system for the simultaneous determination of ammonia, creatinine and urea", Sensors and Actuators, B108 (2005) 700-707

[11] P. Ciosek, I. Grabowska, Z. Brzozska and W. Wroblewski: "Analysis of dialysate fluids with the use of a potentiometric electric tongue", Microchimica Acta, 163 (2008) 139-145

[12] M. Guttierez, S. Alegret and M. del Valle: "Bioelectronic tongue for the simultaneous analysis determination of urea, creatinine and alkaline ions in clinical samples", Biosensors and bioelectronics, 23 (2008) 795-802

[13] G.K. Chandler, J.R. Dodgson and M.J. Eddowes: "An ISFET-based flow injection analysis system for determination of urea: experiment and theory", Sensors and Actuators, B1 (1990) 433-437

[14] J-Q. Wang, J-C. Chou, T-P. Sun, S-K. Hsiung and G-B. Hsiung: "pH-based potentiometric flow injection biosensor for urea", Sensors and Actuators B91 (2003) 5-10

[15] B. Premanode and C. Toumazou: "A novel nano power biosensor for real time monitoring of creatininase and urease in peritoneal dialysis", Sensors and Actuators B120 (2007) 732-735

[16] W. Sant, M.L. Pourciel Gouzy, J. Launay, T. Do Conto, R. Colin, A. Martinez and P. Temple-Boyer: "Development of a creatinine-sensitive sensor for medical analysis", Sensors and Actuators, B103 (2004) 260-264

[17] www.medisensors.com

[18] W. Sant, M.L. Pourciel Gouzy, J. Launay, T. Do Conto, A. Martinez and P. Temple-Boyer: "Development of chemical field effect transistors for the detection of urea", Sensors and Actuators, B95 (2003) 309-314

[19] M.L. Pourciel Gouzy, J. Launay, W. Sant, V. Conédéra, A. Martinez and P. Temple-Boyer: "Development of photo-polymerisable polyvinyl alcohol for biotechnological applications", Sensors and Actuators, B94 (2003) 330-336

[20] P. Bergveld, A. Van der Berg, P.D. Van der Wal, M. Skowronska – Ptasinska, E.J.R. Südholter and D.N. Reinhoudt: "How electrical and chemical requirements for REFETs may coincide", Sensors and Actuators, 18 (1989) 309-327

[21] A. Errachid, J. Bausells and N. Jaffrezic-Renault: "A simple REFET for pH detection in differential mode", Sensors and Actuators, B60 (1999) 43-48

[22] P. Temple-Boyer, J. Launay I. Humenyuk, T. Do Conto, A. Martinez, C. Bériet and A. Grisel: "Study of front-side connected chemical field effect transistors for water analysis", Microelectronics reliability, 44 (2004) 443-447

[23] P. Temple-Boyer, A. Benyahia, W. Sant, M.L. Pourciel Gouzy, J. Launay and A. Martinez: "Modelling of urea-EnFETs for hemodialysis applications", Sensors and Actuators, B131 (2008) 525-532

Biographies

William Sant was born on May 18, 1969. He received his Master's Degree in electronics in 1996 and his Diplome d'Etudes Approfondies in Microelectronics from the Université Paul Sabatier de Toulouse (France) in 1999. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes of the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 2000 and received the PhD degree from the Université Paul Sabatier de Toulouse (France) in 2004. Since then, he has joined the HEMODIA society (France) as an R&D engineer and has been working on the development of ChemFETs microsensors for medical applications.

Pierre Temple-Boyer was born on October 25, 1966. He received his Engineer Master's Degree in electronic engineering from the Ecole Supérieure d'Electricité (Paris – France) in 1990 and his Master's Degree in microelectronics from the Université Paul Sabatier de Toulouse (France) in 1992. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes of the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 1992 and received the PhD degree from the Institut National des Sciences Appliquées de Toulouse (France) in 1995. Since then, as a senior researcher, he has been working on the development of micro- and nanotechnologies.

Eric Chanié is director of Research & development of HEMODIA/CAPTOMED company, specialized in single-use medical devices. He joined the company in 2007. He was before R&D director of an analytical company specialized in Smart Sensing Systems. He brings 15 years of expertise in Development & Design of multi-sensor array technologies including sample preparation, selection and design of appropriate sensing technologies, software development, data processing techniques and artificial intelligence. He received an engineer diploma (1993) from ENSEIRB (National Superior School of Electronic and Radiocommunications in Bordeaux, France) as well as an MBA degree (1994). Eric Chanié was involved in coordination of European Research and Development projects related to artificial olfaction, and was a member of the scientific council of European Network of Excellence GOSPEL (General Olfaction and sensing Projects on a European Level). He holds several patents in this field.

Jérôme Launay was born the 11th of March 1975. He received the degree in electronic engineering from the Institut National des Sciences Appliquées de Toulouse" (France) in 1998. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes from the French

"Centre National de la Recherche Scientifique" (LAAS-CNRS) in 1998 and received the PhD degree from the Institut National des Sciences Appliquées de Toulouse (France) in 2001. In 2002, he became lecturer at the Université Paul Sabatier de Toulouse (France). His research activities include the development of chemical microsensors for the detection in liquid phase.

Augustin Martinez was born the 24th of May 1942. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes from the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 1966 and received his Doctorat d'Etat ès Sciences Physiques from the university of Toulouse (France) in 1976. In 1980, he became Professor at the Institut National des Sciences Appliquées de Toulouse. He has been in charge of the "Microstructures et Microsystèmes Intégrés (M2I)" group from 1992 to 1997 and has been assistant director for the LAAS-CNRS from 1997 to 2003. He is working on the development of chemical sensors.

Tables and figures caption

Table 1: specifications of the SiO_2/Si_3N_4-pH-ChemFET technology

(experimental conditions: V_{DS} = 1 V, I_{DS} = 0.1 mA, pH = 4 – T_a = 25°C)

Figure 1: mass fabrication of pH-ChemFET chips (size: 2.1 x 1.6 mm^2) in silicon foundry

Figure 2: ink jet printing of urease-rich, PVA-based enzymatic layers

for the realisation of the urea-EnFET/pH-ReFET module

Figure 3: integration of the urea-EnFET/pH-ReFET module into a specific flowcell

Figure 4: automated mass fabrication of urea-EnFET-based flow injection microsystems

Figure 5: schematics of the urea on-line monitoring in dialysis conditions

Figure 6: calibration curve of the pH-ChemFET microdevices

Figure 7: analytical response of urea in EDTA-based buffer solutions (pH = 7.4)

Figure 8: analytical response of urea in dialysate solutions (pH = 7.4)

Figure 9: analytical response of the urea microsensor in dialysis conditions

Figure 10: progress of urea concentration in dialysis conditions

Figure 11: calibration curves of the urea-EnFET/pH-ReFET microsensor for different solutions

parameter	typical values
sensitivity	53 ± 2 mV/pH
pH range	1-13
resolution	0.1 pH
linearity	0.99
leakage current	0.4 ± 0.04 μA
threshold voltage	1.54 ± 0.04 V
transconductance	0.41 ± 0.01 mA/V^2

Table 1: specifications of the SiO_2/Si_3N_4-pH-ChemFET technology

(experimental conditions: V_{DS} = 1 V, I_{DS} = 0.1 mA, pH = 4.0 – T_a = 25°C)

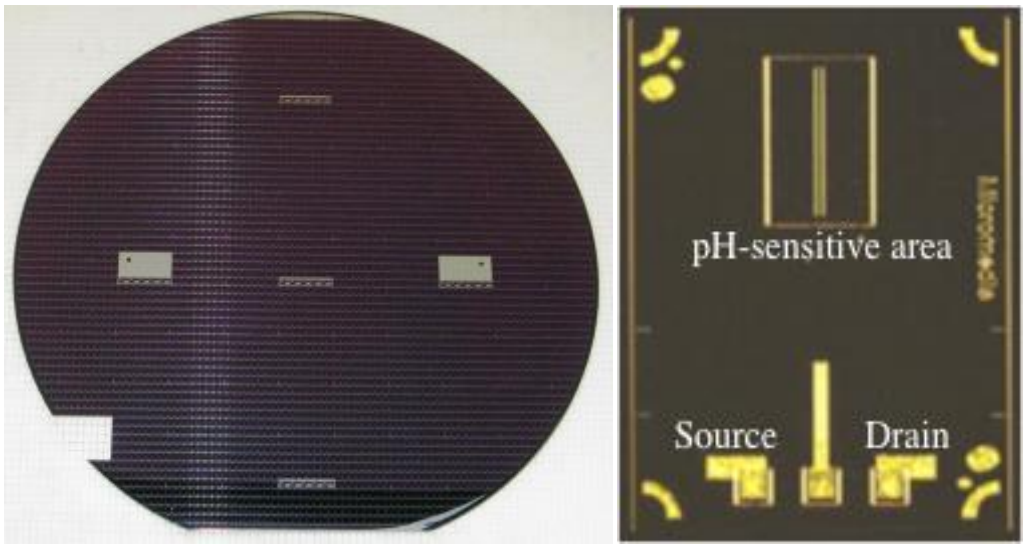

Figure 1: mass fabrication of pH-ChemFET chips (size: 2.1 x 1.6 mm^2) in silicon foundry

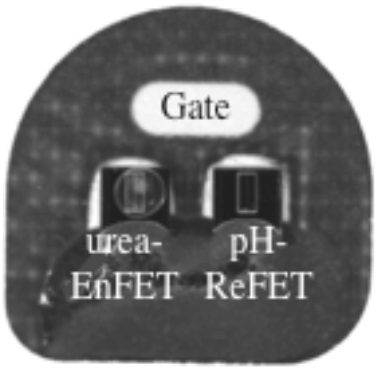

Figure 2: ink jet printing of urease-rich, PVA-based enzymatic layers

for the realisation of the urea-EnFET/pH-ReFET modules

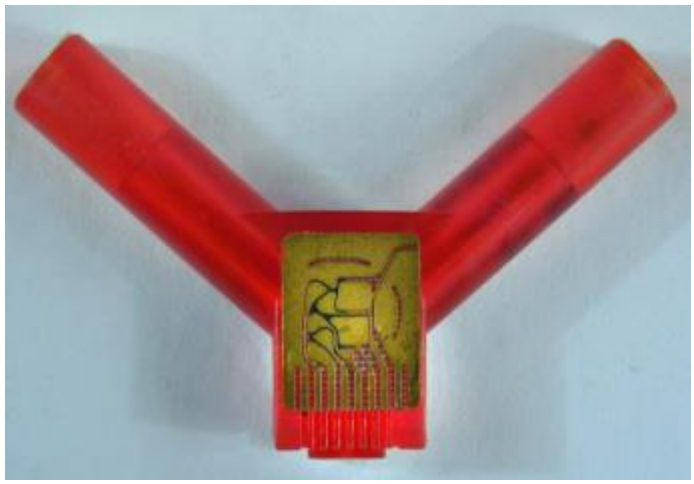

Figure 3: integration of the urea-EnFET/pH-ReFET module into a specific flowcell

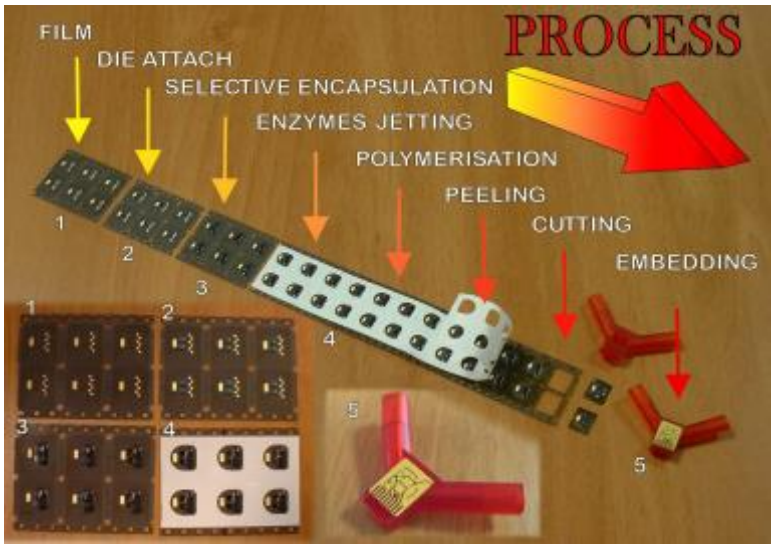

Figure 4: automated mass fabrication of urea-EnFET-based flow injection microsystems

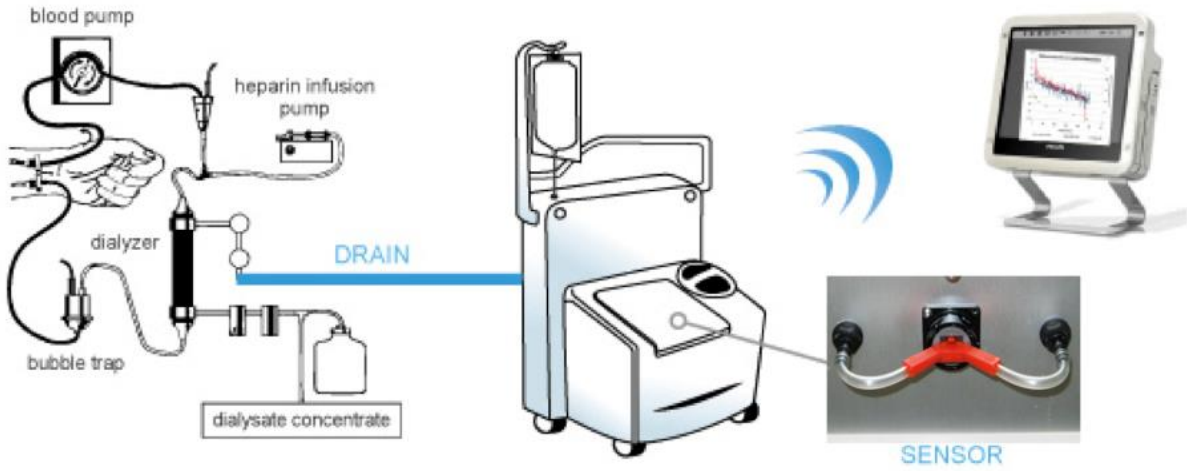

Figure 5: schematics of the urea on-line monitoring in dialysis conditions

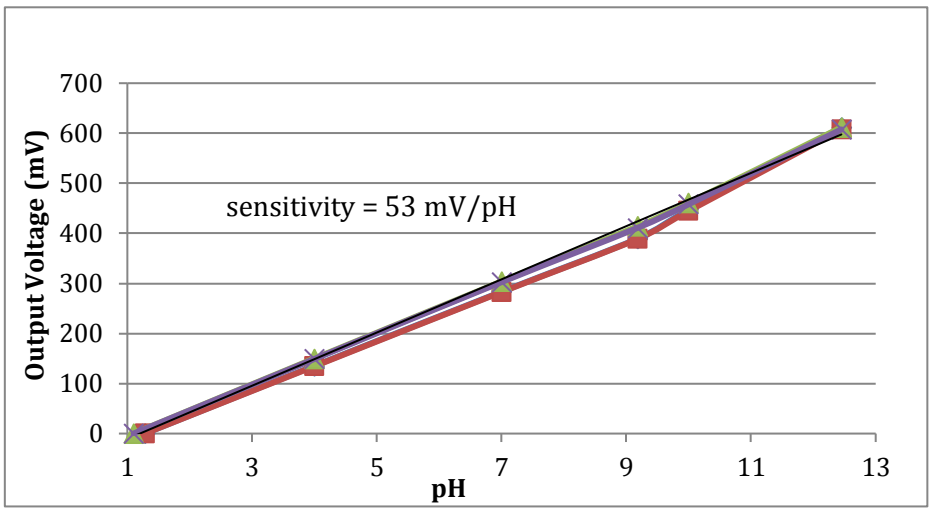

Figure 6: calibration curve of the pH-ChemFET microdevices

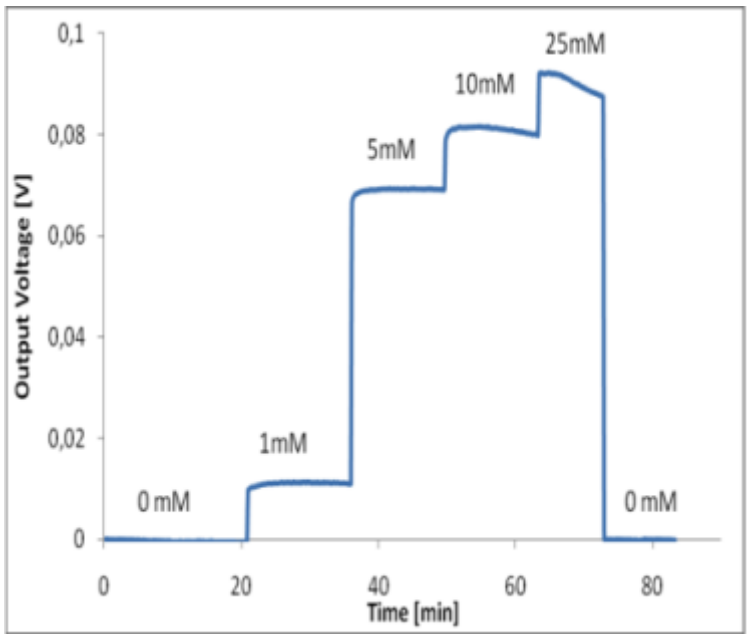

Figure 7: analytical response of urea in EDTA-based buffer solutions (pH = 7.4)

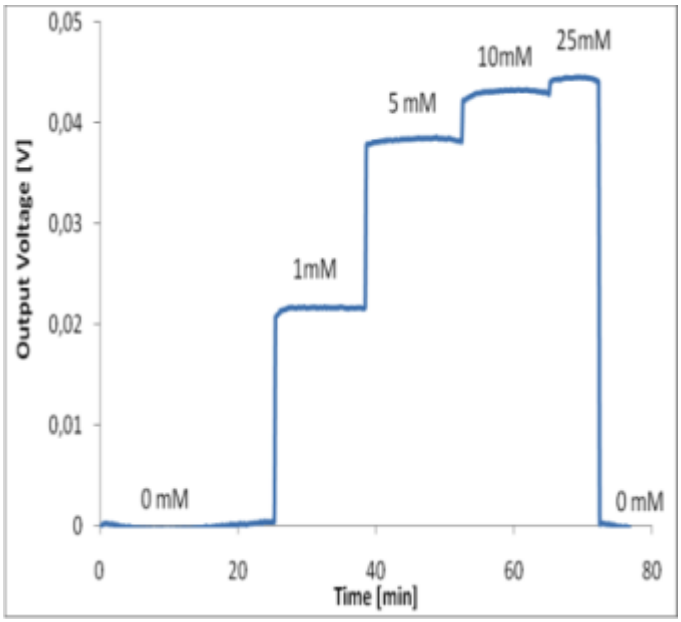

Figure 8: analytical response of urea in dialysate solutions (pH = 7.4)

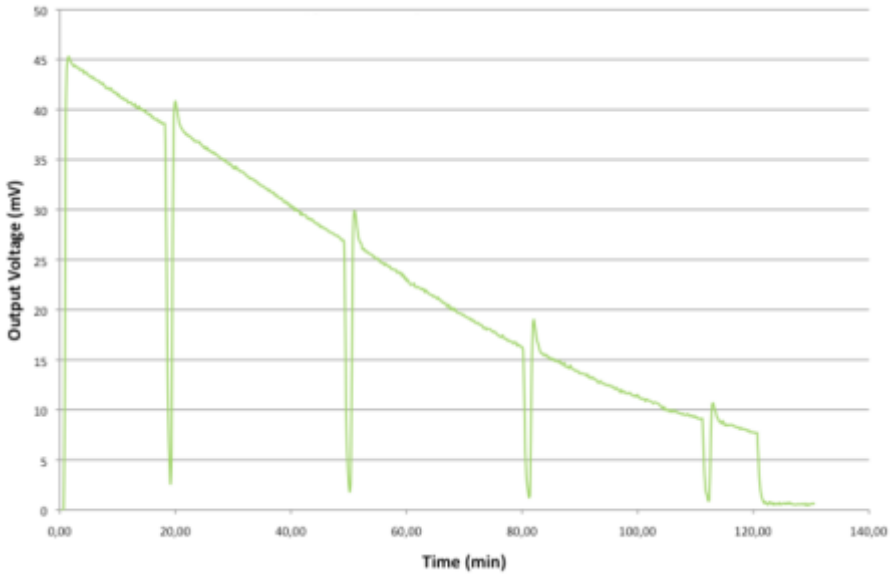

Figure 9: analytical response of the urea microsensor in dialysis conditions

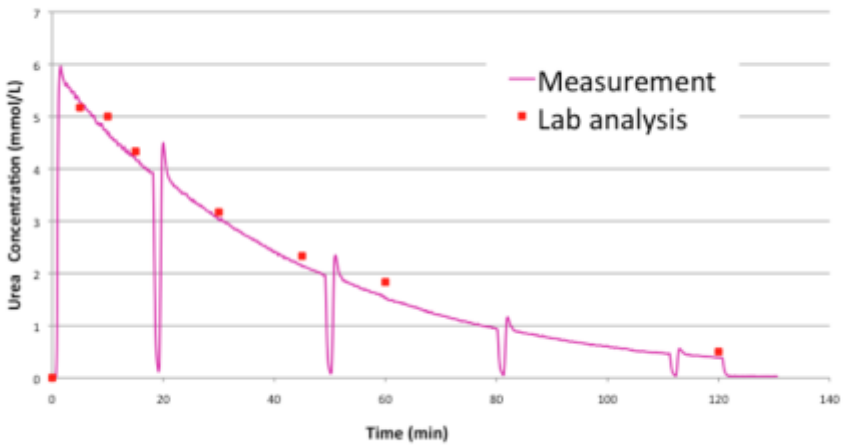

Figure 10: progress of urea concentration in dialysis conditions

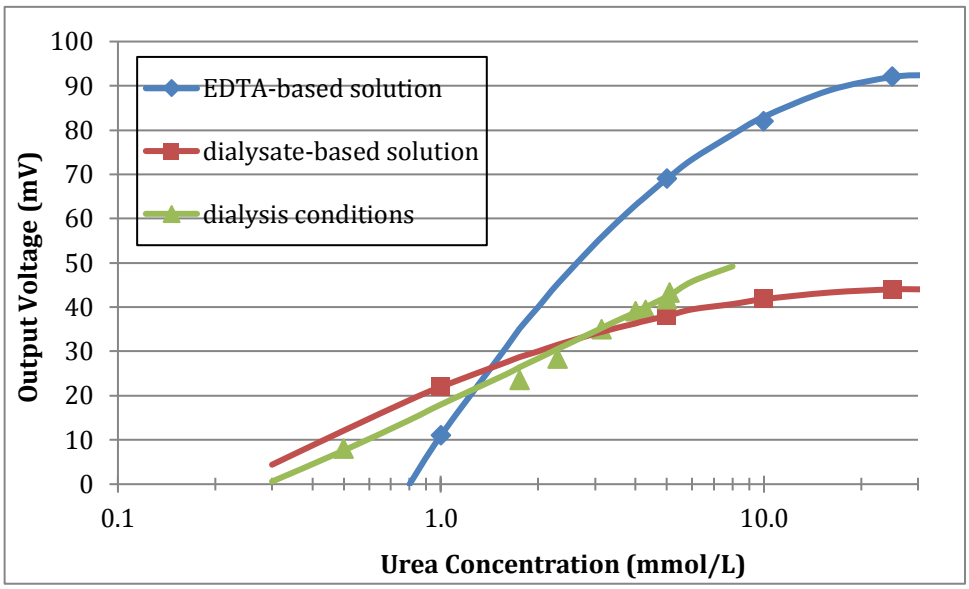

Figure 11: calibration curves of the urea-EnFET/pH-ReFET microsensor for different solutions