

Biocompatible Multielectrolyte Sensors for Artificial Kidney

Julien Fils, Frédéric Revol-Cavalier, Fabrice Navarro, Frédérique Mittler, Gilles Marchand
CEA – LETI MINATEC, 17 rue des martyrs, 38054 Grenoble Cedex 9, France

Abstract. The NEPHRON+ project aims to develop an artificial wearable kidney for hemodialyzed patients. The process and the health status of the patient are monitored measuring several electrolytes and more specifically sodium, potassium and calcium. These sensors have to be reliable for a long lifetime and to be compatible according to the highest ISO standard for medical device. This work presents the design and the integration of such electrochemical platform in an artificial organ.

Keywords: electrochemical sensor, artificial kidney, biocompatible sensor, sodium ISE, potassium ISE, calcium ISE

1. Introduction

The patients affected by renal diseases are monitored and treated in a hospital in a discontinuous manner in preset time-intervals, for patients typically 3 times a week. This results in a variety of medical drawbacks for the patient and is very costly for the health care system. The average life expectancy of an ESRD patient is only 5 (age 45-64) to 9 years (age 20-44). Furthermore, the present haemodialysis method requires a costly in hospital infrastructure with dialyser machines and water processing equipment to supply dialysate fluid. This infrastructure including the required personnel is limited and costly; therefore a bottleneck is created to treat an increasing amount of patients more frequently. The clinical practice takes years to advance and reconfigure since, it is difficult to keep and process data from multiple patients. A possible solution to these problems consists in treating and monitoring renal patients continuously via a wearable artificial kidney (Figure 1), while being mobile anywhere and anytime.

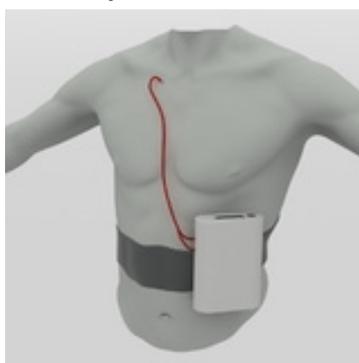


Fig 1: Sketch of the wearable artificial kidney in use

2. The NEPHRON+ project

The work described in this paper is a part of the Nephron+ project, a European Integrated Project which will provide a major leap forward in Renal Care. It aims at a next generation, integrated solution for personalized treatment and management of patients with chronic renal failure. It presents an ideal solution for continuous dialysis outside the hospital offering better blood clearance, while patients can stay mobile and active in social and economic life. It relies on an ICT-enabled wearable artificial kidney for on-body

blood purification. Thus, NEPHRON+ optimizes the renal treatment based on a closed-loop and context aware operation with following elements (Figure 2):

- the in-device autonomous and independent personalized treatment
- the patients' interaction and feedback for local control operation of his/her own treatment while also considering lifestyle aspects and
- the clinicians' monitoring and reconfiguration based on the individual patient's condition and disease evolution
- wider level feedback that incorporates the statistical/intelligent analysis of the treatment policies and the system operation by correlating multiple patients' measurements.

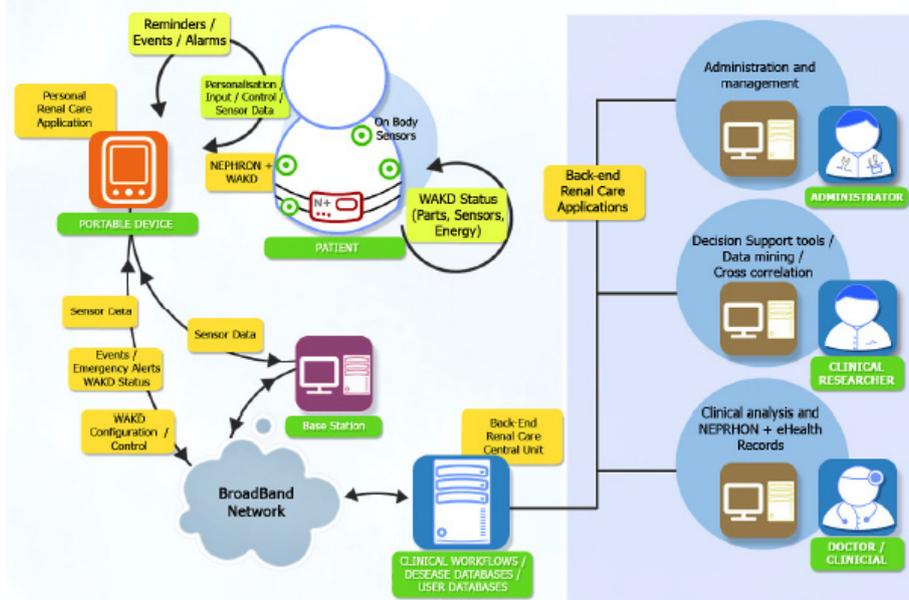


Fig 2: Overview of the NEPHRON+ project

The system allows for real-time, continuous, multiparametric (tele) monitoring of both the patient and the device via innovative sensors. Thus, a set of innovative sensors are integrated into the wearable device and worn by the patient. The continuous data collection allows for early detection of anomalies and trend analysis on the health status of the patient, offering learning curves for improved treatment. The patients benefit from lower costs, higher clearance levels, and more comfort which will result in improvement of their health condition, longer life expectancy and improved economical and social living conditions.

3. Multi-electrolyte sensor

In order to provide a personal monitoring and a feedback of the loop control, many parameters are monitored among them, the electrolytes concentrations (potassium, sodium and calcium) are essential information. In order to embed all these sensors in the device, many improvements have been done to miniaturize the size of the sensors and also of the electronic board which converts the signal in a ready-to-use concentration.

3.1. Electrochemical platform

The sensors are all based on a resting potential measurement. It means that a potential is measured between two electrodes: the first one is a reference electrode - in our specific case an Ag/AgCl electrode - and the second one is an ISE (Ionic Specific Electrode). The ISE membranes are all PVC-based membrane which allows getting a good electrochemical response but also presents a good biocompatibility. The sensors are located on a unique Printed Circuit Board (PCB) platform. This unusual substrate has many advantages. It is *i/* commercially available, *ii/* easy to produce, *iii/* cheap and *iv/* some electronic components can be added as an EPROM to store parameters (calibration curves). This electrochemical platform (Figure 3) is integrated in the filtration circuit of the artificial kidney before and after the sorption unit through two fluidic chambers. The electrochemical sensors are controlled by a unique electronic board to constitute the overall system.

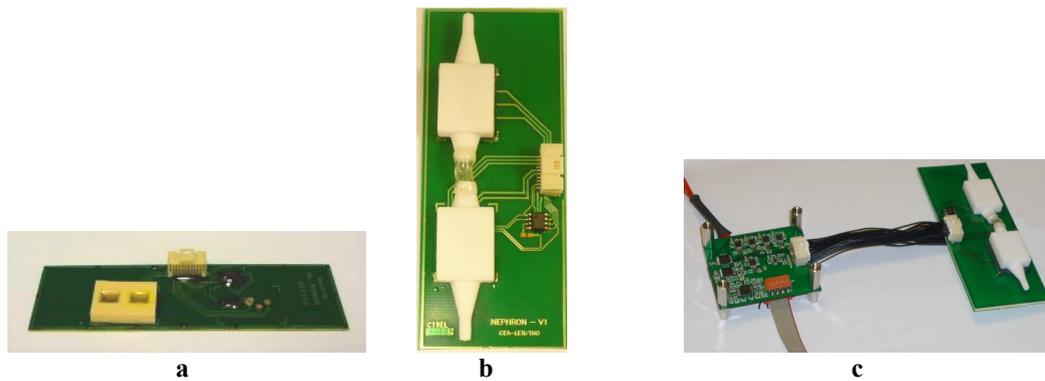


Fig 3: a – Picture of the platform ; b Picture of the platform with the fluidic chambers; c- Picture of the overall system

3.2. Results

The accuracy (deviation from reality), the time response and the precision of each sensor shall allow getting a reliable value of the respective ion. For instance, the sodium monitoring is major issue. Indeed, if its concentration is out of the range, it may cause some heart disorder. In the following figure, the measurements of several sodium concentrations show a good stability (variation less than 1mV per hour) and a response time shorter than one minute.

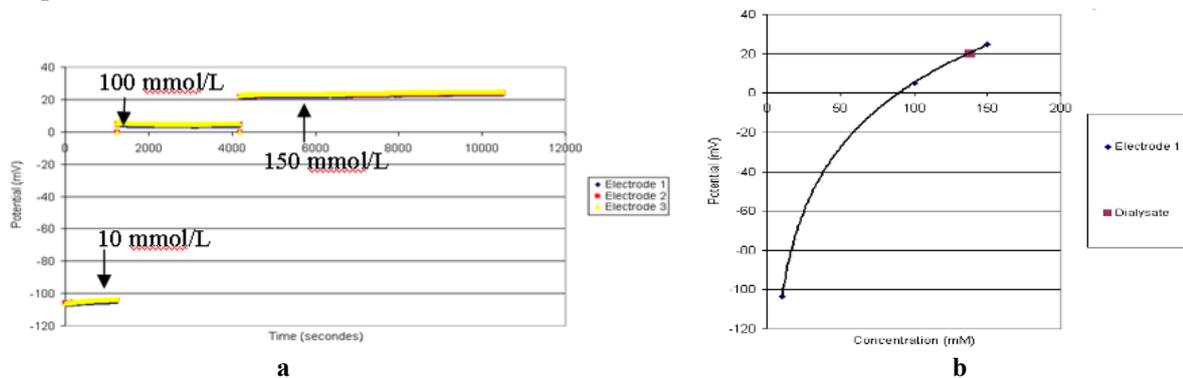


Fig 4: a- Measurement of Na⁺ concentrations with three different electrodes; b- Calibration curve of a DOS based electrode and measurement in dialysate

In order to use the sensor, a calibration curve is needed and is obtained with solutions containing different Na⁺ concentrations. For each concentration, the associated potential is plotted and a logarithmic regression gives the parameters of calibration. This regression is logarithmic since the resting potential is correlated with the Nernst equation. Finally, the potential is measured in the dialysate currently used in hemodialysis and supplied by Dirinco (ACC 2134) (plot “dialysate”). The measured value corresponds to a concentration of 138.5mmol/L while the supplier gives a concentration of 138mmol/L. It means our sensor is able to measure the Na⁺ concentration in the dialysate with reliability.

Table 1: Performances of the electrochemical sensors regarding to the medical requirements

	Na ⁺			K ⁺			Ca ²⁺		
	Salt solution	Dialysate	Requirements	Salt solution	Dialysate	Requirements	Salt solution	Dialysate	Requirements
Dynamic range	10-160mM	130-150mM	At least 135-146mM	2-12mM	1-6mM	At least 3.5-5mM	0.5 - 6mM	0.5-3mM	At least 0.8 - 3mM
Precision	1 mM	1.5 mM	1 mM	0.1 mM	0.1 mM	0.1 mM	<0.1 mM	< 0.1 mM	0.1 mM
Accuracy (deviation from reality)	±1%	±1%	±1%	±1%	±2%	±2%	±1%	±1%	±2%
Response time	<1 minute	< 3 minutes	each minute	<1 minute	< 1 minute	each minute	<1 minute	< 1 minute	each minute

All the performances of the electrochemical sensors developed in the NEPHRON+ project are reported below. The requirements are those needed by the physicians to monitor the health status. As a conclusion, it appears that the sensors fulfill the expected behavior and they are ready to be integrated in the device.

4. Biocompatible sensors

The validation of the platform biocompatibility is another key issue to be addressed, since the sensor is in the close environment to the biological fluid. Cytotoxicity assays are performed according to the guidelines of the ISO standard (ISO10993- 5). In this study, the cytotoxicity assessment of materials is considered in the same time of the ISE design for selecting the safest components for each function (polymer membrane, plasticizer, ion carrier and ion exchange) and reduces thus the overall toxicity risk.

4.1. Protocol

WST-1 assay (Roche) has been performed for different components of ion-selective membranes (Na^+ , K^+ or Ca^{2+}) on murine fibroblasts (NIH-3T3 cell line, adherent cells, ATCC N^oCRL-1658TM). Different conditions have been tested in accordance to the International Organization for Standardization (ISO) 10993 which provides a series of standards for evaluating the biocompatibility of a medical device prior to clinical testing. Basically, materials have been prepared not only for direct contact tests with an exposure time of 24h (1) but also for extraction tests using the cell culture medium as extraction vehicle (2) (see Figure 5). This second approach has been considered for assessing the cytotoxicity of potential degradation products coming from the membrane after their release into the “specific” cell culture medium.

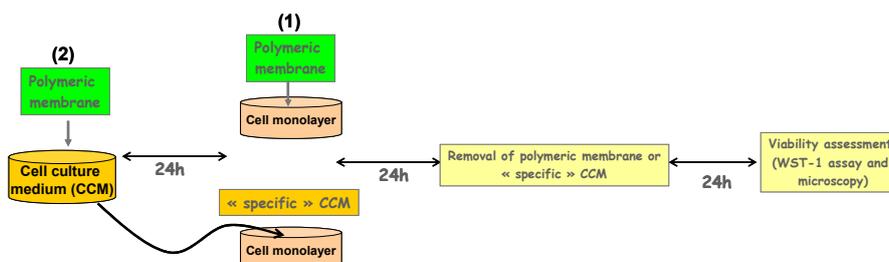


Fig.5: protocol of cytotoxicity assessment

4.2. Results

Whatever the ion carrier and ion exchange required, the ISE relies on a poly(vinyl chloride) (PVC) membrane. Depending on the plasticizer used for the preparation, either 2-nitrophenyloctyl ether (2-NPOE) or dioctyl sebacate (DOS) or dioctyladipate (DOA), the membrane elicits different toxicity profiles. The PVC-based membrane including 2-NPOE induces a severe to moderate levels of toxicity, in direct contact or extraction tests respectively (see Figure 6A). The replacement of 2-NPOE by either DOS or DOA does not modify the electrochemical features (*data not shown*); whereas the corresponding PVC-membrane seems to be better tolerated by cells in culture (more than 75% of viable cells in both conditions) (see Figure 6A).

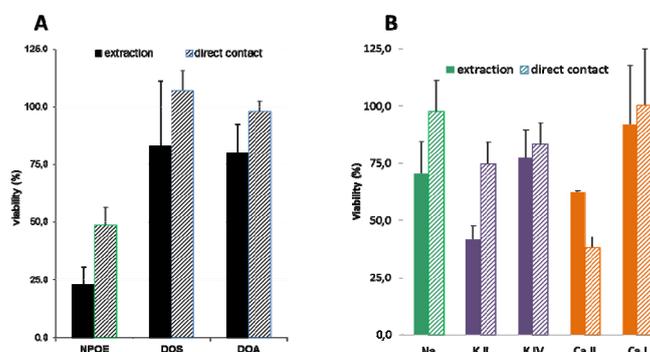


Fig. 6: Graphs showing the cell viability after extraction test (*full colored bars*) or direct contact test (*hatched bars*) for the PVC membrane depending on the plasticizer used (NPOE, DOS, or DOA) (A) or for different ion selective membranes (Na^+ , K^+ or Ca^{2+}) (B). Results are expressed as percent of negative control, a not cytotoxic surface of PDMS.

The cell line was NIH 3T3 and experiments have been performed in triplicate for each condition.

Using a PVC membrane produced with DOA, we have designed different ISE for Na^+ , K^+ or Ca^{2+} according to the ion carrier and the ion exchange used. The Na^+ selective electrode requires 4-tert-Butylcalix[4]arenetetraacetic acid tetraethyl ester and presents only a slight to moderate toxicity in extraction test, while no or marginal toxicity is observed in direct contact test (see Figure 6B). For the K^+ selective membrane preparation, we can use either Potassium ionophore II membrane named K^{II} , or Potassium ionophore IV named K^{IV} . The K^{IV} membrane presents only a slight toxicity in both conditions (extraction or contact direct) likely due to a release of degradation products in the medium. Nevertheless, this release seems to be lower or less toxic for this membrane than for the K^{II} membrane, considering the toxicity observed in extraction test for K^{II} selective substrate. The Ca^{2+} selective membrane can be prepared using either Calcium ionophore II, membrane named Ca^{II} or Calcium ionophore I, named Ca^{I} . This last membrane is better tolerated by cells (more than 90% of viable cells in both conditions) compared to the Ca^{II} membrane (moderate to severe toxicity observed in extraction and direct contact tests respectively).

5. Conclusion

As a conclusion, this paper describes the development of miniaturized electrochemical sensors used for artificial kidney monitoring. We demonstrated that the performances of the developed sensors are in accordance with specifications established by clinicians. Furthermore, the low cytotoxicity was demonstrated for Na^+ , K^+ (K^{IV} membrane) and Ca^{2+} (Ca^{I} membrane) sensors.

6. Acknowledgements

We thank all the partners involved in the NEPHRON+ project and specially TNO (Netherlands) and Nanodialysis (Netherlands) for their participation to the design of the electrochemical platform.

7. References

- [1] O. A. Sadik Zhouet al, Status of Biomolecular Recognition using Electrochemical Techniques – *Biosensors & Bioelectronics*, 2009, **24**, 2749-2765,
- [2] R. Popovtzer, T. Neufeld, E. z. Ron, J. Rishpon, and Y. Shacham-Diamand, Electrochemical detection of biological reactions using a novel nano-bio-chip array, *Sensors and Actuators B: Chemical*, 2006, **119**, 664-672,
- [3] U. Guth, F. Gerlach, M. Decker, W. Oelßner and W. Vonau, Solid-state reference electrodes for potentiometric sensors, *Journal of Solid State Electrochemistry*, 2009, **13**, 27-39
- [4] K. Kimura et al., Effects of -Substituents on Ion Selectivity of Bis(12-crown-4-methyl) Malonates as Neutral Carriers for Sodium Ion-Selective Electrodes, *Anal. Sci.* 1996, **12**, 67
- [5] Bedlechowicz-Sliwakowska I, Lingenfelter P, Sokalski T, Lewenstam A, Maj-Zurawska M., Ion-selective electrode for measuring low Ca^{2+} concentrations in the presence of high K^+ , Na^+ and Mg^{2+} background, *Anal. Bioanal. Chem.* 2006, **385**(8), 1477