Optical sensor for interstitial pH measurements

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Abstract. An optical fiber sensor for measuring the pH in interstitial fluid is described. Microdialysis is the approach followed for extracting the sample from the subcutaneous adipose tissue. The interstitial fluid drawn flows through a microfluidic circuit formed by a microdialysis catheter in series with a pH glass capillary. The pH indicator (phenol red) is covalently immobilized on the internal wall of the glass capillary. An optoelectronic unit that makes use of LEDs and photodetectors is connected to the sensing capillary by means of optical fibers. Optical fibers are used to connect the interrogating unit to the sensing capillary. A resolution of 0.03 pH units and an accuracy of 0.07 pH units are obtained. Preliminary *in vivo* tests are carried out in pigs with altered respiratory function. [DOI: 10.1117/1.2714807]

Keywords: pH; optical sensor; interstitial fluid; microdialysis.

Paper 06179R received Jul. 4, 2006; revised manuscript received Dec. 18, 2006; accepted for publication Dec. 18, 2006; published online Mar. 22, 2007. This paper is a revision of a paper presented at the SPIE conference on Advanced Environmental, Chemical, and Biological Sensing Technologies III, Oct. 2005, Boston Mass. The paper presented there appears (unrefereed) in SPIE Proceedings Vol. 5993.

1 Introduction

The continuous monitoring of interstitial metabolites contained in the adipose tissue of intensive care patients is one of the objectives of the 4-year CLINICIP (closed loop insulin infusion in critically ill patients) European project.

Critically ill conditions may cause atypical physiological symptoms even in people without chronic pathologies.[1] The pH is an informative indicator of the conditions of a living system and can be valuable in determining the physiologic status of critically ill patients.

It is generally measured along with pO_2 (partial pressure of oxygen) and pCO_2 (partial pressure of carbon dioxide) on demand by drawing blood samples and utilizing laboratory instrumentation. The requirement for very frequent and/or continuous monitoring of these parameters has led to the development of optical fiber sensors for blood pH,[2] oxygen,[3] and carbon dioxide.[4] An intravascular catheter for the simultaneous measurement of pH, pO_2, and pCO_2, based on a system designed and tested by Gehrich et al.,[5] was developed by Cardiovascular Devices (CDI). The intravascular sensor consisted of a 0.6-mm catheter containing three 125-μm optical fibers with the sensing chemistry immobilized at their tips. Problems emerged during clinical trials on volunteers in critical care due to possible clot formations around the sensor tips, but mainly due to the so-called wall effect. The latter primarily affects oxygen and carbon dioxide measurements and is caused by a diffusion gradient of these parameters from the blood vessel wall toward its center. These problems implied inadequate performances of the CDI intravascular catheter, which in fact never reached the market. Therefore, CDI developed an extravascular system for extracorporeal blood circuit. In this system, a disposable cartridge containing the chemistry for the pH, pO_2, and pCO_2 and on line with the blood circuit is connected to the interrogating unit via an optical fiber cable. The system is currently commercialized by Terumo[6] and it is mainly used in open-heart operations.

Other intravascular probe systems were proposed by Puritan Bennett, Optex Biomedical, and Diametrics,[7] with a probe structure that is essentially similar to the CDI intravascular probe previously described. The intravascular probe developed by Diametrics (Paratrend) was also widely used for the invasive measurement of human tissues.[8–10] All these systems have been available on the market for a more or less long time, but currently none are available, with the Paratrend system being the last one to be withdrawn from the market in 2005.

Recently, subcutaneous adipose tissue has been proposed as a promising site for the continuous measurement of glucose in diabetic patients,[11–14] and whether this location could also be appropriate for the analysis of pH, pO_2, and pCO_2 is under debate.[15–17] The microdialysis method is becoming more and more important as a reliable way to examine the interstitial space of intact tissue.[18–20] The microdialysis catheter mimics a blood capillary, and molecules characterized by a small molecular weight can diffuse across the microdialysis membrane and equilibrate with a perfusion solution. The resulting fluid samples (dialyzate) are generally collected for off-line analysis by means of external instrumentation properly designed for rapid analysis of small volumes[21–23] (less than microliters), but have also been developed and miniaturized properly for online continuous monitoring.[24,25] This approach is less invasive than the intravascular approaches, but it is also really promising in the cases where the blood loss from diagnostic

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1083-3668/2007/12(2)/024024/7/$25.00 © 2007 SPIE

samples can give rise to critical situations, such as in hospitalized infants or in intensive care patients.[22]

Here, a continuous measurement of pH is proposed, based on the withdrawal of interstitial fluid from adipose tissue by means of a biocompatible microdialysis catheter. The interstitial fluids drawn flow through a microfluidic circuit formed by the catheter in series with a glass capillary with the pH indicator immobilized on its internal wall. Absorption is the optical working principle used for the pH detection. The proposed sensor was carefully characterized in the laboratory. Preliminary *in vivo* tests on animals are described.

2 Materials and Methods

2.1 *Chemical Protocols*

The sensing layer of the pH sensor is constituted by phenol red, which is covalently bound to the glass surface by means of the Mannich reaction.[26] This reaction consists of the condensation of formaldehyde with the primary amines of the silane groups immobilized on the glass surfaces and the active hydrogens of the phenol red. Phenol red (Sigma) was covalently immobilized on a glass capillary (length, 10 cm; ∅, 0.5/1.0 mm) (Drummond Scientific Company), which was connected to a peristaltic pump (Minipuls3-Gilson) by means of PVC tubing (Gilson) for all the functionalization and experimental steps. The glass capillary was previously treated with an acidic solution of 2-(N-Morpholino)ethanesulfonic acid (MES, Sigma) 0.1 M at pH=4.7 for 10 min at 25 °C. Activation of the glass surface was then carried out with a solution of 10% [percent volume in volume (v/v)] 3-aminopropyl-trimethoxysilane (APTS) in acetone (Sigma) for 12 h at 65 °C. The concentration of APTS was varied between 5 and 50% (v/v). After this treatment, it was washed for 10 min with MES to remove the unreacted APTS. Formaldehyde [37% (Sigma)] was then allowed to flow through the capillary for 10 min, and a 10-μM solution of phenol red in MES for a further 10 min. The capillary was left for 12 h at 25 °C, and was then washed with MES for 5 min. Before their utilization, the capillaries were further washed in a continuous flow with the buffer solution at pH 7.0 for 3 to 5 h to remove all excess dye that was not covalently bound.

McIlvaine buffers at different pH values (ionic strength 1 M) were prepared by mixing citric acid and sodium phosphate dodecaidrate in appropriate proportions. Potassium chloride was added to obtain the requested ionic strength.[27]

Buffers at different ionic strengths (0.005 to 1 M) were Britton-Robinson buffers, prepared by mixing acetic acid, phosphoric acid, and boric acid to obtain a 0.04-M solution. The pH and the ionic strength were adjusted with NaOH 0.2N and potassium chloride, respectively.[28] In the microdialysis experiments, we used 5% [percent weight in volume (w/v)] mannitol (Merck) and Ringer solution (Fresenius) containing sodium chloride (8.6 g/L), potassium chloride (0.3 g/L), and calcium chloride (0.33 g/L).

2.2 *Optoelectronic Instrumentation*

An optoelectronic unit for interrogation of the pH sensor immobilized on a capillary was developed. Optical fibers (core diameter, 200 μm) were used to couple the capillary with the unit. The capillary was placed inside a black plastic piece that has precise holes for the two fibers on its lateral surface. These were used to carry the light from the source to the capillary and to collect the modulated light, respectively (Fig. 1). The optoelectronic unit used a light-emitting diode at 590 nm as a source (Ledtronics, L200CWY3KB) and a photodiode as the detector (OPT 101, Burr-Brown). The LED was alternately driven at two current levels so that it emitted at two slightly different wavelengths. The detected signals were processed to measure the slope of the absorption band. The LED was chosen at a wavelength close to the inflection point of the absorption spectrum to obtain the best sensitivity. This signal modulation and data processing made it possible to avoid the interferences coming from source fluctuations, air microbubbles, and any other common-mode down-lead fluctuations.

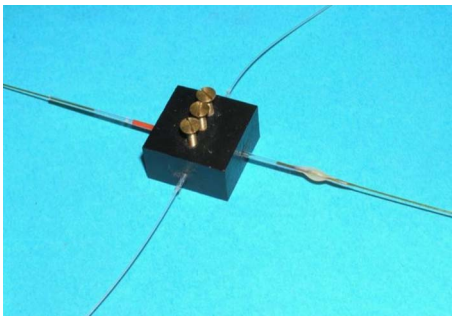

Fig. 1 Photo of the optical flow cell for the interrogation of the pH-sensing capillary.

2.3 *Fluidic Systems*

Two different fluidic systems were used, the first one for the characterization of the sensing capillary in the lab, and the other for the measurement on animals.

In the characterization of the sensing capillary (Fig. 2), the pH sensor was connected to the peristaltic pump, and the fluidic line went from the buffer solutions directly to the sensing capillary and then to the waste container. A six-way selection valve (Upchurch Scientific) was used to change the sample flowing through the fluidic line. PVC tubing (internal diameter 0.5 mm, Gilson) was used for the connections.

Figure 3 shows the setup for microdialysis measurements. The peristaltic pump was used in a push-pull configuration to cause the sample to flow through the microdialysis catheter (CMA 60, CMA Microdialysis AB). Fused silica capillaries (internal diameter 0.32 mm, Postnova Analytics) were used to connect the CMA 60 catheter and the sensing capillary in the tests with animals.

3 Results and Discussion

3.1 *Laboratory Characterization*

Sensing capillaries were manufactured with an APTS concentration that ranges from 5 to 50%. The concentration of the silylating agent plays a key role in the final pH working range of the sensor. The pK of the bound dye was evaluated by means of the theoretical sigmoid curve, according to the equation

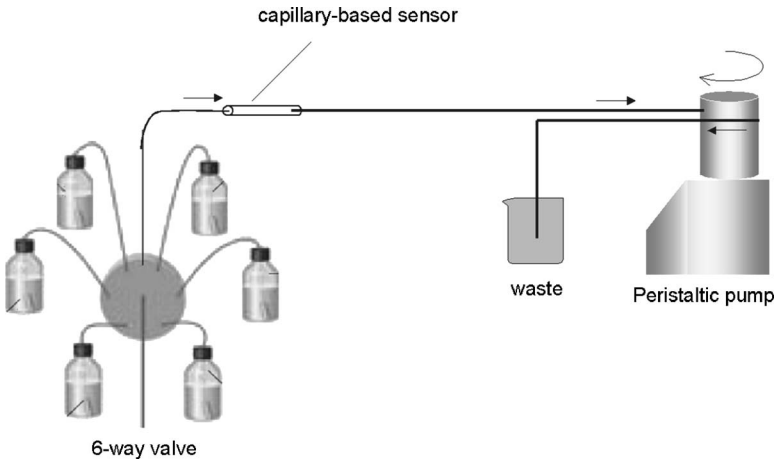

Fig. 2 Setup of the fluidic system for the characterization of pH-sensing capillary.

$$S = S_0 + \frac{a}{1 + \exp\left[-\frac{\mathrm{pH} - \mathrm{pK}}{b}\right]}, \quad (1)$$

where S is the measured signal, pK is $-\log K$, K is the dissociation constant of the immobilized indicator, S_0, b, and a are constants, and S_0 and S_0+a are the values of the measured signal in correspondence with the minimum absorption ($\mathrm{pH} \ll \mathrm{pK}$) and maximum absorption ($\mathrm{pH} \gg \mathrm{pK}$), respectively.

Table 1 shows the pK and the working range of phenol red after immobilization for the different concentrations of APTS used. A shift of the pK toward a lower pH was observed in correspondence of an increase of the APTS concentration.

The capillaries obtained exhibited an excellent sensitivity and stability. Figure 4 shows the long-term response curve of a 30% APTS capillary, and the relative calibration curve ($\mathrm{pK}=5.56$) is shown in Fig. 5. The different pH steps, repeated after 70 h of continuous measurements, evidenced only a small drift. This drift can be ascribed to both a small leakage of the dye from the chemical sensing layer and to the optoelectronic components. The contribution of the sensing chemical layer to the drift is given by the absolute decrease of the signal change between pH 4.0 and 8.0 observed in the two calibration curves carried out at the beginning of the measurement and after 70 h, whereas the shift upward of the baseline can be ascribed to a drift of the optoelectronic components, presumably the LED. Notwithstanding the observed drift, the performance of the sensor in the linear range was still satisfactory with a sensitivity and an accuracy of 0.03 and 0.07 in the linear range. The effect of the drift caused by the leakage of the dye can be diminished by a more prolonged washing of the sensing capillary after its preparation used to remove all the excess of the not covalently bound dye. At the same time, the drift due to the optoelectronic components can be decreased by a better stabilization of the temperature of the sources and of the detector.

On the basis of the clinical requirements for intensive care, which call for a working range of between 6 and 8 pH units, a 10% concentration of APTS was used in the preparation of the capillaries. Figure 6 shows the typical response curve for different pH steps, and the related calibration curve of a 10% APTS sensing capillary is showing in Fig. 7. The fitting with the theoretical curve, as given in Eq. (1), was excellent with a correlation coefficient of 0.999.

Table 2 shows the characteristics shown by the pH sensor in the laboratory characterization as compared with the requirements for its utilization in intensive care units. The reproducibility of the sensing capillary is also quite good. Roughly 50% of the produced capillaries following the procedure described in Sec. 2.1 showed a performance in agreement with the data given in Table 2. A calibration procedure to be performed before each measurement enabled us to render the variability among the different capillaries irrelevant.

An important aspect that must always be considered when analyzing the performance of an optical pH sensor is the effect that a change in the ionic strength causes in the accuracy

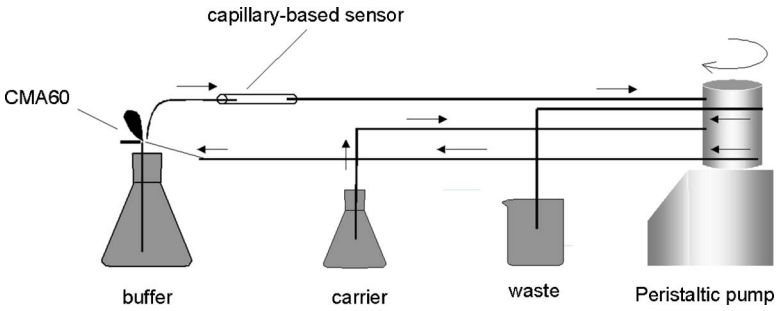

Fig. 3 Setup of the fluidic system for the microdialysis measurements.

Table 1 Characteristics of the pH-sensitive glass capillary for different concentrations of APTS.

APTS Concentration (%)	pK	Working Range
5	7	6–8
10	6.8–7.2	5–8.5
30	5.5–5.8	4–7
50	4.9–5.0	3.5–6.5

of the optical sensor. In fact, there is an intrinsic inaccuracy in any optical measurement of pH, which is based on a measurement of the concentration of hydrogen ions, whereas the pH value is related to their activity. This aspect is very often underestimated, if not completely disregarded, leading to totally wrong accuracies.[29]

The effect of the ionic strength was carefully investigated and five different series of Britton-Robinson buffers at different ionic strengths were prepared (0.005, 0.02, 0.1, 0.5, and 1 M). Figure 8 shows the calibration curves for the five different ionic strengths. The rather extensive differences clearly show that a knowledge of the ionic strength of the interstitial fluid is extremely important to obtain a reliable pH measurement.

3.2 *In Vivo Tests*

After this laboratory characterization, the sensing capillary was connected to the CMA 60 microdialysis catheter, following the configuration already shown in Fig. 2. The utilization of the peristaltic pump in the push-pull configuration minimized the problem of the formation of air microbubbles along the flow line. The tubing of the CMA 60 catheter was connected to the sensing capillary by means of a fused silica capillary (Fig. 9). The use of a fused silica capillary prevents the diffusion of gas from the external environment inside the flow line, which would alter the pH value of the flowing samples, especially in the *in vivo* tests. In fact, in these tests, the drawn samples are characterized by a much higher partial pressure of carbon dioxide in comparison with what is present

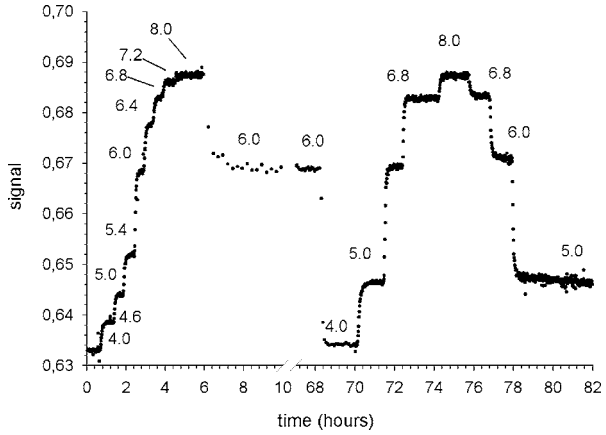

Fig. 4 Long-term response curve of a 30% APTS sensing capillary. The numbers in the graph are the pH values of the McIlvaine buffers.

Table 2 Comparison between the performance of the realised sensor and the requirements for the application in intensive care units.

	Clinical Requirements		
	Minimum	Optimum	Sensor Characteristics
Working range	6.5 to 7.6	6.0 to 8.0	6 to 8 pH units
Resolution	0.03	0.01	⩽0.03 pH units
Accuracy	0.2	0.04	⩽0.07 pH units
Response time	10 min	1 min	⩽5 min
Sampling time	15 min	continuous	5 min
Working temperature	15 to 40 °C	15 to 40 °C	15 to 40 °C
Operation time	3 days	7 days	3 days

Minimum requirements are minimum characteristics that the final sensor(s) should meet to perform the clinical tests and optimum requirements are the ideal characteristics of the sensor(s) that would perfectly satisfy all of the clinical requirements

in the normal atmosphere. The use of gas-permeable tubings would imply a diffusion of the atmospheric CO_2 inside the tubing with a consequent decrease in the dissolved carbonic acid in the dialyzed solution (perfusate) and an increase in pH.

The recovery rate for the pH through the CMA 60 catheter was measured. The recovery rate, which is an indication of the diffusion of a compound through the microdialysis catheter, is given by the ratio between the concentration of the compound in the perfusate considered and its concentration in the analyzed medium. This value clearly depends on the porosity of the membrane (cutoff approx. 20,000 Da) and on the flow rate: the higher the flow rate, the lower the recovery rate, since there is insufficient time for the diffusing material

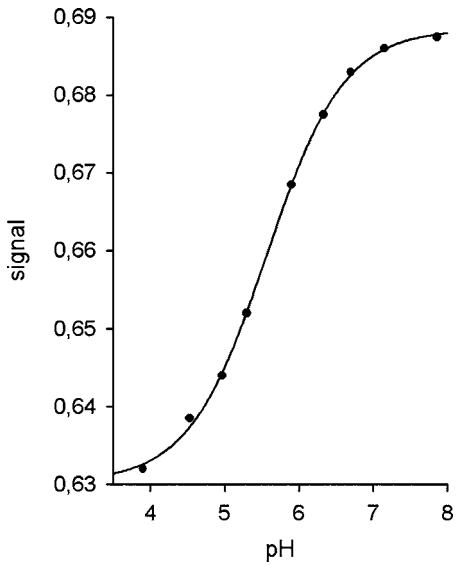

Fig. 5 Calibration curve of the 30% APTS sensing capillary, the response curve of which is shown in Fig. 4.

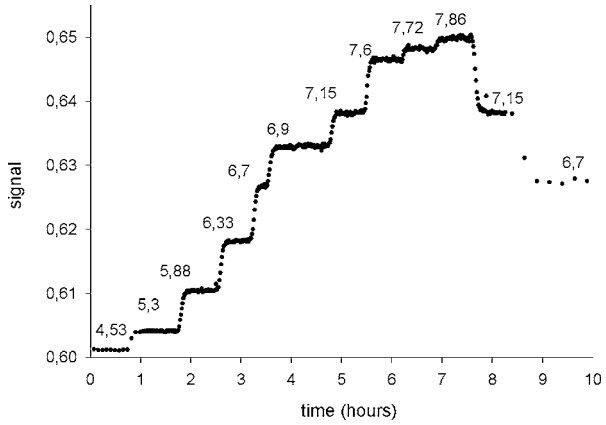

Fig. 6 Response curve of a 10% APTS sensing capillary. The numbers in the graph are the pH values of the McIlvaine buffers.

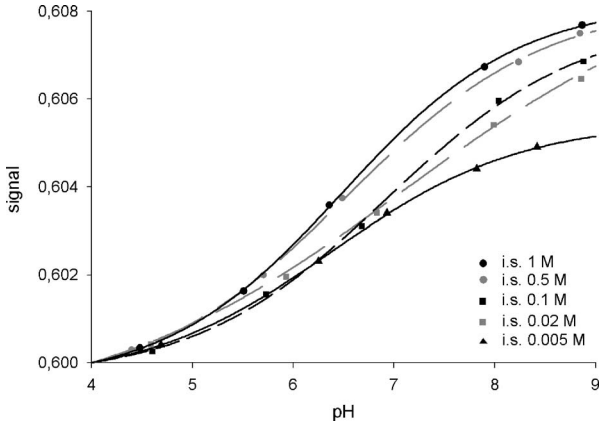

Fig. 8 Calibration curves at different ionic strength obtained with the Britton-Robinson buffers.

through the membrane to equilibrate with the solution that enters the microdialysis catheter, which is generally denoted as the carrier. A solution of 5% mannitol was used as the carrier solution, and the CMA 60 catheter was dipped in buffer solutions at different pH values. The use of mannitol as the carrier solution was justified by the fact that this solution is characterized by the absence of any type of ions. In this way, the pH of the perfusate is completely determined by the ions that diffuse through the membrane of the CMA 60 catheter. The dialyzed sample was collected in a small container and its pH was measured and compared with that of the sample. The pH values of the sample and of the perfusate were determined using a glass electrode. A flow velocity of 5 μL/min was used, which made it possible to obtain, in a reasonable time, a volume of liquid in the container that could be measured with the glass electrode. Table 3 shows the results obtained for different pH steps. As we can see, the recovery rate decreases with an increase in pH, from 100% at a pH of 5.26 down to 72% at a pH of 8.00. This behavior can be ascribed to the asymmetry of the dialysis membrane, which could cause a different inward and outward diffusion of the hydrogen ions. In any case, note that a recovery of 72% at pH 8 corresponds to a change of only 0.14 pH units. The fact that the *in vivo* measurements are carried out with a flow velocity of 1 μL/min (five times slower than the flow velocity utilized), in accordance with the physician's recommendations, implies that the error in the pH measurements due to the recovery of the hydrogen ions through the CMA 60 catheter can be considered negligible.

Dialyzed blood samples were provided by the Institute of Medical Technologies and Health Management of Joanneum Research (Graz, Austria). The pH of the dialyzed samples received was always around 9. As already discussed, this was due to the fact that the carbon dioxide dissolved in the sample passes immediately to the gas phase since the concentration of CO_2 in the atmosphere is very low in comparison with that in blood samples. To have dialyzed blood samples with pH values within the range of interest (6 to 8 pH units), the pH of the dialyzed samples was adjusted with the McIlvaine buffer solutions. Exposure to dialyzed blood did not cause any damage

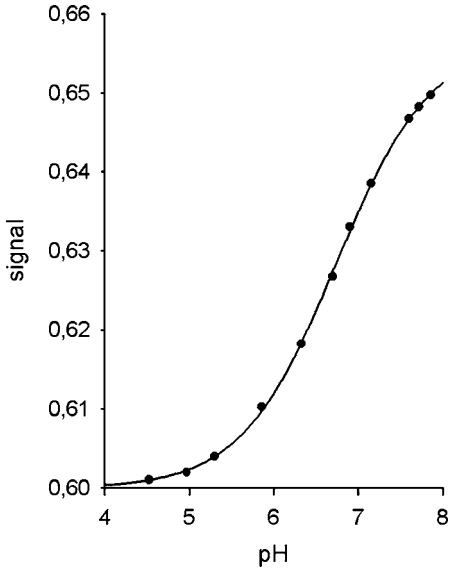

Fig. 7 Calibration curve of the 10% APTS sensing capillary, the response curve of which is shown in Fig. 6.

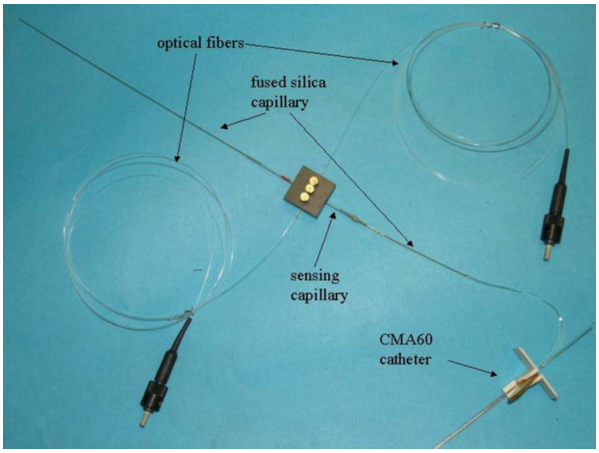

Fig. 9 Particular of the fluidic system for the microdialysis measurements, showing the flow cell, the optical fibers, and the CMA 60 catheter.

Table 3 *In vitro* recovery rate of the CMA 60 for pH at a flow velocity of 5 μL/min.

pH of the Sample	$[H^+]$	pH of the Dialyzed Solution	$[H^+]$	ΔpH	Recovery Rate (%)
5.26	5.49×10^{-6}	5.26	5.49×10^{-6}	0	100
5.84	1.44×10^{-6}	5.80	1.58×10^{-6}	0.04	91.1
6.80	1.58×10^{-7}	6.75	1.78×10^{-7}	0.05	88.9
8.04	9.12×10^{-9}	7.90	1.26×10^{-8}	0.14	72.4

to the sensing layer, which maintained its sensitivity unaltered, as we can see in Fig. 10, which shows two cycles with buffer solutions before and after a 3-h exposure to dialyzed blood.

In vivo tests were carried out at the Ludwig Boltzmann Institut (Lorentz Böhler Krankenhaus) in Vienna, in cooperation with Dr. Martin Kaipel. Measurements were carried out on pigs with an altered respiratory function after the injection of 10 μL/kg of endotoxin. Ringer solution was used as the carrier in these tests.

Figure 11 shows the response curve of the pH sensor over a period of 18 h, the duration of the experiment. The pH tracing is drawn as a broken line joining the small black dots, which are the measured values every 5 min. The pH values of arterial and venous blood, measured with a bench-top analyzer every hour, are also shown. The four dashed lines indicate the times at which the pig was subjected to lung lavages. A monotone decrease during the first 3 h of the experiment was clearly observed, and this trend was in agreement with the critical condition of the pig after the injection of the endotoxin. The noticeable increase in pH after the fourth lung lavage could be ascribed to a temporary recovery of the respiratory function.

The main problem in the *in vivo* pH measurement was the lack of a gold standard with which the obtained measurement could be compared. As already mentioned, no pH sensors are available for *in vivo* tissue measurement in living beings ever since Paratrend was withdrawn from the market.

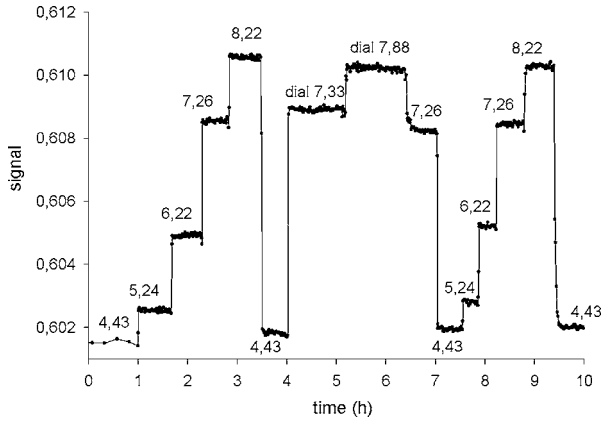

Fig. 10 Effect of a 3-h exposure of the pH sensing capillary to dialyzed blood; the pH values of the buffer and of the dialyzed blood are shown.

The correct working of the sensor was evaluated by comparing the reading of the pH sensor in the presence of McIlvaine solutions at different pH values as carriers before, during, and after the *in vivo* measurement. During the flow of buffers, the flow rate was kept high to maintain the recovery rate practically equal to zero. Figure 12 shows the tracing in the presence of the buffer solutions during the measurement and after the end of the measurement. The values of the detected signal when buffers at pH 5.4, 7.4, and 8.0 flowed through the sensing capillary were practically the same, showing that neither drift nor biological fouling perturbed the measurement.

The fact that the ionic strength of the perfusate is unknown implies that the right calibration curve to be used to obtain the true measured pH value is unknown. Therefore, the *in vivo* pH measurements can currently provide information only on the trend of the pH, but not on the real pH value of the interstitial fluid.

4 Conclusions

A sensor for the measurement of the interstitial pH was developed. This sensor was characterized by an accuracy of 0.07 pH units in the 6 to 8 pH unit range in the laboratory tests. First, *in vivo* measurements were carried out on pigs, and the results were encouraging. Further steps imply (1) the measurement of the concentration of the main ionic species present in the perfusate (e.g., sodium and calcium) from which a rough evaluation of the ionic strength can be made; and (2) the design of a microfluidic line containing both the

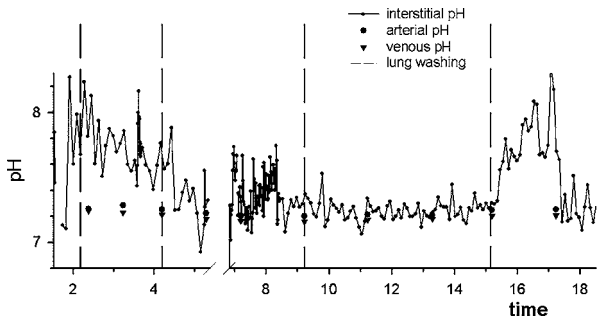

Fig. 11 *In vivo* measurement on a pig with altered respiratory function. Values of arterial and venous blood measured with a bench-top analyzer are also shown. The dashed lines indicate the times at which the pig was subjected to lung lavages.

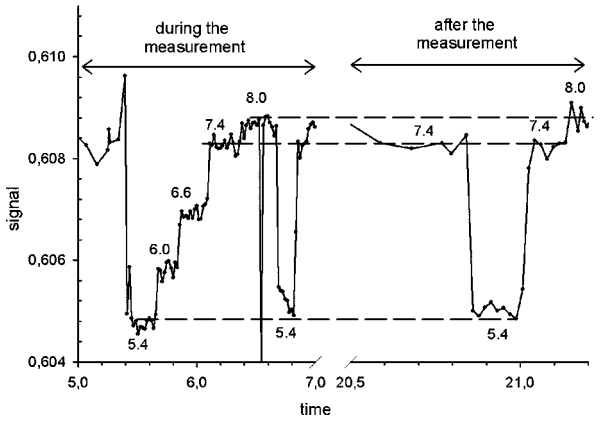

Fig. 12 Tracing of the pH sensor in the presence of the McIlvaine buffers as carriers in the middle of the *in vivo* measurement and at the end of the measurement; numbers in the tracing indicate the pH values of the McIlvaine buffers flowing through the sensing capillary.

pH sensing capillary and an appropriately designed glass microelectrode capable of measuring the correct pH value.

Acknowledgments

This research study was supported by the European Community (EU) within the framework of the 4-year EU funded project under Information Society Technologies (IST) priority, CLINICIP (closed loop insulin infusion for critically ill patients). The authors wish to thank Hans Kohler and Lukas Schaupp, institute of Medical Technologies and Health Management, Joanneum Research, Graz, Austria, for fruitful discussion during characterization of the pH sensor in line with the CMA 60 catheter, and Dr. Martin Kaipel and Dr. Esther Wassermann at the Ludwig Boltzmann Institut, Lorentz Böhler Krankenhaus, Vienna, Austria, for their collaboration during the tests on pigs.

References

1. J. Takala, E. Ruokonen, N R. Webster, M. S. Nielsen, D. F. Zandstra, G. Vundelinckx, and C. J. Hinds, "Increased mortality associated with growth hormone treatment in critically ill adults," *N. Engl. J. Med.* **341**, 785–792 (1999).
2. J. I. Peterson, S. R. Goldstein, and R. V. Fitzgerald, "Fiber optic pH probe for physiologic use," *Anal. Chem.* **52**, 864–869 (1980).
3. J. I. Peterson, R. V. Fitzgerald, and D. K. Buckhold, "Fiber-optic probe for in vivo measurement of oxygen partial pressure," *Anal. Chem.* **56**, 62–67 (1984).
4. G. G. Vurek, P. J. Feustel, and J. W. Severinghaus, "A fiber optic pCO_2 sensor," *Ann. Biomed. Eng.* **11**, 499–510 (1983).
5. J. L. Gehrich, D. W. Lubbers, N. Opitz, D. R. Hansmann, W. W. Miller, J. K. Tusa, and M. Yafuso, "Optical fluorescence and its application to an intravascular blood gas monitoring system," *IEEE Trans. Biomed. Eng.* **2**, 117–132 (1986).
6. www.terumo-us.com.
7. C. M. Ganter and A. Zollinger, "Continuous intravascular blood gas monitoring: development, current techniques, and clinical use of a commercial device," *Br. J. Anaesth.* **91**(3), 397–407 (2003).
8. W. E. Hoffman, F. T. Charbel, G. Edelman, and J. I. Ausman, "Brain tissue oxygen pressure, carbon dioxide pressure, and pH during hypothermic circulatory arrest," *Surg. Neurol.* **46**, 75–79 (1996).
9. B. Venkatesh, R. Meacher, M. Muller, T. Morgan, and J. Fraser, "Monitoring tissue oxygenation during resuscitation of major burns," *J. Trauma: Inj., Infect., Crit. Care* **50**, 485–494 (2001).
10. B. R. Soller, P. O. Idwasi, J. Balaguer, S. Levin, S. A. Simsir, T. J. Vander Salm, H. Collette, and S. O. Heard, "Noninvasive, near infrared spectroscopic-measured muscle pH and pO_2 indicate tissue perfusion for cardiac surgical patients undergoing cardiopulmonary bypass," *Crit. Care Med.* **31**(9), 2324–2331 (2003).
11. F. J. Service, P. C. O'Brien, S. D. Wise, S. Ness, and S. M. LeBlanc, "Dermal interstitial glucose as an indicator of ambient glycemia," *Diabetes Care* **20**, 1426–1429 (1997).
12. D. C. Klonoff, "Noninvasive blood glucose monitoring," *Diabetes Care* **20**, 433–437 (1997).
13. J. P. Bantle and W. Thornas, "Glucose measurement in patients with diabetes mellitus with dermal interstitial fluid," *J. Lab. Clin. Med.* **130**, 436–441 (1997).
14. M. Ellmerer, M. Haluzik, J. Blaha, J. Kremen, S. Svacina, W. Toller, J. Mader, L. Schaupp, J. Plank, and T. R. Pieber, "Clinical evaluation of alternative site glucose measurements in patients after major cardiac surgery," *Diabetes Care* **29**(6), 1275–1281 (2006).
15. O. Soyemi, M. Shear, M. Landry, D. Anunciacion, and B. Soller, "*In vivo*, noninvasive measurement of muscle pH during exercise using near infrared spectroscopy," in *Smart Medical and Biomedical Sensor Technology III*, B. M. Cullum and J. C. Carter, Eds., *Proc. SPIE* **6007**, 60070N (2005).
16. C. G. Cooney, B. C. Towe, and C. R. Eyster, "Optical pH, oxygen and carbon dioxide monitoring using a microdialysis approach," *Sens. Actuators B* **69**, 183–188 (2000).
17. F. Baldini, A. Giannetti, A. A. Mencaglia, A. Bizzarri, M. Cajlakovic, and C. Konrad, "Interstitial pH, pO_2 and pCO_2 controlled by optical sensors," in *Advanced Environmental, Chemical and Biological Sensing Technologies III*, T. Vo-Dinh, R. A. Lieberman, and G. Gauglitz, Eds., *Proc. SPIE* **5993**, 599309 (2005).
18. M. Muller, "Science, medicine, and the future: microdialysis," *Br. Med. J.* **324**, 588–591 (2002).
19. L. Schaupp, M. Ellmerer, G. A. Brunner, A. Wutte, G. Sendlhofer, Z. Trajanovski, F. Skrabal, T. R. Pieber, and P. Wach, "Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion," *Am. J. Physiol. Endosc.* **276**, 401–408 (1999).
20. M. Lafontan, and P. Arner, "Application of *in situ* microdialysis to measure metabolic and vascular responses in adipose tissue," *TIPS* **17**, 309–313 (1996).
21. N. R. Ekberg, N. Wisniewski, K. Brismar, and U. Ungerstedt, "Measurement of glucose and metabolites in subcutaneous adipose tissue during hyperglycemia with microdialysis at various perfusion flow rates," *Clin. Chim. Acta* **359**(1–2), 53–64 (2005).
22. F. A. M. Baumeister, B. Rolinski, R. Busch, and P. Emmrich, "Glucose monitoring with long-term subcutaneous microdialysis in neonates," *Pediatrics* **108**(5), 1187–1192 (2001).
23. D. Street, J. Bangsbo, and C. Juel, "Interstitial pH in human skeletal muscle during and after dynamic graded exercise," *J. Physiol. (London)* **537**, 993–998 (2001).
24. A. Pasic, H. Koehler, L. Schaupp, T. R. Pieber, and I. Klimant, "Fiber-optic flow-through sensor for online monitoring of glucose," *Anal. Bioanal. Chem.* **386**, 1293–1302 (2006).
25. H. M. Heise, U. Damm, O. Vogt, and V. R. Kondepati, "Towards reagent-free blood glucose monitoring using micro-dialysis and infrared transmission spectrometry," *Vib. Spectrosc.* **42**(1), 124–129 (2006).
26. M. Arend, B. Westermann, and N. Risch, "Modern variants of the Mannich Reaction," *Angew. Chem., Int. Ed.* **37**, 1044–1070 (1998).
27. T. C. McIlvaine, "A buffer solution for colorimetric comparison," *J. Biol. Chem.* **49**, 183–186 (1921).
28. H. T. S. Britton and R. A. Robinson, "Universal buffer solutions and the dissociation constant of veronal," *J. Chem. Soc.* 1456–1462 (1931).
29. F. Baldini, "Critical review of pH sensing with optical fibres," in *Advanced Chemical, Biochemical and Environmental Fiber Sensors X*, R. A. Lieberman, Ed., *Proc. SPIE* **3540**, 2–9 (1999).