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GLUCOSE AND LACTATE BIOSENSORS COUPLED WITH MICRODIALYSIS PROBE FOR CONTINUOUS MONITORING

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ABSTRACT: Several microdialysis probes coupled to a microcell, where an amperometric biosensor for glucose or lactate was inserted, have been tested to evaluate the stability of the biosensor response for *in vivo* monitoring of glucose and lactate levels. Microdialysis probes with a molecular weight cut off ranging from 6000 to 20000 Daltons (Da) were compared. A good sensitivity, reproducibility and stability of the signal were noticed for both systems with different biosensors.

RESUMO Várias sondas para microdiálise, acopladas à uma microcélula e contendo biosensores amperométricos para glicose ou lactato, foram testadas para avaliar a resposta *in vivo* para monitoramento de níveis de glicose ou lactato. Foram comparadas várias microsondas com limites de peso molecular entre 6000 e 20000 Daltons (Da). Foi observada uma boa sensitividade, reprodutibilidade e estabilidade do sinal para ambos os sitemas com vários biosensores.

KEYWORDS glucose, lactate, microdialysis probe, flow through analysis

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TABLE 1

CHANGES IN SOME BIOCHEMICAL PARAMETERS OF PATIENTS WITH VARIOUS LIVER DISEASES

Disease	Number of patients	LP	Bilirubin	GPT	TG	GSH	CL
Control	37	8,6 ± 3,8	1,05 ± 0,8	26,5 ± 3,5	136 ± 12	47,4 ± 4,3	53,7 ± 6,1
Alcoholic hepatitis	11	10,8 ± 5,4	5,8 ± 3,7	38,5 ± 4,1	243 ± 62	32,5 ± 1,6	67,4 ± 7,5
Viral hepatitis	35	21,9 ± 3,2 +	9,3 ± 4,1	496,3 ± 24,3 +	315 ± 24	36,9 ± 2,8	74,5 ± 4,8
Acute viral hepatitis	15	66,8 ± 8,4	19,9 ± 2,6 +	532,7 ± 36,5	428 ± 31 +	24,6 ± 3,9 +	85,8 ± 6,4 +
Chronic hepatitis	14	11,3 ± 3,8	3,8 ± 1,6	47,5 ± 5,2	278 ± 21	53,8 ± 6,1	64,7 ± 5,4
Cyrrhosis	8	17,5 ± 4,3	15,7 ± 4,2	36,8 ± 3,6	236 ± 45	29,6 ± 4,9	42,5 ± 5,8
Hepatic coma	10	7 ± 9,5	21,5 ± 8,5	530,2 ± 34,5	410 ± 30	28,5 ± 4,5	84,6 ± 5,4

LP - lipid peroxides **GSH** - glutathione **GPT** - glutamate:pyruvate-transaminase
CL - chemiluminescence **TG** - triglycerides

Parameters have been expressed in this way :

LP - nmole/ml
Bilirubin, TG, GSH - mg %
CL - pulse/minute/0,1 ml ser

+ = significant differences compared to control for $p < 0,01$

INTRODUCTION

Microdialysis is a new technique for continuos monitoring[1-3], based on the principle of dialysis : in a dialysis tube filled with a liquid (carrier flow), chemical substances diffuse in the direction of the lowest concentration due to the gradient concentration.

The technique presumes the continuos removing of chemical substances from any compartment without removing any liquid. Because the dialysis tube is a micro-tube (100-200 μm inner diameter) and the flow rate is very low (25 μL/min) the concentration is not altered by amount taken out.

This technique was first used for measurements of neurotransmitter in the brain and had already been coupled to liquid chromatography[4-6].

Although, the technique had been developed in some laboratories[7-13], several problems unsolved are still remaining.

One of the newest application of microdialysis is for continuos *in vivo* monitoring[14].

In our case the microdialysis technique has been coupled with a glucose or lactate electrochemical biosensors to obtain an instrument for continuous *in vivo* glucose or lactate monitoring[15].

The system works as follows: a buffer solution is pumped at a constant flow rate into a thin dialysis hollow fiber immersed in the sample; the probe retrieves glucose or lactate and other compounds of low molecular weight from the sample. Subsequently, it flows into a wall-jet cell provided with a glucose or lactate biosensor, that will monitor the glucose or lactate level.

We compared *in vitro* several microdialysis probes with different molecular weight cut-off (MWCO) and one sterilized thin dialysis hollow fiber. The main purpose of this study was the control of stability response of biosensor for glucose and lactate, taking into account that this technique can be succesfully used *in vivo* monitoring.

The study was motivated due to several advantages offered by this system: the sensitivity of biosensors can be checked frequently, the hollow fiber used as microdialysis probe can be sterilized, the process of *in vivo* implantation can be controlled and reactions such inflammation and clotting can be monitored and eliminated. Moreover, as was presented in a previous paper[15], the system can be miniaturized as for a wearable glucose or lactate monitor (Glucoday).

We report in this paper our results obtained for *in vitro* measurements for continuous monitoring of glucose and lactate.

EXPERIMENTAL

Materials and apparatus

Glucose oxidase (GOD; EC 1.1.3.4. from Aspergillus niger, type VII, 132000 U/g) was obtained from Sigma Chemical. A GOD-immobilized-nylon net membrane was prepared as described in a previous paper[16].

Lactate oxidase (LOD E.C. 1.1.3.2 from Pediococcus species, 35000 U/g,) was also obtained from Sigma Chemical. The LOD was immobilized by covalently bonding on pre-activated Immobilon-AV affinity membranes (Milipore, Bedford, MA, USA) following the procedure described by Villarta et al[17].

The buffer solution, Dulbecco's physiological buffer (pH=7.4), was prepared in doubly distilled water. All chemicals were of analytical grade.

The glucose stock solution (0.5 M + 0.1% Katon), was prepared with β-D(+) glucose from Farmitalia Carlo Erba (Milano, Italy) in buffer, allowed to equilibrate overnight and suitable diluted. Also, we prepared a stock solution of glucose (0.5 M) in albumin 3%. Stock lactate solution (0.1 M + 0.1% Katon) was prepared with L(+) lactic acid (Li salt) obtained from Sigma Chemical and freshly prepared every three days in Dulbecco's buffer. The solutions were stored at 4°C when not in use.

Cellulose acetate (53% acetyl) and polivinyl acetate of high molecular weight were obtained from Farmitalia Carlo Erba. For casting the cellulose membrane a precision gauge tool was used. This membrane, with about 100 Da MWCO, was prepared as in a previous works[18-20].

The CMA/10 Microdialysis probe (i.d. 400 μm, wall thickness 60 μm and a MWCO 20000 Da) was obtained from CMA/Microdialysis (Stockholm, Sweden). To assemble microdialysis probes manually we used several kinds of hollow fiber: 1) Filtral 12 AN 69 HF polyacrylonitrile sodium methallyl sulphonate (i.d. 200 μm and MWCO approximately 20000 Da) was obtained from Hospal Industrie (Meyziev, France); 2) Spectra/Por Hollow fibers, regenerated cellulose (i.d. 215 μm, wall thickness 18 μm MWCO 6000 Da); 3) Spectra/Por Hollow fibers, regenerated cellulose (i.d. 150 μm, wall thickness 9 μm MWCO 9000 Da); and 4) Spectra/Por *in vivo* Microdialysis Hollow fibers, regenerated cellulose (i.d. 150 μm, wall thickness 15 μm) were obtained from Spectrum Medical Industries Inc (Los Angeles, CA). We inserted into all Spectrum hollow fibers a gold-plated 50 μm-diameter tungsten wire obtained from Goodfellow (Cambridge, UK) to avoid the collapse.

Silicone tubing (i.d. 0.300 mm, o.d., 0.630 mm, wall thickness 0.165 mm) from A-M systems was used to connect hollow fibers to flow system.

Assembling of the sensor

The wall-jet flow cell used in our work[21] included three separates electrodes: The working electrode (platinum disk with diameter of 1.6 mm), the reference (Ag/AgCl) and the auxiliary electrode. A thin (20 μm) membrane of cellulose acetate was stretched over the platinum electrode surface: it removes the electrochemical interference (uric acid, ascorbate etc.) with its nominal MWCO 100; this has the effect that ascorbic and uric acid do not reach the electrode surface while hydrogen peroxide passes through easily. A nylon net (thickness 100 μm), with the immobilized glucose oxidase enzyme or an Immobilon membrane with lactate oxidase immobilized was placed over the electrode area. Sleeve of suitable diameter was used to stretch both the membranes on the Pt electrode surface.

Procedures

The peristaltic pump drives the carrier solution, with a constant flow rate through the microdialysis probe (with 10 mm length), immersed in glucose or lactate standard solutions. A steady-state current is obtained. The standard solutions, where the microdialysis probe was immersed, were manually changed.

The optimization of the functional parameters have been described elsewhere[14,15,22]. The diagram of the flow system for in vitro experiments is shown in Figure 1.

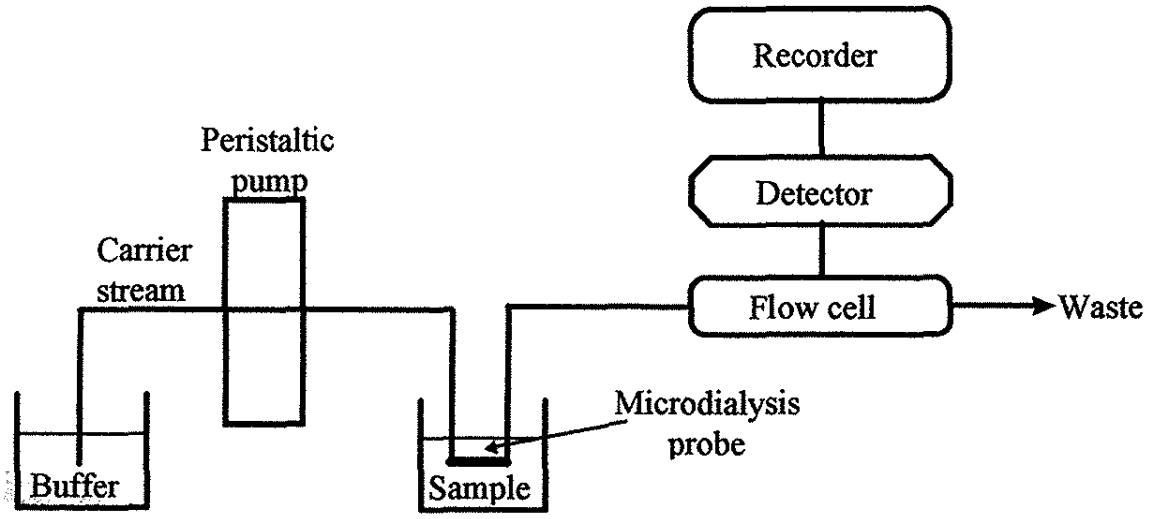

Figure 1. Schematic diagram of the flow system.

RESULTS AND DISCUSSION

The analyte recovery through a microdialysis membrane is a mass transport controlled process. There are three regions were mass transport occurs that must be considered: the sample solution, the dialysis membrane and the solution inside the fiber lumen (i.e. dialysate). Transport in the dialysate is normally considered an important unless the perfusion rate is very low, e.g. < 0.3 μL/min.

At the beginning we checked the performances of the glucose and lactate flow cells without microdialysis probes. For glucose and lactate results are reported in previous papers[14,23]. For lactate we obtained a good linearity (0.1 - 0.5 mM, y=20,7086x+0.72, R=0.9876) and good reproducibility.

The optimum parameters were chosen to be pH=7.4 (Dulbecco's physiological buffer), flow rate 25 μL/min and t=25°C. It had been demonstrated in other studies[14,23,24] that at 37°C the signal (current) obtained with microdialysis probes is about 30% higher and the linearity range is slightly reduced. This is explained by the variation of diffusion coefficient of glucose or lactate through the microdialysis probes.

We compared *in vitro* several microdialysis probes with different MWCO (6000, 9000, 20000 Da) and a microdialysis probe for in vivo measurements.

The dialysis hollow fibers were assembled in laboratory, consisting of a small pieces of a single hollow fiber (1 - 2 cm length) connected with the two ends of Teflon tubes and solded with epoxy glue. We used the manually assembled microdialysis probes which showed better robustness and were easier to handle.

For glucose measurements the fourth microdialysis probe showed almost similar results in spite of different materials and different MWCO. Figure 2a shows the linear response for glucose system with microdialysis probes: 1- MWCO 20000, 1cm length and 2 - microdialysis probe for in vivo experiments. In Figure 2b are presented the linear responses for the same system but for glucose samples prepared in 3% albumin.

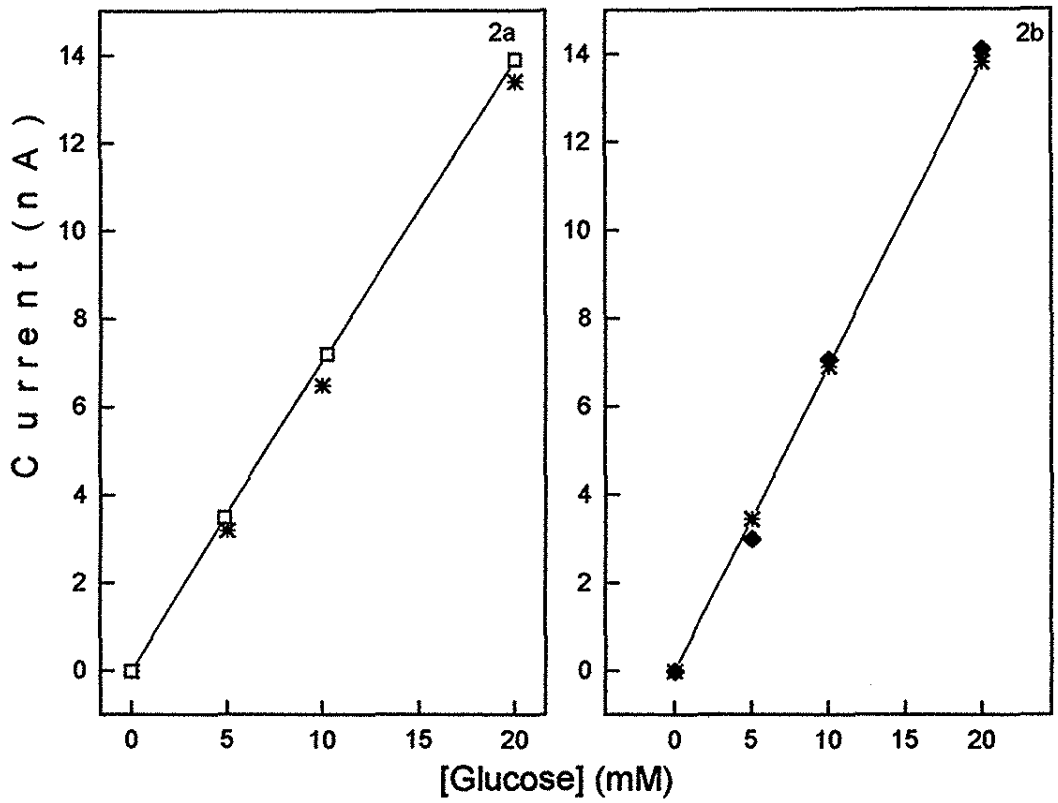

Figure2. The linear response of glucose biosensor coupled with microdialysis probes (1 cm length); **2a** - microdialysis probe with MWCO 20000 Da (□ glucose samples prepared in buffer and * glucose samples prepared in albumin 3%); **2b** - microdialysis probe for *in vivo* measurement (• glucose samples prepared in buffer and * glucose samples prepared in albumin 3%);

As can be observed the results for microdilaysis probe with MWCO 20000 and microdialysis probe for *in vivo* measurements, demonstrate a good linearity for 1 - 20 mM range in both kind of glucose samples (made in buffer and in albumin). The only difference appears for slope values that is higher for system with 20000 MWCO microdialysis probe (0.67) than that for microdialysis probe for *in vivo* (0.47). An explanation of this behavior could be the difference in the geometry or type of materials. For the other microdialysis probes

(6000 and 9000 MWCO) we also obtained, as expected, a good linearity and low differences between the slope values of the calibration curves.

We noticed that for the same concentrations of glucose samples made in albumin 3%, the biosensor gives the values for the current, meaning that albumin does not affect (influence) the response of biosensor (see Fig. 2). The same results were obtained for all kinds of microdialysis probes.

An important study was to check the stability of the biosensor response for a long period as in the case of continuos monitoring. For this reason, the signal (current) was recorded during 15 hours for systems with all four microdialysis probes for both kind of glucose samples. The stability of biosensor response for 10 mM glucose solution prepared in buffer and in albumin 3 % is presented in Figure 3a and 3b respectively. The signals obtained with 20000 MWCO microdialysis probe (Fig. 3a) and microdialysis probe for *in vivo* (Fig. 3b) present small fluctuations due to random variations of the experimental parameters, i.e. stirring conditions.

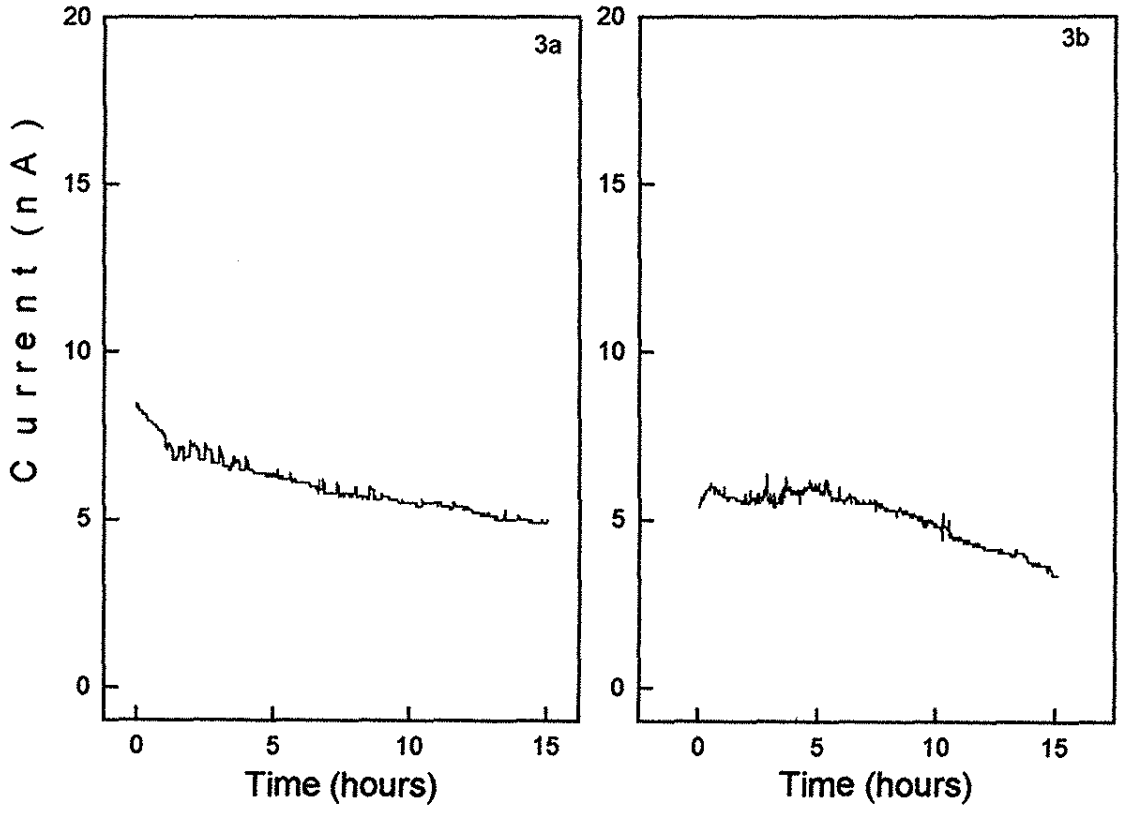

Figure 3. Stability of biosensor response for 10 mM glucose samples; **3a** - glucose sample made in buffer and microdialysis probe with MWCO 20000; **3b** - glucose sample made in albumin 3% and microdialysis probe for *in vivo*.

The slight decrease in time of the signal comes from the consuming of glucose from the samples and not from the change of enzyme activity.

As regards the decrease of the signal during the monitoring of glucose from albumin, observed for microdialysis for *in vivo* measurements, it can be explained only due to the degradation of albumin with time (the measurement was made *in vitro*) and the covering of the hollow fiber.

For *in vivo* monitoring we have to take into account all the factors that can affect the signal such as: the position and the place of the microdialysis probe, the involuntary muscle contractions near the sampling point and if the patient is affected by another disease e.g. Parkinson's disease.

The linearity of the response of the lactate biosensor coupled with microdialysis probe for *in vivo* is presented in Figure 4. A good linearity for 1 - 10 mM range (y=1.0651x+0.54, R=0.9996) is shown as we expected according to other paper[22].

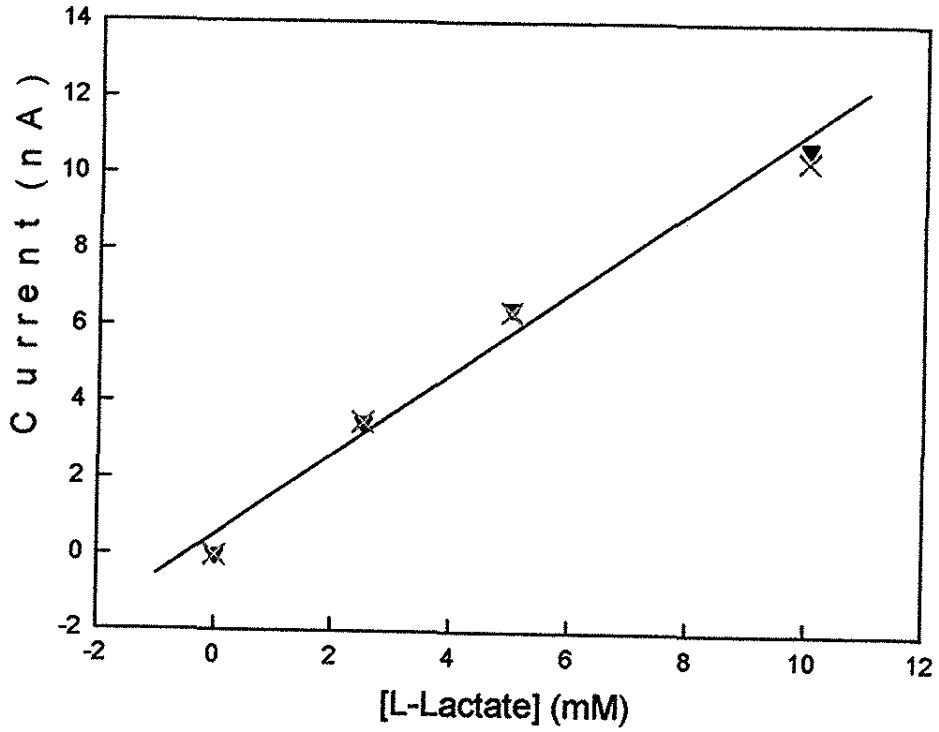

Figure 4. Calibration curve for lactate biosensor coupled with microdialysis probe for *in vivo* monitoring; ▼ values obtained before a continuous monitoring and x values obtained after a period of 12 hours of continuous measurement.

In fact, the main purpose of this research was to check the stability of biosensor response for continuous monitoring. This results from possible applications of this technique in routine clinical chemistry determinations, on-line monitoring of bioprocesses or quality control work.

Figure 5 shows the stability of biosensor response for 5 mM lactate solution with two microdialysis probes monitoring for 10 hours; one of them has MWCO 20000 and the other is used for *in vivo* experiments. As for glucose, all the measurements for lactate were made *in vitro*.

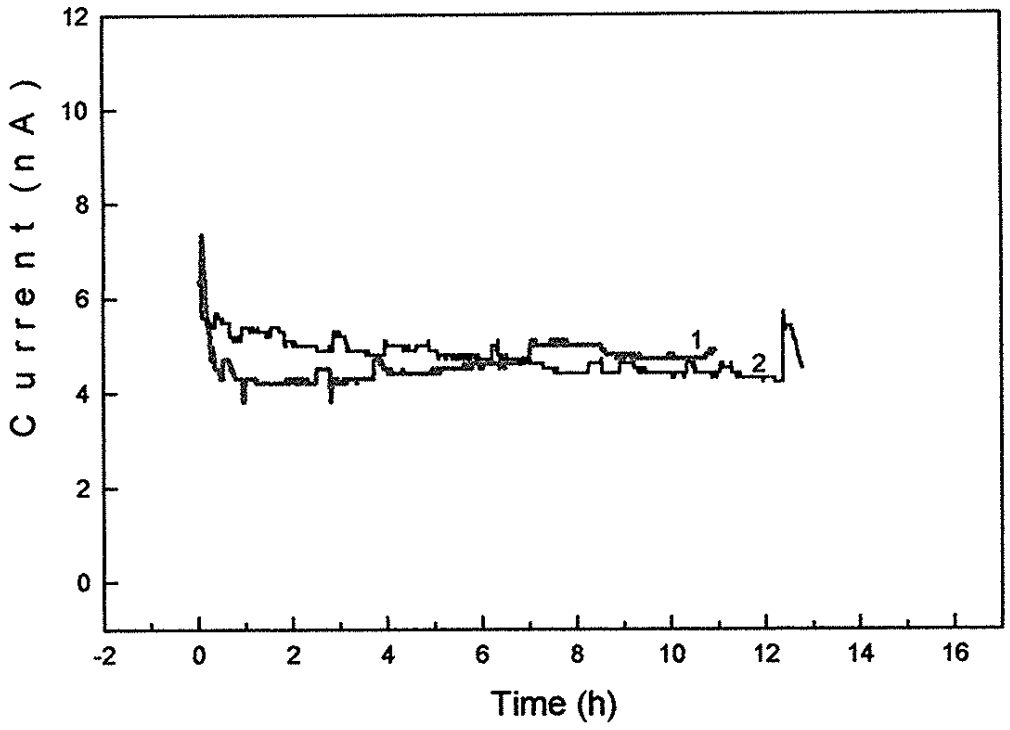

Figure 5. Stability of biosensor response for 5mM lactate with microdialysis probes
1 - for in vivo monitoring and **2** - MWCO 20000 Da

The small variation that we observed in case of glucose system has the same reasons (experimental parameters). After the continuous measurement we checked once again the linearity of the biosensor response which is presented in Figure 4. The same values for the slope obtained in this case proved a good reliability of this system making it suitable for *in vivo* determinations.

CONCLUSIONS

In conclusion, from the stability point of view the coupling of microdialysis with glucose or lactate biosensor in form of wall-jet cell is suitable for measuring glucose and lactate *in vivo*, without any preliminary sample pre-treatment. The glucose system monitors the variations of glucose concentration at the site where the "probe" is inserted. The lactate system

can be used for measurements in sport medicine and in cardiology knowing that lactate is an important metabolite to monitor and control.

The instrument "Glucoday" developed by Ampliscientifica (Milano, Italy) represents a wearable monitor, a sort of metabolic control and it is the first commercial apparatus based on coupling of microdialysis with a biosensor flow cell.

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