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Short communication

# An assessment of calibration and performance of the microdialysis system

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**Abstract**

To improve the reliability of microdialysis measurements of tissue concentrations of metabolic substances, this study was designed to test both the performance and the internal validity of the microdialysis methods in the hands of our research group. The stability of the CMA 600 analyser was tested with a known glucose solution in 72 standard microvials and in 48 plastic vials. To evaluate if variation in sampling time makes any difference in sample concentration (recovery), sampling times of 10, 20 and 30 min were compared in vitro with a constant flow rate of 1 μl/min. For testing of sampling times at different flow rates, an in vitro study was performed in which a constant sample volume of 10 μl was obtained. With the no net flux method, the actual concentration of glucose and urea in subcutaneous tissue was measured. The CMA 600 glucose analysis function was accurate and stable with a coefficient of variability (CV) of 0.2–0.55%. There was no difference in recovery for the CMA 60 catheter for glucose when sampling times were varied. Higher flow rates resulted in decreased recovery. Subcutaneous tissue concentrations of glucose and urea were 4.4 mmol/l and 4.1 mmol/l, respectively. To conclude, this work describes an internal validation of our use of the microdialysis system by calibration of vials and catheters. Internal validation is necessary in order to be certain of adequate sampling times, flow rates and sampling volumes. With this in mind, the microdialysis technique is useful and appropriate for in vivo studies on tissue metabolism.


*Keywords:* Validation; Calibration; No net flux; Microdialysis; Pig

## 1. Introduction

The microdialysis technique was introduced in the beginning of the 1970s as a technique for measurement of the dynamic release of substances from animal brain [1–4]. Since then, the technique has been widely implemented and was introduced in human studies in 1987 [1,3].

The microdialysis technique has been used for continuous sampling of low molecular weight compounds including glucose, lactate, pyruvate, glycerol, glutamate and urea, as well as for sampling of pharmacological agents in the extracellular space [1,3,5,6]. For many drugs and endogenous ligands, specific receptors are localized in peripheral compartments rather than in serum. Therefore, interstitial concentrations, rather than concentrations in blood, determine their biologic effects [7]. The microdialysis technique is based on passive diffusion of substances along their concentration gradients, from the extracellular fluid into the dialysate. The microdialysis catheter (shown in Fig. 1) allows free diffusion of small solute molecules with a weight between 5 and 30 kDa (kilo Dalton) [1,3,5].

The term recovery is often used in microdialysis measurement and it reflects the ratio between the concentration obtained in the microdialysate and the real concentration in the extracellular fluid surrounding the probe. Recovery of a specific agent through a probe is dependent on probe location, concentration gradient, molecular weight, probe membrane permeability, probe area, perfusion fluid properties and molecular charge [1,3,8].

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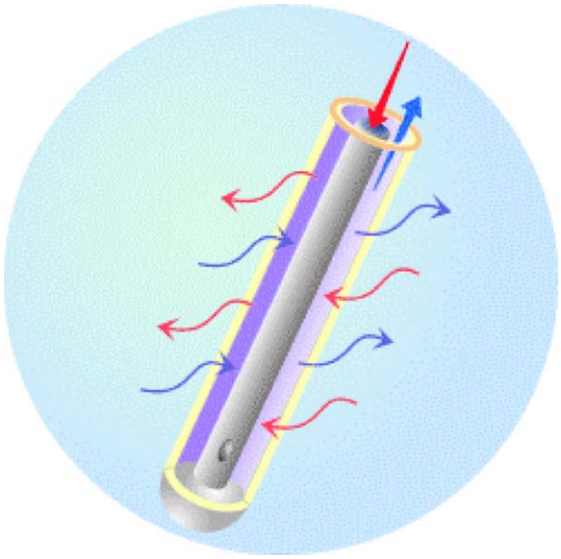

Fig. 1. A microdialysis catheters with semipermeable membrane. Shown with permission from CMA Microdialysis, Solna, Sweden.

Microdialysis sampling is often performed under nonequilibrium conditions and it shows only fractions of unbound concentrations in the extracellular space [8]. In order to correctly assess the interstitial fluid concentration of any substance by microdialysis, the catheter must be calibrated [5,8,9]. To find out whether or not a substance is able to cross the dialysis membrane, an in vitro calibration should be performed before the in vivo experiment [1,3,5].

To control recovery in different interstitial spaces, an in vivo calibration should be performed in situ before the start of the experiment [3,5,8,9]. The no net flux method (NNF-method) is a commonly used method for calibration. The NNF-method provides an option to determine the true interstitial concentration defined by the zero net flux point where concentrations in the dialysate and the perfusate are the same. To obtain the zero net flux point, the interstitial space should be perfused with a least four predefined concentrations [3,5,8,10,11]. The NNF-method is demanding and requires the metabolism to be stable [10].

The general aim was to evaluate the microdilaysis system both for the catheter/probe and the CMA 600 analyser, and to learn more about the microdialysis measurement process to avoid problems in future experiments.

## 2. Materials and methods

The experimental protocol consists of three different parts. The first part comprises a validation of the analysis method and an in vitro calibration of time for sampling and flow rate. The second part is an in vitro calibration test of the catheter after animal experiments. The third part is a subcutaneous in vivo calibration for glucose and urea in seven pigs with the NNF-method.

The term perfusate referred to the solution which is injected and flows proximally in the microdialysis catheter. The perfusate in this case is Ringer's solution, but can be replaced by another solution if indicated in order to allow diffusion of selected substances. The term dialysate referred to the solution, once diffusion of extracellular substances has occurred into the microdialysis catheter.

### 2.1. Validation

To evaluate the stability of the CMA 600 analyser (CMA Microdialysis, Sweden), 10 μl of glucose with a known concentration of 4.91 mmol/l were collected in each of 72 standard microvials (CMA Microdialysis, Sweden) and in 48 plastic vials (CMA Microdialysis, Sweden). Glucose concentrations in all vials were immediately analysed by the CMA 600 analyzer. In the subsequent experiments, plastic vials were used.

### 2.2. In vitro calibration

To evaluate if recovery is dependent on the time of sampling, we placed a CMA 60 (CMA Microdialysis, Sweden) catheter connected to a CMA 102 microdialysis pump (CMA Microdialysis, Sweden) in a known glucose solution. The syringe contained CMA perfusion fluid (CMA Microdialysis, Sweden).

The flow rate was 1 μl/min and sampling times were either 10, 20 or 30 min. Ten samples were collected for each sampling time.

To test for the relation between recovery and flow rate, a CMA 60 catheter was placed in a known glucose solution. The volume of the dialysate was held constant at 10 μl. The flow rate was 0.3, 0.5, 1, 2 or 5 μl/min. 10 samples were collected for each flow rate. To obtain a constant dialysate volume of 10 μl sampling times varied between 33, 20, 10, 5 and 2 min, respectively. The results that was presented as relative recovery by gain (in percent) was calculated as [dialysate]/[concentration of the known solution] × 100.

### 2.3. Probe check

The probe membrane function of 14 CMA 20 microdialysis probes was tested in vitro after experimental studies in animals. The probes were washed out with distilled water during the night after the primarily experiment, with a flow rate of 0.3 μl/min. The following day the probes were placed in a glucose solution with a known concentration of 5 mmol/l. The check always started with 30 min equilibrium period with a flow rate of 2 μl/min. The CMA 20 microdialysis probes were then tested with a flow rate of 2 μl/min and with a sampling time of 10 min. Each probe was tested in vitro with three separate collection periods and the mean values for glucose concentration were calculated. A coefficient of variance (CV%) was calculated for each probe's three measurements. The recovery percentage was calculated

from the mean glucose concentrations for each probe. The mean CV and CV range for the 14 probes were also calculated.

### 2.4. In vivo calibration with the no net flux method

This part of the protocol was approved by the Umeå Review Board for ethical animal use. One CMA 20 probe was inserted subcutaneously, in an anesthetised pigs that was being studied as part of another experiment. An equilibration period of 60 min was allowed to elapse. For the experiment, a CMA 102 microdialysis pump was used. The microsyringe contained Ringer's solution (Fresenius kabi, Norway) with different concentration of glucose and urea (0.5, 2, 5 or 10 mmol/l). Before the sampling, each concentration of glucose and urea had an equilibrium period of 30 min. The time interval for sampling was 10 min. The microdialysate (perfusate) flow rate was 2.0 μl/min. Three samples where collected. A linear regression analysis was performed, for the relationship between inflow concentration of urea and glucose in the perfusate (Cin) and the difference in (net) concentration of glucose and urea between inflow and outflow (Cout). The real concentration of urea and glucose in the interstitial (subcutaneous) space could then be calculated using linear regression analysis. If there was no net flux ($y = 0$ in Figs. 3 and 4), we infer that the interstitial concentration for these substances was the same as in the perfusate. The probe was checked after the experiment, as described above.

### 2.5. Analysis

For analyses of the microdialysis samples, a CMA 600 analyzer (CMA Microdialysis, Sweden) was used. CMA 600 is a clinical chemical analyser that uses enzymatic reagents and colorimetric measurements of the microdialysis samples.

### 2.6. Statistics

Microsoft Excel was used for analysis with the Student's *t*-test and $p < 0.05$ was used to determine significant differences between grouped measures.

## 3. Results

### 3.1. Validation

Results from the CMA 600 analyzer with standard and plastic vials with a known glucose solution of 4.91 mmol/l resulted in mean values between 4.38 and 4.48 mmol/l (Table 1). Values in Table 1 show a coefficient variance (CV) between 0.27 and 0.55%. The CMA 600 analyzer showed no significant difference in glucose measurement over one batch of 24 samples.

Table 1
Glucose in standard (1) and plastic (2) vials

| Vial | Mean (mmol/l), $n = 24$ | CV (%) |
|---|---|---|
| 1 | 4.40 | 0.27 |
| 1 | 4.48 | 0.45 |
| 1 | 4.42 | 0.32 |
| 2 | 4.38 | 0.39 |
| 2 | 4.39 | 0.55 |

CV: coefficient of variance.

Table 2
In vitro examination of CMA 60 microdialysis catheter in a glucose solution (4.41 mmol/l) with different sampling time 10, 20 and 30 min

| Sampling time (min) | Mean (mmol/l), $n = 10$ | CV (%) |
|---|---|---|
| 10 | 4.41 | 3.6 |
| 20 | 4.46 | 2.68 |
| 30 | 4.54 | 1.21 |

CV: coefficient of variance.

Table 3
In vitro examination of flow rate with a CMA 60 microdialysis catheter in glucose solution

| Flow rate (μl/min) | Mean relative recovery by gain (%) | CV (%) |
|---|---|---|
| 0.3 | 108 | 3.54 |
| 0.5 | 103 | 1.54 |
| 1 | 100 | 3.61 |
| 2 | 93 | 4.98 |
| 5 | 68 | 3.43 |

CV: coefficient of variance.

### 3.2. In vitro calibration

Results from different sampling times of 10, 20 and 30 min are presented in Table 2. There were only minimal differences in mean values between groups and CV were between 1.21 and 3.6%.

The mean value for relative recovery decreased when the flow rate increased as shown in Table 3. The lowest values were observed at a flow rate of 5 μl/min. Fig. 2 shows that

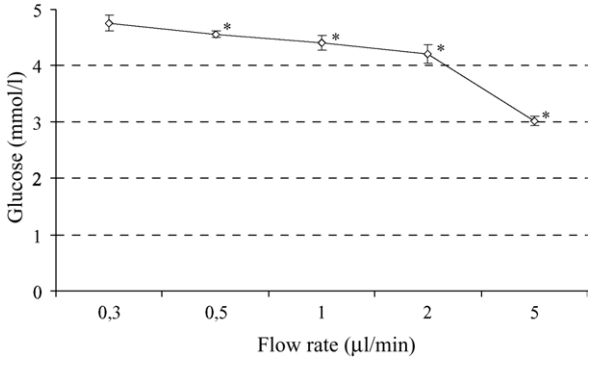


Fig. 2. In vitro experiment with CMA 60 catheters in glucose solution. The glucose concentration in relation to the flow rate. Data are presented in mean and 95% confidence interval for glucose, $n = 10$ in 0.3, 0.5, 1 and 5 μl/min and $n = 9$ in 2 μl/min. (*) Significant difference.

Table 4
Control of CMA 20 microdialysis probe after animal experimental

| | CMA 20 |
|---|---|
| Mean CV (%) | 5.07 |
| Max CV (%) | 17.06 |
| Min CV (%) | 0.51 |
| Mean recovery (%) | 35.31 |
| Number (*n*) | 14 |

CV: coefficient of variance.

mean glucose concentrations obtained from sampling at different flow rates were significantly different from each other.

### 3.3. Probe check

Data on mean recovery and variation (CV) are described in Table 4. The most prominent difference between the probes was that one probe had a high CV max of 17.06%.

### 3.4. In vivo calibration with the no net flux method

Four different glucose and urea concentrations of 0.5, 2, 5, 10 mmol/l were successfully infused. Figs. 3 and 4 demonstrate a linear relationship between different inflow concentrations of urea and glucose in the perfusate (*x*-axis) and the net concentration differences of glucose and urea between inflow and outflow (*y*-axis). From that regressions analysis an approximation (no net flux) of glucose and urea was made by interpolation (Figs. 3 and 4). The zero net flux point was 4.4 mmol/l for glucose and 4.1 mmol/l for urea.

## 4. Discussion

For microdialysis results to be reliable there must be clear knowledge and understanding concerning technical aspects of microdialysis sampling and measurement. It is important to consider the appropriateness of probe membrane type and size, along with its sampling location, sampling time and flow rate. In our porcine experimental work, some initial inconsistent results during the introduction of microdialysis into our laboratory made us focus on calibration and measurements of the microdialysis system.

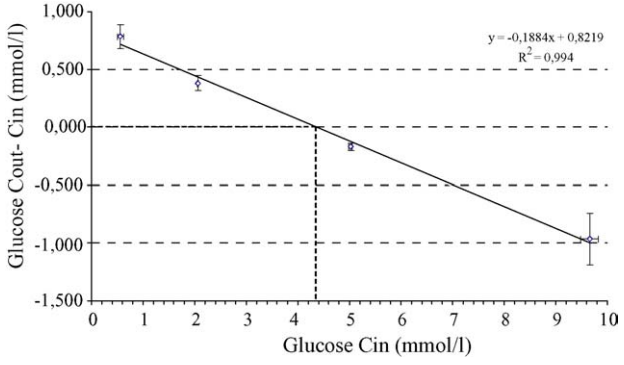


Fig. 3. The linear relationship between glucose concentration in and the function of glucose concentration out against glucose concentration in with the NNF-method. The zero net point gives a glucose value on 4.4 mmol/l. Data are presented as mean and ±S.E.M.

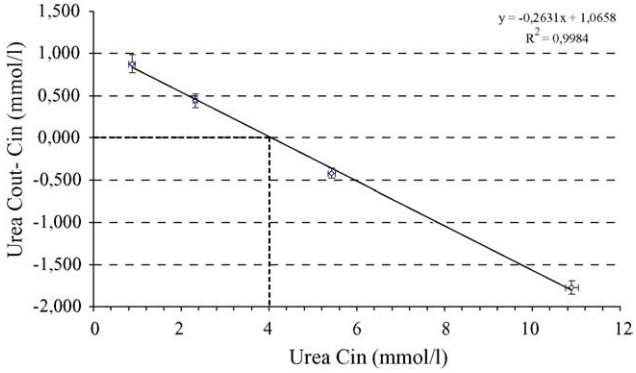


Fig. 4. The linear relationship between urea concentration in and the function of urea concentration out against urea concentration in with the NNF-method. The zero net point gives a urea value on 4.1 mmol/l. Data are presented as mean and ±S.E.M.

During this work, we have learned much about the microdialysis system by measurement of the CMA 600 analyser and by studies on effects of variation of sampling time and flow rate. This knowledge had led to fewer problems for our group when microdialysis is used. The next step was to improve an in vitro calibration method for the catheters and finally the NNF-method was used to find out true values of glucose and urea in subcutaneous tissues in pigs.

Dependent on, the experimental design, for instance clinical verses animal models, different microdialysis pumps, probes and vials are used. Differences in handling and in design between standard vials and plastic vials potentially can influence the results. The present study between different vials showed that variations in results were rather small and the highest CV was 0.55% for plastic vials. The test also showed that the CMA 600 analyzer is stable over time for one batch containing 24 samples. Since the CMA 600 is very sensitive for air bubble it is much easier to prevent air bubbles in plastic vials than in standard vials.

When a microdialysis experiment is planned, it is important to make sure that an adequate volume is collected for all planned analyses. To evaluate if variation in sampling time makes any difference in sample concentration (recovery) sampling times of 10, 20 and 30 min were compared in vitro. There were only small variations when sampling times were altered. Surprisingly, glucose concentration were higher (4.46 and 4.53 mmol/l) than the known glucose solution (4.41 mmol/l) when sampling times of 20 and 30 min were used. This might be due to the fact that plastic vials are open during the experiments and that evaporation during longer sampling times result in more concentrated solutions. For optimal experimental results it is important that sampling time is balanced against flow rate. For testing of sampling times against different flow rates (0.3, 0.5, 1, 2 and 5 μl/min) an in vitro study was performed. Constant sample volume of 10 μl was always obtained. The glucose concentration

decreased significantly when flow rate was increased. Flow rates between 0.3, 0.5, 1, 2 μl/min are acceptable in terms of variations and recovery. However, recovery at a flow rate of 5 μl/min is to low. For studies on rapid metabolic changes in tissues, results from this part of the study support the conclusion that a flow rate of 2 μl/min with a sampling time of 10 min is suitable.

After in vivo experiments in pigs, each probe was tested in vitro. Depending on exchange to the vials and results of an in vitro test, the probe was reused in the next animal. The CMA 20 probe is designed for basic research and guaranteed for single use though with proper care they have been reused several times in our hands. The CMA 20 probe gave a stable diffusion volume throughout the experiments.

Since the relative recovery might vary between tissues, it is recommended that an in vivo calibration is performed [3,5,7,8,9,11]. An in vivo calibration makes it possible to find out the real concentration of a substance. There are different calibration methods that can be used. In this study the NNF-method was used. From the linear regression analysis the concentration of subcutaneous glucose and urea could be estimated [3,8]. The value for glucose was 4.4 mmol/l and 4.1 mmol/l for urea. If the true concentration of a substance is estimated with rehabilitee the relative recovery can be calculated.

To conclude, this work shows that it is important to validate the microdialysis system by calibration of catheters. It is also important to consider sampling times, flow rates and sampling volumes. With this in mind, the microdialysis technique is useful and appropriate for in vivo studies on tissue metabolism.

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