CAPSULES
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To Calibrate or Not to Calibrate

A Question of Experimental Need

Microdialysis sampling is a powerful technique for investigating biochemical events in the extracellular fluid of virtually any tissue, organ or biological fluid. Development of the technique from a long-term dialysis sac implantation, through push-pull cannulas, to the present continuous sampling method was accomplished largely by researchers in the neurosciences. Today, microdialysis sampling is a standard technique in the neurosciences and its success has led to the extension of microdialysis sampling to applications in general pharmacokinetic, toxicology, and ADME studies. Some of the characteristics and advantages of microdialysis sampling are summarized in T1.

Microdialysis sampling is accomplished by implanting a probe consisting of a hollow-fiber dialysis membrane into the organ or biological fluid of interest. The short length of dialysis fiber is affixed to pieces of narrow bore tubing which serve as inlet and outlet tubes. A solution, termed the perfusate, is pumped slowly through the probe. The perfusate is an aqueous solution which closely matches the ionic composition and pH of the surrounding sample matrix. For sampling in vivo from tissue, the sample matrix is the extracellular fluid (ECF). When the perfusate is correctly matched to the sample matrix, there should be no net exchange of ions or water across the membrane.

Microdialysis is a diffusion controlled process. The perfusion rate through the probe is generally in the range of 0.5 to 5.0 µL/minute. At this flow rate there is no net transport of liquid across the dialysis membrane. The driving force for mass transport is the concentration gradient existing between the ECF and the fluid within the microdialysis membrane. Low molecular mass compounds diffuse into (recovery) or out of (delivery) the probe. Large molecules such as proteins and small molecules bound to proteins are excluded by the membrane. Those molecules passing through the membrane are swept along by the perfusate and exit the probe. The solution leaving the probe, called the dialysate, is collected for analysis.

Table 1. Microdialysis sampling for pharmacokinetic, toxicology, and ADME studies: a technique complementary to classical methods.

Characteristic of microdialysis sampling	*Advantages accrued*
No net change in fluid volume of surroundings	Continuous sampling possible Good temporal resolution
Minimal perturbation of surrounding tissue	More representative of normal physiology Long-term sampling in awake animal
Protein-free samples without further cleanup or extraction	No further enzymatic action on the analyte Quantitation of only the "free fraction" of analyte No loss of analyte due to sample cleanup Direct on-line coupling to analytical system
Pre-experiment microdialysis samples possible	Animal serves as its own control Fewer animals required overall
Samples reflect the extracellular fluid composition	Multiple analytes can be profiled in each sample

Microdialysis sampling is not typically performed under equilibrium conditions, so the concentration of the analyte determined in the dialysate is some fraction of its actual concentration in the surrounding sample matrix. The relationship between the analyte concentration in the dialysate and that in the sample matrix may be thought of as the extraction efficiency (EE) of the probe. Dialysis membrane properties and surface area, analyte properties, diffusion rate in the membrane and in the sample matrix, temperature, probe geometry, perfusion flow rate, processes, and physiological conditions are among the factors that influence the extraction efficiency. Fortunately, under normal conditions of microdialysis sampling, these parameters remain constant so that although equilibrium is not established, a steady-state is rapidly achieved. Thus, the EE of the probe for a given set of parameters is constant and the direction of the net flux of the analyte across the membrane is determined by the concentration gradient of the analyte. In practice, the probe's EE may be determined by a recovery experiment or a delivery experiment.

Determining the EE in vitro by either a recovery or a delivery experiment is straightforward since the analyte concentration is known (standard solution) or can be determined directly (by analysis of the dialysate sample). The determination of probe EE in vivo, especially by recovery experiments, is considerably more challenging. In vivo, the probe collects analytes from the extracellular fluid (ECF) of the tissue in which it is implanted. The concentration of analyte in the surrounding sample matrix is in most cases unknown. Furthermore, the parameters influencing probe efficiency are not the same in vivo as in vitro so EEs determined in vitro are not necessarily valid in vivo.

Considerations for In Vivo Calibration

To calibrate or not to calibrate — that is a question of experimental need. Think about what you really want to know. The information sought from microdialysis sampling falls into one of three general cases: [1] comparing two states or conditions, [2] obtaining order of magnitude values or patterns, or [3] knowing absolute, accurate ECF levels in the target tissue. Let's examine each case in more detail and consider what calibration, if any, is necessary to obtain the needed information.

Case 1: The need to compare two states such as basal versus excited or formulation A versus formulation B. In these cases changes in analyte concentration provide sufficient information. F1 is an example of this case. The figure is from Zhou et al. who observed changes in glutamate and aspartate concentrations in rat brain stimulated by increasing the potassium ion level around the probe [1]. Here one does not need an exact EE value but rather a constant EE over the time course of the experiment. Upon implantation of the probe, there will be some perturbation of the surrounding tissue. While such

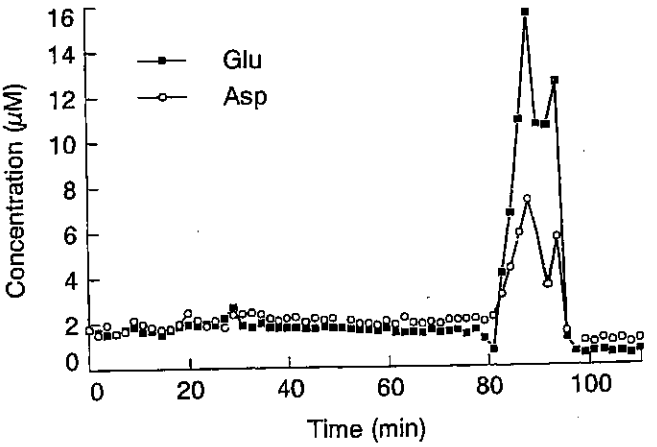

Figure 1. Changes in concentration of neuroactive amino acids in rat brain stimulated by increased K^+ administered via microdialysis probe [1].

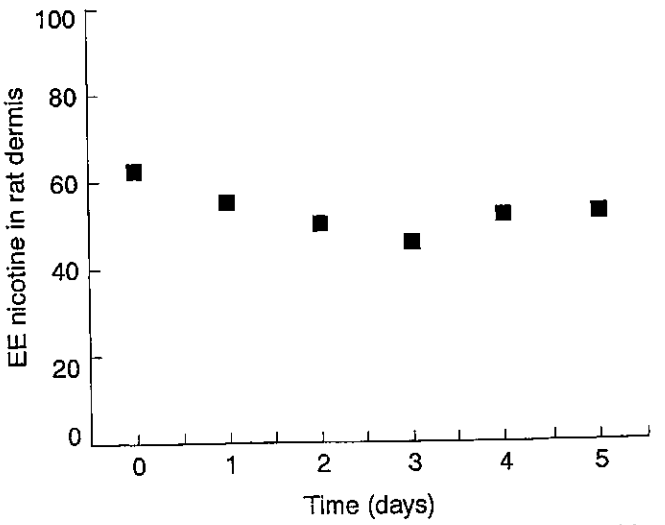

Figure 2. In vivo delivery of nicotine via probe implanted in the dermis of a rat [2].

perturbation may affect probe EE, it usually persists for only a short time, on the order of an hour. Although changes in probe EE may occur with time in long-term experiments (probes in place longer than 3 days) the EE is often fairly consistent over several days (see F2) [2]. For days 1-5, $EE_{delivery}$ falls within a 5% range, a narrow enough range to be acceptable for many applications.

Case 2: The need to know the order of magnitude of the analyte concentration in ECF. In other words, is the analyte present at 10 ng/mL or 100 ng/mL? In vitro calibration may be sufficient since it is within two to three times the in vivo value. F3 shows the distribution of $EE_{in\ vivo}$ / $EE_{in\ vitro}$ for 30 linear probes. Phenol was the analyte of interest and the in vivo EEs were determined by delivery in liver [3]. Using the mean (0.87) or the median (0.91) ratio as an in vivo correction factor for an in vitro calibration would be a reasonable first estimate for many probes. Such estimates would error by a factor of less than two for about 90% of the probes. The concentration of analyte in the in vivo dialysate, corrected for the in vitro EE, will be within the order of magnitude of the analyte concentration in ECF for probes where the ratio is as low as 0.5, using the in vitro EE without adjusting for the ratio.

Another situation in which in vitro calibration may be used directly for in vivo, is when sampling from moving fluid such as bile or blood. Stenken et al. have shown that when the linear flow rate of sample around the membrane is greater than or equal to 0.211 cm/s, the diffusion layer around the probe collapses to such an extent that diffusion through the membrane becomes the limiting factor and in vivo EE will be the same as in vitro at a given temperature [4]. Linear flow rate in a blood vessel or bile duct exceeds 0.211 cm/s, so EE determined in vitro in a stirred or flowing sample at 37° should be applicable in vivo in these cases.

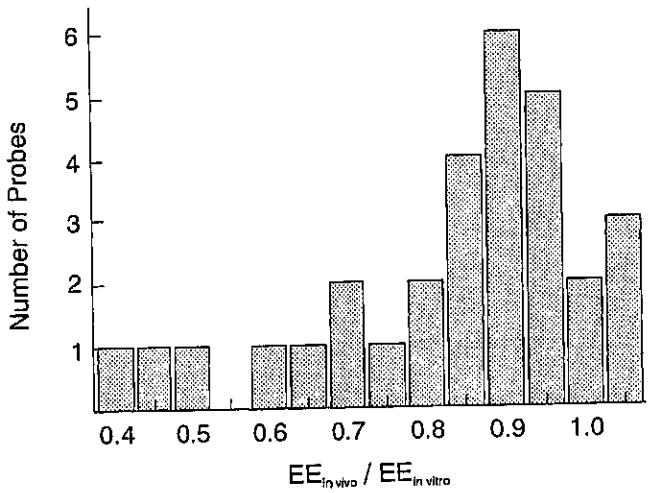

Figure 3. Distribution of $EE_{in\ vivo}$ / $EE_{in\ vitro}$ for 30 probes (0.05 intervals) [3].

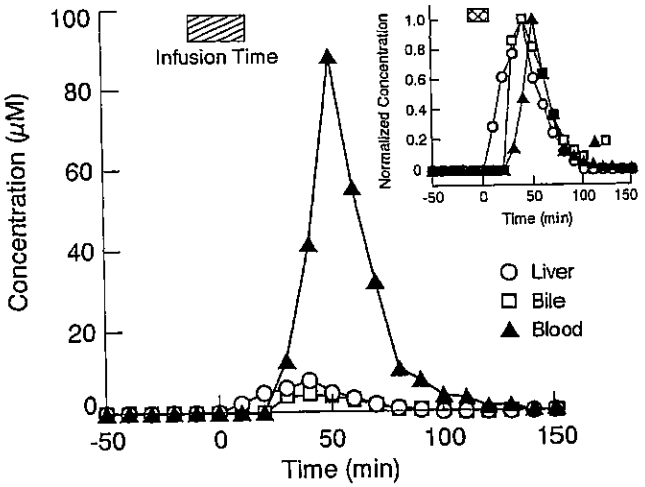

Figure 4. Concentration-time profile of phenyl-glucuronide in liver, blood, and bile sampled simultaneously by three microdialysis probes [5].

Case 3: The need for accurate in vivo concentration values will generally require that EE be determined in vivo for the analyte in the target tissue. F4 illustrates this case. Here, a concentration-time profile for a metabolite (phenyl-glucuronide) during and following a 20 minute i.v. infusion of the parent compound (phenol) is desired [5]. To obtain actual tissue concentrations of the metabolite, an in vivo calibration for the probe's EE of the metabolite from liver tissue would be necessary.

Possible techniques for in vivo calibration include very low flow rate [6; 7], zero net flux [8; 9], "retrodialysis" [10-13], and in vivo delivery of the analyte [3; 14]. The complexity of calibration necessary depends on the degree of accuracy needed.

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