CAPSULES
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Ultrafiltrate and Microdialysis DL Probe In Vitro Recoveries: Electrolytes and Metabolites

Purpose

UF ultrafiltration and DL microdialysis probes are well-suited for sampling interstitial concentrations of ions and metabolites in peripheral tissue [1-3]. In addition to in vivo sampling, ultrafiltration probes can be used for a quick and efficient method of sample cleanup, in which biological fluids such as blood or urine are used. The first step in utilization of membrane sampling techniques is to determine the recovery characteristics of the probes in vitro.

Recovery refers to the amount of analyte obtained through the probe membrane. There are two measures of recovery: **Absolute Recovery** and **Relative Recovery**. **Absolute recovery** is the total amount of material removed from the system through the probe. **Relative Recovery** is the concentration of the analyte in the solution obtained from the outflow of the probe relative to the concentration in the solution or tissue being sampled. (It is expressed as a percentage.) During in vivo sampling, relative recovery may be important if the concentration of the analyte in the tissue is low, if the sensitivity of the assay is low, or if only very small samples can be taken. Absolute recovery may be a concern if one is removing substances such as neurotransmitters, which may be involved in feedback mechanisms.

Recovery is affected by a number of factors. Microdialysis recovery depends on membrane surface area, chemical and physical characteristics of the membrane, temperature, the perfusion rate, sample matrix, and chemistry of the analyte. Factors affecting ultrafiltration recovery are: membrane characteristics, temperature, sample matrix, and chemistry of the analyte. Because of the sample matrix effect, which is present in both UF and MD sampling, it is not possible to directly extrapolate in vitro recoveries to in vivo results. However, performance of in vitro recovery studies prior to in vivo studies are important to validate that the analyte crosses the membrane and that there is no interaction of the analyte with the probe materials. Also, one can get an indication of the concentrations to be expected and the sensitivity of the analytical methods that will be needed.

Methods

All recovery studies were done in a stirred solution at 37°C. The probes tested in this study were the UF-3-12 probe (MF-7023) and the DL-5 probe (MF-7051). Probes were soaked overnight in distilled water. This procedure removes the protective glycerin coating from the outside of the fibers. The probes were then placed in fresh distilled water. The UF probe was attached to the mini-pump (MF-5200) and pumped at a flow rate of approximately 300 µL/hr. Perfusate was pumped through the DL probe using the BAS Bee microdialysis syringe pump (MF-1001) and the Bee Hive variable flow rate controller (MF-1020) at a rate of 2 µL/min. Nano-pure water was used as the perfusate for electrolyte recovery studies. Ringer's solution was used as the perfusate for the metabolites. The probes were flushed for one hour and the collected samples were used as blanks. The probes were then placed in the test solution. Four, one hour samples were collected. Test solution samples were collected before the start of the recovery study and after each probe sample. Since the first sample was diluted by the dead volume liquid from the flush samples, samples 2 to 4 were used to calculate recovery.

For each analyte, three concentrations were used: one representing the normal physiological concentration, and the other two representing the pathologically high and low levels. These three solutions encompassed the range of concentrations that could be found in in vivo sampling.

Analyte Analyses

Sodium and potassium were analyzed with ion selective electrodes. Chloride, lactate, calcium, magnesium, and phosphorus were analyzed spectrophotometrically. Glucose was analyzed by the BAS LC method using a glucose oxidase immobilized enzyme reactor and a "wired" peroxidase electrode [4].

Results and Discussion

For each probe in each solution, the recoveries were calculated by dividing the concentration of the probe sample by the average of the test solutions obtained immediately before and after the probe sample. This compensated for any possible changes in test solution concentration due to evaporative losses or unequal solvent and solute removal.

F1 shows the recoveries for one UF probe in the three different glucose test solutions. The sample labeled zero is the flush solution. The concentration of the analyte in this solution should be zero. If a non-zero concentration were obtained, it would indicate analyte carry over from a pervious study. Recoveries from the first sample can sometimes appear low. This results from rinse solution that is left over in the dead volume of the probe and tubing when the probe is switched from the flush to the test solution. Variations of recoveries in samples 2 to 4 should only occur randomly from the variability in assay method. Any significant differences between samples 2 to 4 would indicate interaction of the test compound with probe materials. Interactions of test compounds and probe materials could add a bias to the results.

It is also necessary that recoveries be the same for the physiological and pathological concentrations that are sampled during in vivo tests with these probes. F2 shows the sodium recovery for each UF probe for each concentration tested.

The recoveries of each analyte are summarized in T1. Both UF and DL probes have high recoveries, making either probe suitable for in vivo studies of these analytes.

References

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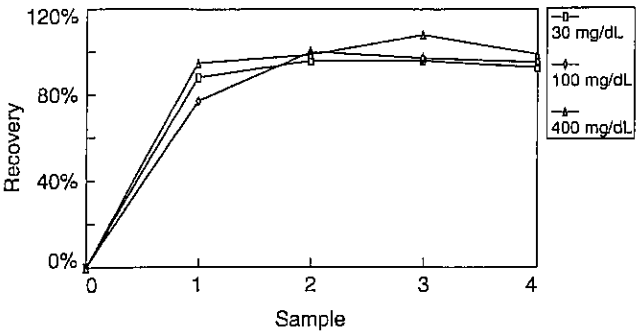

Figure 1. Recovery of one UF probe in solutions of three different concentrations. Sample zero is in saline solution with no glucose. At sample 1, the probe is placed into the test solution. Samples 2 to 4 are used to calculate recovery for the probe.

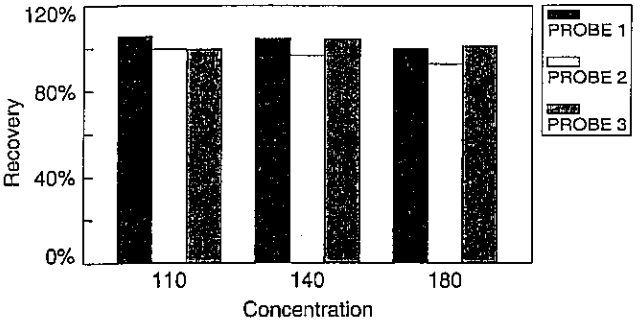

Figure 2. Sodium recoveries for three UF probes at three concentrations spanning the range of physiological and pathological concentrations. Recoveries are the same, within experimental error, for each probe at each concentration.

Analyte	UF-3-12	DL-5
Sodium	101% ± 2%	101% ± 2%
Potassium	94% ± 13%	106% ± 4%
Calcium	100% ± 3%	97% ± 3%
Magnesium	99% ± 7%	101% ± 7%
Chloride	96% ± 4%	95% ± 7%
Phosphorus	102% ± 4%	99% ± 2%

Table 1. In vitro recoveries.

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