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

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UF ultrafiltration and DL microdialysis probes are well-suited for sampling interstitial concentrations of ions and metabolites in peripheral tissue (1,4). The first step in utilization of membrane sampling techniques is to determine the recovery characteristics of the probes in vitro.

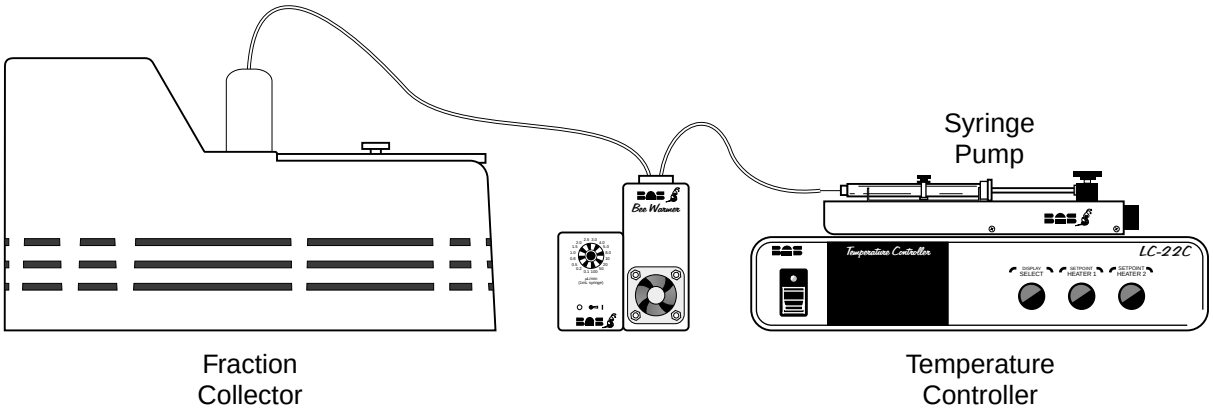
Recovery refers to the amount of analyte obtained through the probe membrane. There are two measures of recovery: **Absolute Recovery** and **Relative Recovery**. **Absolute recovery** is the total amount of material removed from the system through the probe. **Relative Recovery** is the concentration 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 or may not be a concern. If one is removing a small amount of a substance, which is present in large quantities, there is probably little physiological effect. However, if the substance being removed is present in low concentrations, removal could alter the physiological processes being studied. For example, if one were removing glucose from the subcutaneous tissue, the glucose would be replaced very quickly from the blood circulating through the capillaries. However, if one were studying neurotransmitters in the brain, removing significant amounts may alter feedback mechanisms and invalidate the phenomenon being studied. In this case one wants to minimize absolute recovery.

In this study in vitro recovery was determined for sodium, potassium, chloride, glucose and lactate with ultrafiltration (UF) and dialysis loop (DL) microdialysis probes. These analytes are commonly measured in routine blood tests. They are altered in many disease processes and their concentrations may also be affected by pharmacological agents. These analyte concentrations are also altered under a variety of other conditions (Glucose is utilized and lactate is produced by muscular contractions). Electrolyte shifts can occur during altitude changes and from the conditions of microgravity during space flight. Membrane probes provide a useful tool for studying these changes in animal models ranging from small rodents to large mammals. In the future, membrane probes may prove useful for on-line monitoring of these variables in clinical situations.

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 which will be needed.

F1

The in vitro recovery apparatus provides temperature control and stirring for the test solution.



T1

Test concentrations for in vitro recovery.

Analyte	Low	Normal	High
Sodium	110 meq/L	140 meq/L	180 meq/L
Potassium	2 meq/L	4 meq/L	7 meq/L
Chloride	70 meq/L	100 meq/L	130 meq/L
Lactate	1 mM	5 mM	10 mM
Glucose	30 mg/dL	100 mg/dL	400 mg/dL

Methods

The instrumentation for the in vitro recovery is illustrated in ***F1***. Since temperature affects the probe recovery, in vitro studies should be done at the temperature at which the probes will ultimately be used. For in vivo studies, the temperature is usually 37 °C. In order to prevent concentration gradients from developing during the study, the solution should be stirred. The heating stirring unit has a well that is large enough to hold a 20 mL scintillation vial. This is a conveniently sized container for in vitro recovery studies. The container is small enough so that excessive amounts of expensive analytes are not required, and it is large enough so that concentration changes in MD and volume changes in UF do not significantly affect results. The temperature is controlled by a BAS LC-22C Temperature Controller.

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 in the recovery apparatus and held in place with tubing clips. 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 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. ***T1*** lists the test concentrations for this study.

Analyte Analyses

Sodium was analyzed with the Cardy Compact Ion Meter (Horiba, Japan) for sodium (C-122), and potassium was analyzed with the Cardy Compact Ion Meter for potassium (C-131). Chloride was analyzed spectrophotometrically using the Sigma Kit 461-3. Lactate was analyzed using the Sigma Kit 735-10. Glucose was analyzed by the BAS LC method using a glucose oxidase immobilized enzyme reactor and a "wired" peroxidase electrode (5,6).

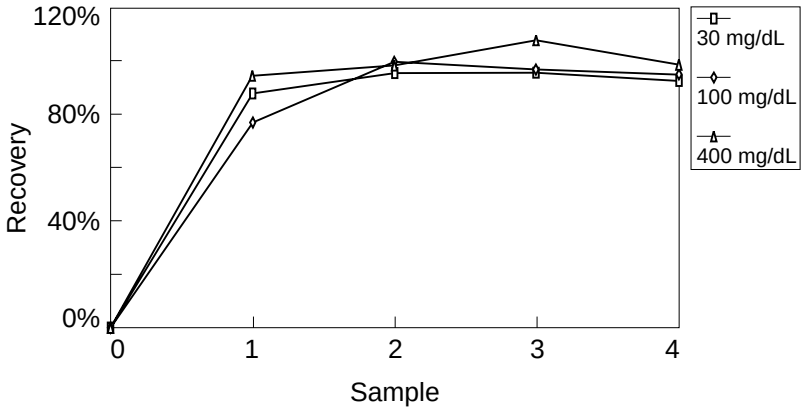
Results and Discussion

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.

F2 shows the recoveries for one UF probe in the three different glucose test solutions. The sample labeled zero is the flush solution.

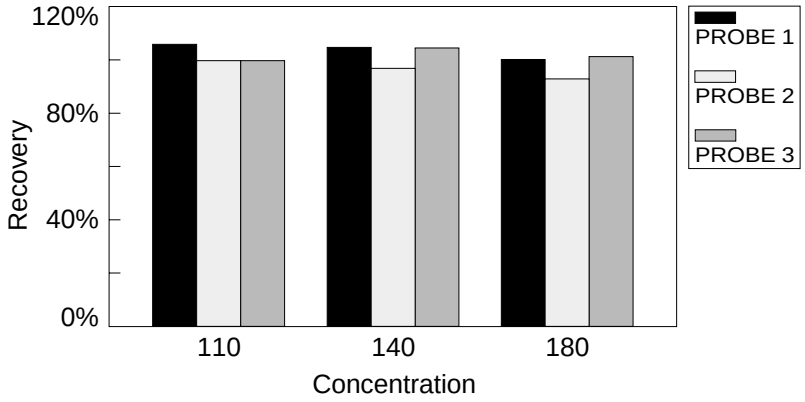
F2

Recovery of one UF probe in solutions of three different glucose 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.



F3

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.



T2

In vitro recoveries.

Analyte	UF-3-12	DL-5
Sodium	101% ± 2%	101% ± 2%
Potassium	94% ± 13%	106% ± 4%
Chloride	96% ± 4%	95% ± 7%
Glucose	99% ± 3%	96% ± 3%
Lactate	94% ± 5%	95% ± 7%

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 previous study. Recoveries from the first sample can sometimes appear low. This is caused from the 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 desirable, but not absolutely necessary, for probe recoveries to be the same in all probes of a given type. Recovery studies can be done on individual probes before their intended use, but if one can be confident that in vitro recoveries of all probes are the same, this step can be eliminated.

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. ***F3*** shows the sodium recovery for each UF probe for each concentration tested.

For each analyte, two-way ANOVAs without replication (α=5%) were done at each concentration on probe by sample, and on each probe for sample number by concentration. For sodium, glucose and lactate, there were no significant effects of the sample, probe or concentration for ultrafiltration or microdialysis probes at the p = 0.1 level. For potassium, there was no significant effect of concentration or sample for ultrafiltration or microdialysis probes, but there was a significant difference between probes. For chloride, there was no significant effect of any of the factors in the analysis of the UF probes. For the MD probes, there was no significant effect of the sample or probe but there was a significant effect of concentration.

The recoveries of each analyte are summarized in ***T2***. Both UF and DL probes have high recoveries, making either probe suitable for in vivo studies of these analytes. Choosing a probe for an in vivo study would be made on factors other than recovery. These include the tissue being sampled, the duration of the study, the flow rate, and the sample size required.

During the course of these recovery studies, it became obvious that the Cardy meters and commercial ion selective electrodes were not ideal for use with ultrafiltration and microdialysis in vivo samples. Meters which express concentrations in ppm do not have the sensitivity to detect significant physiological changes. A difference of 1 ppm may be as much as 5 meq/L and therefore, physiological changes could be missed or lost in the random variations of the meters. Because the samples collected from in vivo probes tend to be small in volume, conventional ion selective electrodes may be too large for reliable measurement. Therefore, with the idea for making microdialysis

and ultrafiltration techniques useful for electrolytes, we are exploring the development of miniature electrodes suitable for use with membrane probe samples.

Acknowledgement

This research was funded by NASA research grant: NAGW 4525.

References

1. *E.M. Janle, P.T. Kissinger, Microdialysis and Ultrafiltration Sampling of Small Molecules and Ions from In Vivo Dialysis Fibers, AACC TDM/Tox 14 (1993) 159-165.*
2. *E.M. Janle, S.R. Ash, Comparison of Urea Nitrogen and Creatinine Concentrations in Dog Plasma and Ultrafiltrate Samples. Current Separations 12 (1994) 169-171*
3. *E.M. Janle, P.T. Kissinger, J.F. Pesek, Short interval monitoring of glucose in Zucker Diabetic Fatty (ZDF) rats. Current Separations 14 (1995) 58-63.*
4. *M.C. Linhares, P.T. Kissinger, Determination of Endogenous Ions in Intercellular Fluid Using Capillary Ultrafiltration and Microdialysis Probes. J. Pharmaceutical and Biomedical Analysis 11 (1993) 1121-28.*
5. *E.M. Janle, S.R. Ash, W.E. Zopp, P.T. Kissinger, Determination of Glucose in Microliter Samples of In Vivo Ultrafiltrates and Microdialysates Using Amperometric Flow Injection Analysis With an Enzymatic Reactor. Current Separations 12 (1993) 14-16.*
6. *L. Yang, E.M. Janle, T. Huang, J. Gitzen, P.T. Kissinger, M. Vreeke, A. Heller, Applications of "Wired" Peroxidase Electrodes for Peroxide Determination in Liquid Chromatography Coupled to Oxidase Immobilized Enzyme Reactors. Anal. Chem. 67 (1995) 1326-31.*