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Comparison of recovery and delivery in vitro for calibration of microdialysis probes

Yeping Zhao, Xiaozhong Liang, Craig E. Lunte *

Department of Chemistry and Center for Bioanalytical Research, University of Kansas, Lawrence, KS 66045, USA

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Abstract

One approach to calibrating a microdialysis probe in vivo is to determine the amount of a test compound that diffuses out of the membrane relative to the amount in the perfusate (i.e., the probes delivery) and assume that the same relation exists for the amount of analyte that will diffuse into the probe relative to the concentration in the sample (i.e., the probes recovery). This concept was systematically tested in vitro with a wide range of compounds, microdialysis probe configurations, dialysis membrane types, and solution conditions. Recovery was found to depend on the identity of the analyte, the type of dialysis membrane, the membrane length, the perfusion flow rate, and the perfusate pH. Recovery was independent of the analyte concentration. No statistically significant difference was found between the recovery and delivery of a given compound with a given probe under any of the conditions tested.

Keywords: Microdialysis sampling; Membranes; Calibration

1. Introduction

Microdialysis sampling has become an important technique for studying neurochemical release in the brain [1] and is gaining acceptance in sampling from peripheral tissues [2–6]. Microdialysis sampling is accomplished by implanting a short length of hollow fiber dialysis membrane at the site of interest. This fiber is slowly perfused with a sampling solution. If a concentration difference exists between the probe lumen and the surrounding tissue small molecules will diffuse in or out of the microdialysis probe. If the concentration of the compound is higher in the tissue than in the perfusate, net mass transport will be into the probe. This is termed a recovery experiment. On the other hand, if the concentration is higher in the perfusate than in the surrounding tissue net mass transport will be out of the probe. This is termed a delivery experiment.

The perfusate flow rate, although typically in the range of 1 to 5 μl/min, is typically too fast for equilibration to occur between the perfusate and surrounding tissue. Rather the concentration determined in the dialysate is some fraction of the actual tissue concentration. The ratio of the dialysate concentration to the tissue concentration is termed the recovery of the microdialysis probe and is the calibration factor needed for quantitative microdialysis sampling. While the recovery of a microdialysis probe can readily be determined in vitro using samples of known concentration, it is not possible to

* Corresponding author.

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directly determine recovery in vivo because the sample concentration is not known. Unfortunately, the recovery of a microdialysis probe is a function of the sample matrix and therefore recoveries determined in vitro are generally not valid in vivo. Several approaches for determining the recovery in vivo have been described [7–10]. All of these methods require several hours to perform during which the in vivo concentration must remain constant and are therefore not generally applicable. Olson and Justice [11] have reported an in vivo calibration procedure for following transient concentrations. This method relies on interanimal reproducibility to overcome the time constraints of the other calibration procedures.

Another approach to calibration is to determine the delivery of the analyte and assume that the recovery is identical. The delivery is the ratio of the concentration lost from the perfusate relative to the initial perfusate concentration when the sample concentration is zero. Because determination of delivery requires only the perfusate concentration and the dialysate concentration, it is readily determined for both in vitro and in vivo experiments. If the relationship between the delivery and recovery can be established, determination of the delivery in vivo would provide the necessary recovery value for calibration of the microdialysis probe.

While there are examples of both recovery and delivery being determined for specific analytes, no systematic examination of this relationship has been reported. In this report, we investigate the recovery and delivery in vitro of a wide range of compounds under a variety of solution and sampling conditions.

2. Experimental

2.1. Reagents

Antipyrine, 3-aminophenol, phenol, acetaminophen, resorcinol, vanillic acid, syringic acid, acetylsalicylic acid, *p*-coumaric acid, 3,5-dimethoxy-4-hydroxycinnamic acid (synapic acid), 4-aminosalicylic acid, (±)-phenylpropanolamine, procaine, theophylline, caffeine, and arterenol were obtained from Sigma (St. Louis, MO). All chemicals were of reagent grade or better and used as received.

2.2. Microdialysis system

Flexible cannula microdialysis probes [3] and linear microdialysis probes [5] were constructed as described previously. The length and type of membrane were varied as described in the results. Three types of membrane were used. Cuprophan (CUP-1) is a regenerated cellulose membrane with a molecular weight cut-off (MWCO) of 12 kD and dimensions of 222 μm o.d. and 200 μm i.d. DOW is a cellulose acetate membrane with a MWCO of 5 kD and dimensions of 250 μm o.d. and 232 μm i.d. PAN is a polyacrylonitrile membrane with a MWCO of 29 kD and dimensions of 340 μm o.d. and 240 μm i.d.

Microdialysis sampling was performed with a CMA/100 microinjection pump (Bioanalytical Systems, West Lafayette, IN) connected to the microdialysis probe with PTFE tubing. The microdialysis probe was immersed in a 60 ml beaker containing the sample. The beaker was placed in a dry heat block maintained at 37°C. Dialysates were collected in 250 μl polypropylene microcentrifuge tubes.

2.3. Determination of recovery

A standard solution of the analytes of interest in Ringer's solution at pH 7.4 was placed in a 60 ml beaker. The beaker was thermostatted at 37°C using a dry heat block. The solution was stirred using a stir plate placed under the dry heat block. A microdialysis probe was placed in the standard solution and perfused with Ringer's solution at a rate of 1 μl/min unless otherwise specified. The system was first flushed for 45 min then dialysate was continuously collected over 20 min intervals. Dialysate samples were collected until six sequential samples exhibited no trend in concentration and had no greater than 5% R.S.D. upon chromatographic analysis. The recovery was determined as the ratio of the concentration of the analyte in the dialysate to the concentration in the standard solution. According to:

$$R = \frac{C_d}{C_s} \times 100$$

where C_d is the concentration in the dialysate and C_s is the concentration in the sample.

Table 1
Properties of the compounds used in this study

Compound	Solubility (mg/ml water)	pK_a (25°C)	Charge form (at pH 7.4)	Ref. (pp)
Acetaminophen	14.3	9.5	Neutral	[15] 849
Antipyrine	1000	1.5	Neutral	[15] 872
Caffeine	16.7	0, 3.6	Neutral	[15] 421
Phenol	76.9	10	Neutral	[15] 488
Resorcinol	1000	9.5, 10.1(20°C)	Neutral	[15] 959
Arterenol	400	8.6, 9.8, 12	Cation	[15] 819
3-Aminophenol	25	9.0, 8.9 (21°C)	Cation	[16] 75; [17] 455
(±)-Phenylpropanolamine	400	9.4 (20°C)	Cation	[15] 895
Procaine	1000	9	Cation	[15] 925
Theophylline	8.33	< 1, 8.6	Cation	[15] 1011
4-Aminosalicylic acid	1.67	1.8, 3.6	Anion	[15] 343
Acetylsalicylic acid	3.33	3.5	Anion	[15] 361
p-Coumaric acid	< 1		Anion	[16] 2565
Sinapic acid			Anion	
Syringic acid	< 1		Anion	[18] 7–352
Vanillic acid	1.16	4.52	Anion	[16] 9840

2.4. Determination of delivery

A system identical to that described for the recovery determinations was used except that a Ringer's solution at pH 7.4 was used as the sample. For determination of delivery, the perfusion solution contained known concentrations of the analytes. A microdialysis probe was placed in the Ringer's solution and perfused with the analyte solution in Ringer's solution at 1 μl/min unless otherwise specified. The system was first flushed for 60 min then dialysate was continuously collected over 20 min intervals. Dialysate samples were collected until six sequential samples exhibited no trend in concentration and had

Table 2
Concentration dependence of recovery and delivery

Compounds	%Recovery ($n = 3$)				%Delivery ($n = 3$)			
	25 μM	50 μM	100 μM	300 μM	25 μM	50 μM	100 μM	300 μM
Acetaminophen	80.1 ± 1.8	77.2 ± 3.5	79.4 ± 1.2	80.9 ± 0.1	82.0 ± 1.7	76.8 ± 1.6	82.2 ± 3.6	78.4 ± 1.5
Antipyrine	76.7 ± 2.0	81.4 ± 1.4	81.5 ± 2.3	80.6 ± 1.5	85.8 ± 1.5	81.4 ± 2.2	83.4 ± 0.9	82.9 ± 1.4
Caffeine	83.2 ± 1.2	86.9 ± 1.1	85.0 ± 1.7	86.0 ± 1.1	86.3 ± 0.9	82.4 ± 2.1	84.5 ± 0.9	84.1 ± 1.3
Phenol	90.3 ± 0.3	83.9 ± 2.5	88.4 ± 0.7	90.7 ± 0.9	92.6 ± 2.8	88.3 ± 0.8	94.1 ± 1.5	89.2 ± 1.9
Resorcinol	86.6 ± 1.5	82.4 ± 2.9	85.7 ± 1.4	87.9 ± 0.2	87.4 ± 1.5	83.3 ± 5.3	88.0 ± 3.0	82.7 ± 2.5
Arterenol	80.6 ± 3.4	73.1 ± 2.8	65.5 ± 0.8	74.0 ± 0.4	88.7 ± 1.7	69.5 ± 2.3	76.1 ± 1.3	73.9 ± 2.1
3-Aminophenol	91.8 ± 0.5	91.7 ± 1.4	92.2 ± 0.4	91.5 ± 0.7	90.9 ± 1.1	90.5 ± 1.2	89.4 ± 0.5	90.5 ± 0.3
(±)-Phenylpropanolamine	82.1 ± 1.8	83.9 ± 0.8	79.5 ± 1.2	81.3 ± 0.3	81.3 ± 1.6	78.3 ± 0.9	79.0 ± 1.2	77.1 ± 0.3
Procaine	78.2 ± 1.8	78.5 ± 1.1	75.2 ± 1.2	74.8 ± 0.4	78.1 ± 1.5	74.9 ± 1.1	75.9 ± 0.8	73.6 ± 2.1
Theophylline	84.0 ± 1.3	90.1 ± 1.2	88.6 ± 1.8	86.3 ± 1.1	88.1 ± 0.9	83.6 ± 2.2	85.6 ± 0.8	86.3 ± 1.2
4-Aminosalicylic acid	85.6 ± 1.0	85.9 ± 1.9	85.5 ± 0.2	85.7 ± 1.3	86.5 ± 1.2	86.1 ± 1.3	84.8 ± 0.5	86.4 ± 0.2
Acetylsalicylic acid	69.2 ± 1.5	73.4 ± 1.2	75.0 ± 2.3	72.2 ± 1.4	81.2 ± 1.0	76.8 ± 1.9	79.6 ± 0.7	80.2 ± 1.5
p-Coumaric acid	78.9 ± 1.6	77.8 ± 1.1	74.2 ± 1.3	75.1 ± 0.4	85.4 ± 2.0	82.6 ± 1.0	83.9 ± 1.0	78.5 ± 0.6
Sinapinic acid	75.5 ± 1.6	65.3 ± 1.7	69.2 ± 1.1	69.8 ± 0.1	80.9 ± 1.9	74.9 ± 0.7	76.8 ± 1.6	69.9 ± 0.5
Syringic acid	76.9 ± 1.0	77.5 ± 2.5	74.5 ± 0.3	78.1 ± 1.7	75.5 ± 1.4	74.3 ± 2.0	72.1 ± 0.5	72.3 ± 0.3
Vanillic acid	79.1 ± 0.9	79.3 ± 2.2	78.4 ± 0.3	79.6 ± 1.6	81.1 ± 1.8	80.2 ± 2.1	76.0 ± 0.4	77.0 ± 0.3

Table 3
Ratio of recovery to delivery

Compounds	Ratio
Acetaminophen	0.99 ± 0.04
Antipyrine	0.96 ± 0.04
Caffeine	1.01 ± 0.03
Phenol	0.97 ± 0.04
Resorcinol	1.00 ± 0.05
Arterenol	0.96 ± 0.10
3-Aminophenol	1.02 ± 0.02
(±)-Phenylpropanolamine	1.03 ± 0.03
Procaine	1.01 ± 0.04
Theophylline	1.02 ± 0.04
4-Aminosalicylic acid	1.00 ± 0.02
Acetylsalicylic acid	0.93 ± 0.04
p-Coumaric acid	0.93 ± 0.04
Sinapic acid	0.93 ± 0.06
Syringic acid	1.04 ± 0.04
Vanillic acid	1.01 ± 0.04

Values are mean ± S.D., $n = 12$.

no greater than 5% R.S.D. upon chromatographic analysis. The delivery was determined as the ratio of the loss in concentration from the perfusate to the initial concentration in the perfusate. According to:

$$D = \frac{C_p - C_d}{C_p} \times 100$$

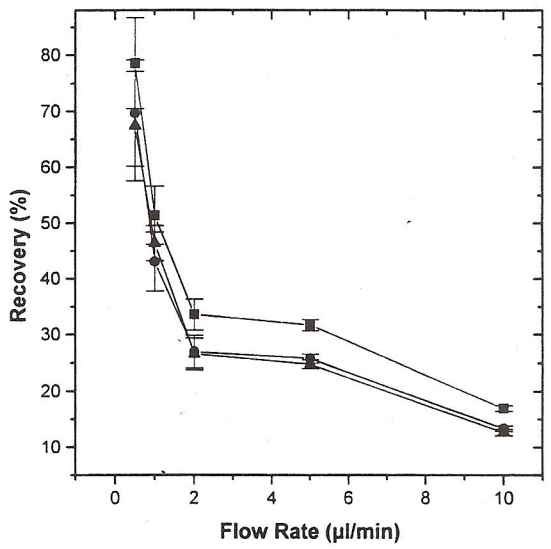

Fig. 1. The relationship between recovery and perfusate flow rate. Symbols are: ■, 3-aminophenol; ▲, caffeine; ●, theophylline.

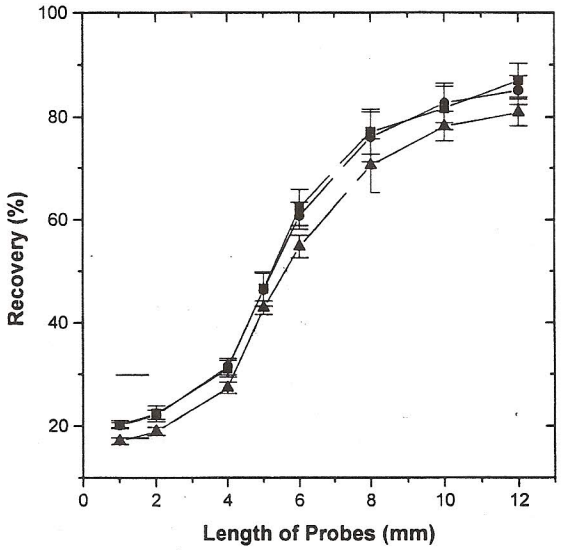

Fig. 2. The relationship between recovery and probe length. Symbols are: ■, acetaminophen; ▲, vanillic acid; ● caffeine.

where C_d is the concentration in the dialysate and C_p is the concentration in the perfusate.

2.5. Chromatographic system

Dialysis samples were analyzed by HPLC. The chromatographic system consisted of a Shimadzu LC-6A pump with an SCL-6A system controller (Shimadzu, Columbia, MD). Detection was by absorbance at 225 nm using a Shimadzu PD-6AV UV–visible detector connected to a Datajet Integrator (Spectraphysics, San Jose, CA). A 5 µl sample loop was used for all experiments. Separation was achieved on a Sepstik ODS 3 µm particle microbore column (100 × 1 mm) from Bioanalytical Systems

Table 4
Recovery/delivery ratio versus perfusion flow rate

Flow rate (µl/min)	3-Aminophenol	Theophylline	Caffeine
0.5	1.05 ± 0.11	1.08 ± 0.12	1.10 ± 0.11
1	1.06 ± 0.06	1.10 ± 0.05	1.00 ± 0.04
2	0.98 ± 0.04	0.90 ± 0.02	0.90 ± 0.03
5	0.95 ± 0.01	0.92 ± 0.01	0.93 ± 0.01
10	0.98 ± 0.04	0.95 ± 0.02	0.96 ± 0.03

Values are mean ± S.D., $n = 3$.

using a mobile phase of 0.05 M ammonium phosphate buffer, pH 2.5, with 15% (v/v) acetonitrile. A flow rate of 50 μl/min was used in all experiments.

3. Results and discussion

3.1. Concentration dependence of recovery and delivery

The test compounds were chosen to provide a wide range of hydrophobicities and charge states (Table 1). Recovery and delivery of all test compounds were determined over the concentration range of 25 μM to 300 μM using a flexible microdialysis probe with a 10 mm CUP-1 membrane. Recoveries of the test compounds varied from 92% to 65% and deliveries varied from 94% to 69% (Table 2). The data were evaluated by the one-way ANOVA method. All p-values were greater than 0.3 indicating no significant difference between recoveries (or deliveries) for a given compound at different concentrations. Statistical analysis also showed no significant difference between the recovery and delivery of a compound. The ratio of recovery to delivery for the sixteen test compounds is shown in Table 3.

3.2. Flow rate and membrane length dependence of recovery and delivery

As has been reported previously [12–14], the recovery is a function of the perfusion flow rate and length of the dialysis membrane. As shown in Fig. 1, the recovery decreases with increases flow rate in an approximately exponential fashion. However, as seen from the values in Table 4, the ratio of recovery to delivery is independent of flow rate. Recovery increases with increasing length of the dialysis membrane as shown in Fig. 2. As the recovery approaches 100% increasing the length of the membrane becomes increasingly less effective at increasing the recovery. The asymptotic approach to 100% recovery is expected as the concentration in the dialysate approaches that in the sample. The reason for the nonlinear behavior at short membrane length is less obvious. It is thought to arise from turbulent flow at the inlet and outlet where the dialysis membrane is joined to the connecting tubing increasing mass transport in the dialysate in these regions. This effect becomes dominant at shorter lengths of membrane while a longer lengths a laminar flow regimen dominates. As with flow rate, the ratio of recovery to delivery is independent of the length of the dialysis membrane (Table 5). Therefore, variation of the

Table 6
Effect of dialysis membrane type on %recovery

Compound	Cuprophan	DOW	PAN
Acetaminophen	46.6±3.3	40.3±2.3	37.6±1.2
Caffeine	46.4±3.2	39.1±2.2	30.2±0.9
Phenol	75.7±4.8	65.9±1.8	43.5±1.6
Resorcinol	63.7±3.8	50.8±0.8	47.6±0.7
Arterenol	48.7±2.0	35.2±2.1	19.6±2.1
Theophylline	57.4±4.0	42.6±1.4	28.8±0.8
p-Coumaric acid	57.2±2.7	48.9±0.8	25.7±0.9
Syringic acid	43.9±5.9	25.4±0.7	10.9±0.8
Vanillic acid	42.9±1.3	25.1±2.3	12.5±0.5

Values are mean±S.D., $n = 3$.

Table 5
Recovery/delivery ratio versus dialysis membrane length

Length (mm)	Acetaminophen	Caffeine	Vanillic acid
1	1.04±0.01	1.34±0.02	0.97±0.01
2	0.86±0.02	0.90±0.01	0.88±0.01
4	0.97±0.06	1.02±0.06	0.99±0.04
5	1.04±0.04	1.00±0.04	1.00±0.03
6	0.93±0.04	0.93±0.03	0.91±0.03
8	1.01±0.07	1.02±0.08	1.03±0.08
10	0.99±0.08	1.00±0.09	1.02±0.06
12	1.03±0.06	1.03±0.06	1.01±0.10

Values are mean±S.D., $n = 3$.

Table 7
Recovery/delivery ratio for different dialysis membrane types

Compound	Cuprophan	DOW	PAN
Acetaminophen	1.04±0.04	0.95±0.03	0.97±0.02
Caffeine	1.00±0.04	0.96±0.03	0.95±0.02
Phenol	1.03±0.02	1.04±0.04	0.92±0.06
Resorcinol	0.99±0.09	0.95±0.03	0.99±0.02
Arterenol	1.09±0.07	0.97±0.06	0.86±0.05
Theophylline	1.01±0.09	0.94±0.02	0.93±0.02
p-Coumaric acid	0.98±0.03	1.08±0.04	0.90±0.04
Syringic acid	0.92±0.07	0.93±0.02	0.91±0.01
Vanillic acid	1.00±0.03	0.81±0.04	0.86±0.04

Values are mean±S.D., $n = 3$.

Table 8
Effect of microdialysis probe geometry on %recovery

Compound	Probe length					
	10 mm		6 mm		3 mm	
	Flexible	Linear	Flexible	Linear	Flexible	Linear
Acetaminophen	58.1 ± 5.5	78.6 ± 3.9	26.4 ± 1.5	58.2 ± 2.6	18.6 ± 2.4	36.0 ± 1.3
Caffeine	58.3 ± 4.6	83.6 ± 2.9	28.0 ± 1.6	65.2 ± 2.7	20.7 ± 1.7	46.5 ± 2.3
Phenol	68.6 ± 4.8	94.2 ± 2.9	40.7 ± 2.2	90.8 ± 3.0	28.7 ± 3.3	84.7 ± 2.6
3-Aminophenol	66.9 ± 4.1	89.8 ± 3.5	35.4 ± 2.8	75.5 ± 2.8	25.2 ± 1.7	54.0 ± 2.2
Theophylline	57.0 ± 4.3	83.8 ± 3.2	28.5 ± 2.0	65.5 ± 4.1	20.3 ± 2.1	41.8 ± 1.8
Syringic acid	51.5 ± 4.0	73.4 ± 2.6	22.2 ± 1.3	52.3 ± 1.6	15.9 ± 1.8	32.0 ± 1.1

Values are mean ± S.D., $n = 3$.

perfusion flow rate and length of the dialysis membrane can readily be used to adjust recovery to a desired value.

3.3. Dialysis membrane dependence of recovery and delivery

The different membrane materials available have slightly different chemistries. While all are hydrophilic, PAN is highly negatively charged, DOW has a slight negative charge, and CUP-1 is neutral. The PAN membrane also has a much thicker wall (ca. 50 μm) than either the CUP-1 or DOW (ca. 10 μm) membranes. However, the MWCO of the PAN membrane (29 kD) is nearly six times that of the DOW membrane (5 kD) and over twice that of the CUP-1 membrane (12 kD). In addition, PAN membranes have asymmetrical pores while the cellulose based membranes have symmetrical pores. The recovery of test compounds that included anionic, cationic, and neutral species at pH 7.4 were determined for flexible probes with 10 mm lengths of the various membrane types. For all compounds the recovery decreased in the order CUP-1 > DOW > PAN demonstrating the importance of membrane thickness on recovery (Table 6). The lower recoveries for the DOW membrane illustrate that the MWCO of the membrane is important even for relatively small molecules due to the non-equilibrium nature of microdialysis sampling. In addition, the recovery of anionic compounds was considerably reduced for the PAN membrane relative to neutral or cationic compounds. This trend was also seen for the DOW membrane although to a lesser extent. These observations show the influence of the membrane chemistry on recovery. It is insufficient to view the dialysis membrane as a simple inert diffusion barrier, rather interactions do occur between the membrane and solutes. Again, although significant differences are observed in the recovery using different membrane types, no significant difference was observed between the recovery and delivery for a given membrane (Table 7).

3.4. Comparison of flexible and linear microdialysis probe geometries

A number of microdialysis probe geometries have been described for sampling from various sites. The most commonly used are the cannula type (represented by the flexible design in this study) and the linear type. Using the same length of CUP-1 dialysis fiber, linear microdialysis probes consistently gave higher recoveries than flexible probes (Table 8). This may be a result of the larger volume in the fiber for linear probes relative to flexible probes. In the flexible probe design the inner cannula extends well into

Table 9
Recovery/delivery ratio for linear probes

Compound	10 mm	6 mm	3 mm
Caffeine	0.97 ± 0.01	1.00 ± 0.07	0.92 ± 0.05
Phenol	0.96 ± 0.00	0.97 ± 0.02	0.95 ± 0.01
3-Aminophenol	0.98 ± 0.01	0.98 ± 0.02	0.94 ± 0.04
Theophylline	0.97 ± 0.01	0.99 ± 0.03	0.90 ± 0.06
p-Coumaric Acid	0.96 ± 0.03	0.98 ± 0.09	0.95 ± 0.12
Syringic acid	0.96 ± 0.01	1.03 ± 0.06	0.88 ± 0.08

Values are mean ± S.D., $n = 3$.