Microdialysis - principles of recovery

Recovery - definition of terms

The dialysing properties of the microdialysis probe can be expressed as its recovery for a particular substance.

By comparing the concentration of the substance in the microdialysis probe effluent with the concentration of the medium it is possible to calculate the recovery of the substance.

Relative recovery will approach 100% as the flow rate approches zero, and decrease as the flow rate increases. It is commonly expressed in percent.

Absolute recovery is defined as the mass of a substance recovered during a defined time period . It is zero when the flow rate is zero, and will reach a maximum at higher flow rates, as shown in Fig. 1.

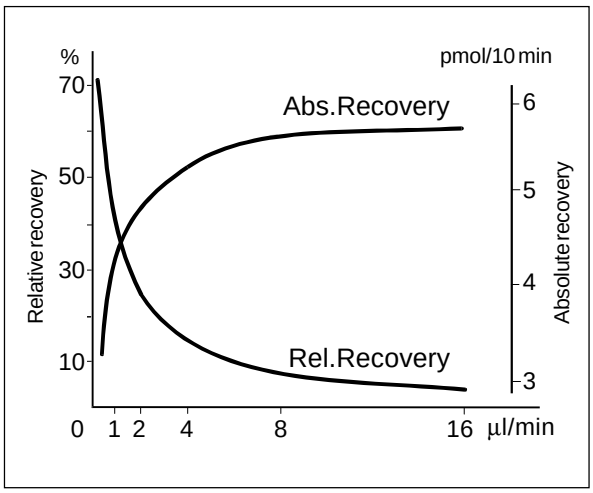

*Fig.1. The **relative** and **absolute** recoveries for Dopamine as a function of flow rate. **CMA/10 Microdialysis Probe,** 4 mm membrane.*

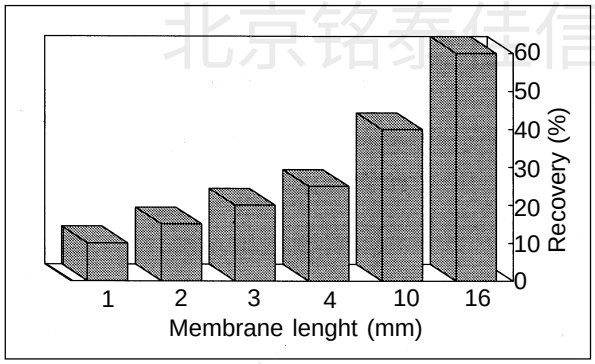

*Fig.2. Effect of **membrane length** on recovery Experimental conditions: **CMA/12 Microdialysis Probes** Temperature: +20 ° C Flow rate 2 µl/min Test substance: Dopamine*

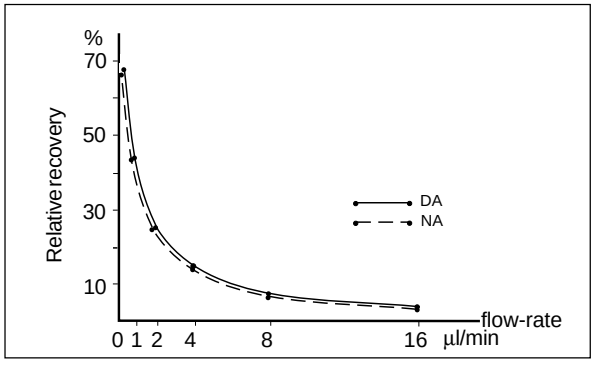

*Fig.3. Effect of flow rate on recovery Experimental conditions: **CMA/12 Microdialysis Probe** with 4 mm membrane, Temperature: +20 ° C Test substances: Dopamine, Noradrenaline*

The recovery of substances in vitro is depending upon:

- length and diameter of the membrane (larger area - higher recovery)
- flow rate
- temperature
- molecular weight of the substance
- molecular shape of the substance
- molecular charge
- binding to the membrane and tubing

Other factors such as pH of the medium and degradation of the substance may also affect the recovery.

Before measuring endogenous substances, it is always advisable to perform an in vitro experiment, to establish the dialysing properties of the microdialysis probe for the particular substance(s) of interest.

It is also possible to study the condition of the probe from day to day by in vitro test.

APPLICATION NOTE

Recovery experiment

Materials:
CMA/10 Microdalysis Probe 4 mm membrane
CMA/100 Microinjection Pump
CMA/130 In Vitro Stand
CMA/140 Microfraction Collector
Microsyringe 1 ml
Perfusion liquid: Ringer´s solution 147 mM Na^+, 2.4 mM Ca^{2+}, 4 mM K^+, 155.6 mM Cl-, pH 6.0
Medium: Catecholamines, 10^{-6} M

Three samples were collected from the microdialysis probe effluent, in 300 µl polyethylene vials in 10 min fractions, and compared with samples taken from the medium.

The samples were analyzed by liquid chromatography with electrochemical detection. See Fig. 4.

The medium consisted of catecholamines dissolved in Ringer, at a concentration of 10^{-6} M in an Eppendorf vial in the CMA/130 In Vitro Stand.

The CMA/10 Microdialysis Probe was prepared according to the instruction in the package.
The probe was perfused with 2 µl/min. for 30 min. to equlibrate the system before starting the collection of samples.

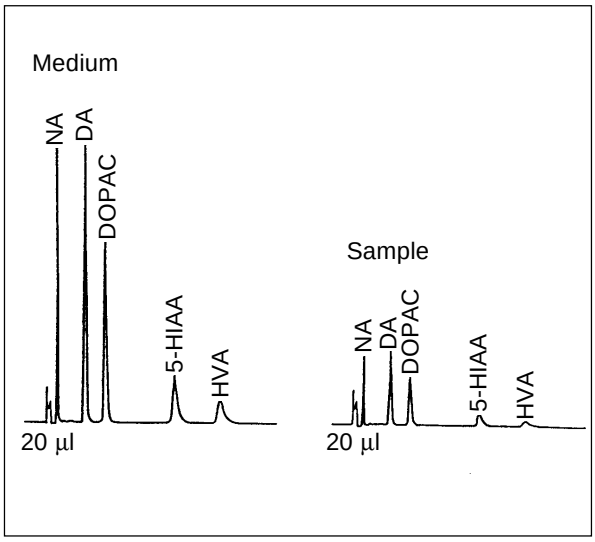

Fig.4.

In Vitro recovery +20 °C

Substance	Membrane length 2mm	3mm	4mm
Dopamine	14%	19	24
DOPAC	15	19	24
5-HT	13	17	24
5-HIAA	14	17	24
HVA	13	19	24
Noradrenaline	14	18	23
Na^+	30	39	49
K^+	48	61	71
Pyruvate	26	38	45
Lactate	23	34	39
Hypoxanthine	17	23	31
Inosine	11	17	21
Guanosine	12	16	21
Adenine	15	21	29
Adenosine	10	14	19
Glucose	13	18	22
Acetylcholine	17	24	27
Choline	20	26	31
Aspartate	13	16	24
Asparagine	14	17	25
Glutamate	13	16	24
Serine	12	14	22
Glutamine	14	16	24
Taurine	18	22	27
Tyrosine	14	16	20
GABA	17	21	25
α-aminobutyric acid	16	20	30
Tryptophan	14	19	27
Methionine	14	18	27
Valine	14	18	26
Phenylalanine	14	18	26
Isoleucine	13	18	26
Leucine	14	19	26
Insulin		4	
Glucagon		1	
Somatostatin			6
VIP			6

Substance	Membrane length 2mm	5mm
Angiotensin	9.4%	19
AVP	9.1	18.3
β-Endorphin	1.4	3
Bombesin	8.1	16.6
CCK-8	6.2	12.7
CRF	1.6	
Dynorphin 1-17	3.3	6.5
LHRH	8	15.6
[Leu]enkephalin	10.5	20.9
[Met]enkephalin	13	24.8
Neurotensin	6.3	12
NPY	0.7	1.5
Oxytocin	8.6	16.4
Substance K	9.1	18
Substance P	7.5	15.5
TRH	11.8	19.4

Tab.1. In vitro recovery.
Flow rate 2 µl/min. Temperature: +20 °C

Molecular weight and recovery

Linear relationship between the log % recovery (in vitro) shown by a CMA/10 Microdialysis Probe and the molecular weight of the substance sampled indicates an exponential relationship between these two factors.

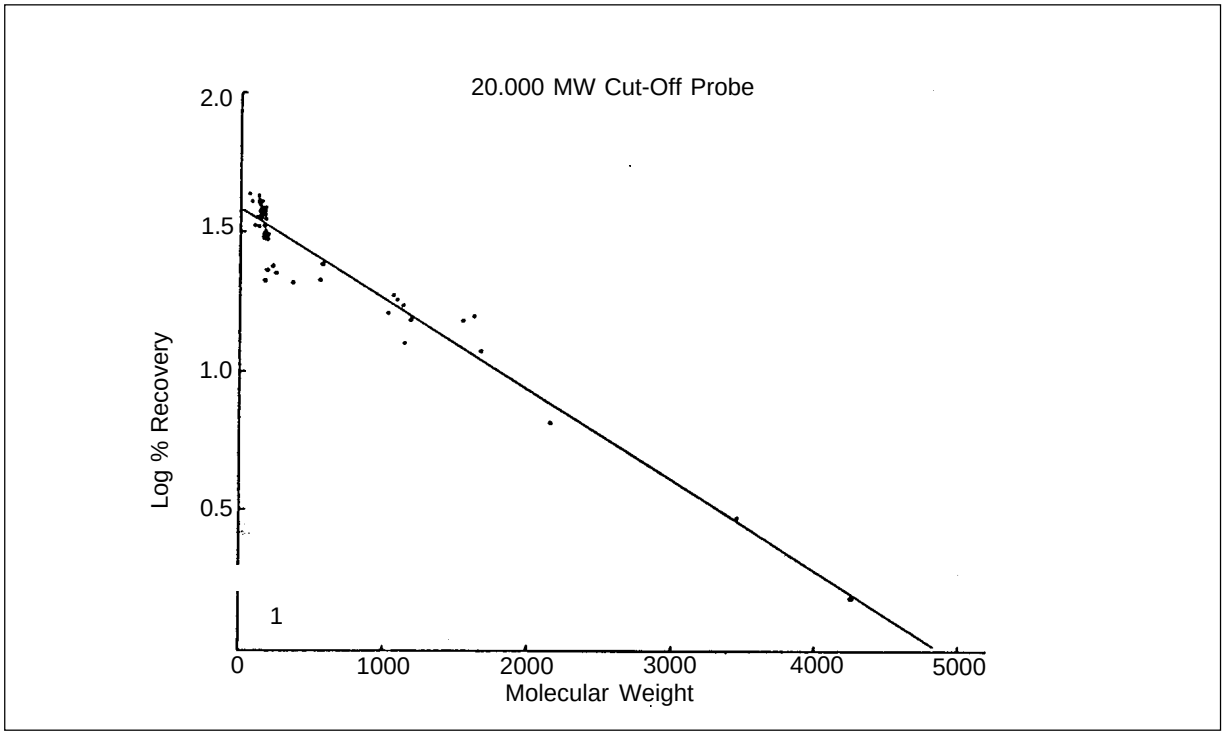

Fig. 5. Recoveries of 42 different substances are plotted. Recovery is minimal at approximately 5000 MW, even though the membrane´s nominal cut-off is 20 000. CMA/10 Microdialysis Probe, 5 mm membrane. Flow rate 2 µl/min.

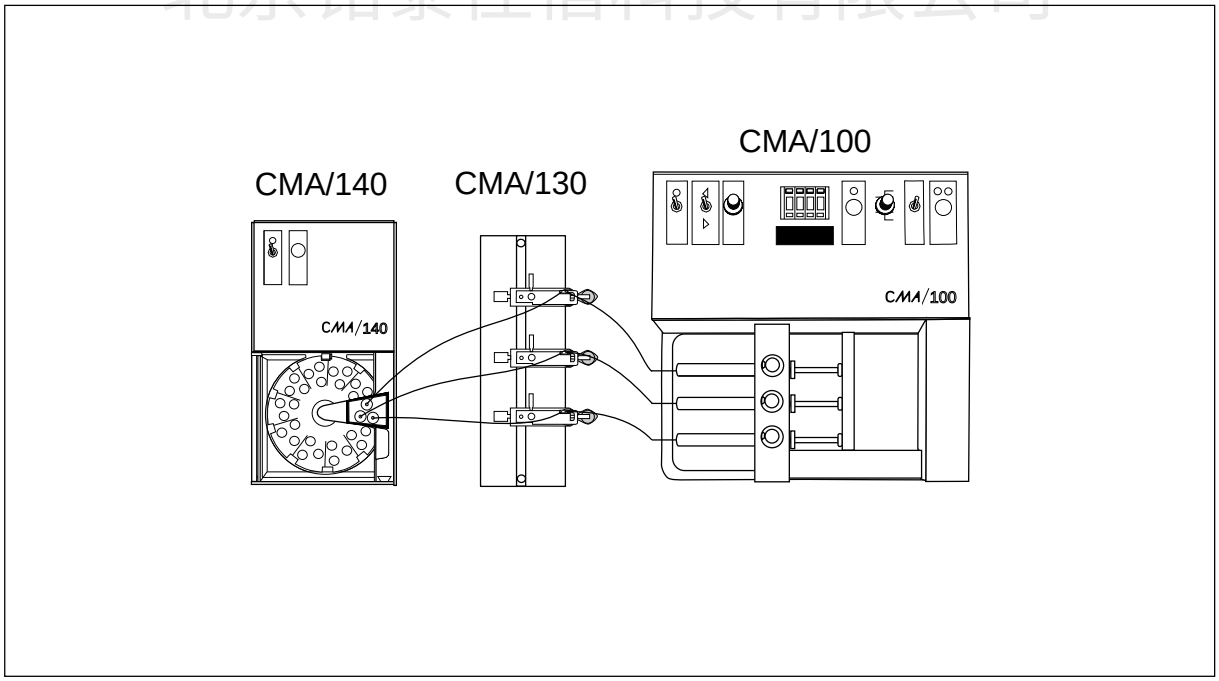

Fig. 6. A typical setup for an in vitro recovery experiment.

(Data on peptides in Tab. 1. and Fig. 5. kindly provided by Dr. K. Kendrick, A.F.R.C. Institute on Animal Physiology and Genetics Research, Babraham,Cambridge U.K.)

If you require further details on Microdialysis procedures, HPLC analysis, instrumentation or bibliography, please do not hesitate to contact:

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