Electroosmotic Sampling. Application to Determination of Ectopeptidase Activity in Organotypic Hippocampal Slice Cultures.

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Abstract. The SI includes the derivation procedure of enzyme rate based on GGFL production or
YGGFL loss.

SUPPORTING INFORMATION

1. The moles of internal standard (IS) collected

$$mol_{IS} = U \times t_s \times [IS] \quad (1)$$

Where U is the sampling flow rate, t_s is sampling time and $[IS]$ is the initial concentration of IS in bath.

$$mol_{IS} = A_{IS}/f_{IS} \quad (2)$$

A_{IS} is the peak area of IS and f_{IS} is a calibration factor. Thus, U can be expressed as follows,

$$U = A_{IS}/([IS] \times t_s \times f_{IS}) \quad (3)$$

2. Exposure time of peptide to tissue, t_e

$$t_e = V_t/U \quad (4)$$

V_t is the volume of tissue sampled. By substituting Eq. (3) and Eq. (2) into Eq. (4), we obtained

$$t_e = V_t \times f_{IS} \times t_s \times [IS]/A_{IS} \quad (5)$$

and

$$t_e = V_t \times t_s \times [IS]/mol_{IS} \quad (5a)$$

3. The moles of substrate/product consumed/produced per unit time with initial rate assumption

$$k = v_{max}/(K_m + [s]) \quad (6)$$

$$\frac{\Delta[s]}{\Delta t} = -k[s] = ([s] \times v_{max})/(K_m + [s]) \quad (7)$$

Thus, the concentration of substrate leaving the tissue $[s]_o$ can be expressed as follows,

$$[s]_o = [s]_i - k \times [s]_i \times t_e \quad (8)$$

or

$$[s]_o = [s]_i(1 - kt_e) \quad (9)$$

$[s]_i$ is the concentration of substrate entering into the tissue.

From Eq. (8) or Eq. (9), we obtained

$$k = (1 - \frac{[s]_o}{[s]_i})/t_e \quad (10)$$

$$[s]_o = \frac{mol_{s,o}}{mol_{IS}/[IS]} \quad (11)$$

Here $mol_{s,o}$ is the moles of substrate leaving the tissue. When substituting Eq. (5a) and (11) into Eq. (10),

$$k = \frac{mol_{IS}}{[IS] \times V_t \times t_s} - \frac{mol_{s,o}}{[s]_i \times V_t \times t_s} \quad (12)$$

Therefore, from the decrease of YGGFL (the substrate) we have

$$k = \frac{A_{IS}}{f_{IS} \times [IS] \times V_t \times t_s} - \frac{A_{s,o}}{f_s \times [s]_i \times V_t \times t_s} \quad (13)$$

In Eq. (13), $[IS]$, $[s]_i$, V_t and t_s are given experimental conditions. Here we assume the sampling volume in the tissue is a cylinder. A_{IS} , $A_{s,o}$, f_{IS} and f_s can be obtained from the analysis of chromatographs of standard and sample solutions. Thus, k can be calculated.

Similarly, from the increasing of GGFL (the product) we have

$$k = \frac{A_p}{f_p \times [s]_i \times V_t \times t_s} \quad (14)$$

We note that the sampled volume is not actually a cylinder, however comparison of rates on the same basis will not be affected by the multiplicative error caused by using an estimated volume.