Enhanced microdialysis recovery of some tricyclic antidepressants and structurally related drugs by cyclodextrin-mediated transport

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The enhanced microdialysis relative recovery (RR) of some hydrophobic tricyclic drugs (imipramine, desipramine, amitriptyline, carbamazepine and promethazine) is discussed. Enhanced RR was achieved by including a binding agent [β-cyclodextrin (β-CD) or 2-hydroxypropyl-β-cyclodextrin (HP-β-CD)] in the microdialysis perfusion fluid to form inclusion complexes with the drugs, which increases the analyte flux through the membrane material. The maximum effect of the RR increase for all the drugs studied was observed using a commercially available polycarbonate–polyether (PC) membrane. With a 4 mm PC membrane and 4.41 mmol l^{-1} (0.5% w/v) β-CD included in the microdialysis perfusion fluid (0.9% saline, pH 7.4) at a flow rate of 0.5 μl min^{-1}, RR enhancements over controls were as follows: carbamazepine 136, imipramine 268, desipramine 298, amitriptyline 634, and promethazine 987%. Increasing β-CD [up to 17.63 mmol l^{-1} (2% w/v)] or HP-β-CD [up to 32.5 mmol l^{-1} (5% w/v)] concentration in the microdialysis perfusion fluid enhanced carbamazepine RR three (β-CD) to four (HP-β-CD) times compared to controls through PC microdialysis membranes. The PC membrane gave enhanced RR values that were twice those for cuprophan or AN-69 membranes. Enhanced RR with cyclodextrins was successfully applied to sampling from a protein solution containing desipramine in a 4% w/v bovine serum albumin solution. These results suggest that addition of cyclodextrins to microdialysis perfusion fluids may be used to increase microdialysis RR during blood sampling.

Introduction

Microdialysis is an effective tool for obtaining protein-free samples from the extracellular fluid (ECF) of tissues[1] or from other complex biological matrices.[2] This sampling method is widely applied in neurochemistry, neurophysiology and pharmacology.[3–5] Microdialysis sampling is a diffusion-based separation process in which analyte flux is controlled by the concentration gradient across the semi-permeable membrane. This flux affects the extraction fraction (E_d)[6] and the relative recovery (RR)[7] of the microdialysis probe and RR are defined as

$$E_d = \frac{C_{inlet} - C_{outlet}}{C_{inlet} - C_{sample}}$$

$$RR = \frac{C_{outlet}}{C_{sample}} \quad (1)$$

where C_{inlet} is the inlet concentration of the analyte in the perfusion fluid, C_{outlet} is the outlet concentration of the analyte in the perfusion fluid and C_{sample} is the analyte concentration in the sample matrix far away from the microdialysis probe. E_d equals RR when C_{inlet} is zero.

E_d is dependent upon different parameters such as perfusion fluid flow rates and tissue diffusive and kinetic properties.[6] Because the perfusion fluid continuously flows through the microdialysis membrane, diffusion of analytes through the membrane occurs under non-equilibrium conditions. This constraint during microdialysis sampling under normal sampling conditions makes it impossible to achieve greater than 100% E_d *in vitro* or *in vivo*.[8]

We have recently described an approach to enhance microdialysis E_d for ibuprofen, an extensively protein-bound drug.[9] Enhanced E_d for ibuprofen was achieved by adding β-cyclodextrin (β-CD) to the microdialysis perfusion fluid. β-CD enhances mass transport into the microdialysis probe by reacting with the analyte, thus trapping it as an inclusion complex with β-CD. This binding reaction causes the unbound concentration (C_L) of the analyte in the dialysate to decrease at the inner membrane wall which increases the analyte concentration gradient across the membrane, the analyte flux through the membrane and the microdialysis relative recovery or extraction fraction. Microdialysis E_d is generally considered to be identical during bi-directional analyte mass transport in microdialysis. In other words, E_d from a recovery experiment where the analyte diffuses from the sample to the dialysate equals E_d for a delivery experiment where the analyte diffuses from the dialysate to the sample. This has been shown to be true *in vitro* during normal microdialysis sampling conditions.[10] Since the binding reaction with cyclodextrin can give an E_d greater than 1, it is possible that when C_{inlet} is a non-zero number that E_d will not be identical in both sampling directions. For the above-mentioned reasons and to prevent confusion in the microdialysis literature, the experiments described here will be referred to as enhanced relative recovery (RR) rather than enhanced E_d.

It is known from the chemical engineering and separations literature that diffusion combined with chemical reaction will increase flux through interfaces.[11,12,13] A preliminary mathematical explanation for this enhanced RR process has been derived previously:[9]

$$\frac{dC_L}{dx} = \frac{2\pi D_{mem}(C_0 - C_L)}{Q \ln(R_o / R_i)} - \frac{\pi R_i^2 k C_L}{Q} \quad (2)$$

In this equation, the change in the free, unbound concentration of the analyte, C_L, *versus* the position of the perfusion fluid in the dialysis probe, x, is related to different experimental conditions which include the following: D_{mem} (cm^2 s^{-1}), the analyte diffusion coefficient through the porous, polymeric dialysis membrane; C_0 (mmol l^{-1}), the external analyte concentration; Q (cm^3 s^{-1}), the volumetric flow rate through the

microdialysis probe; R_o and R_i (cm), the outer and inner radii of the probe membrane, respectively; and k (s^{-1}), a first order formation rate constant between the analyte and β-CD. The ratio of the kinetic rate constant to the volumetric flow rate in the second term illustrates that as the perfusion flow rate is increased, the ability of the binding reaction with β-CD to increase flux through the membrane becomes diminished. The effect of flow rate on enhanced E_d has been described previously.[8] In addition to β-CD increasing the analyte flux by coupling diffusion with chemical reaction, the β-CD inclusion complex of the drug would decrease the ability of the drug to diffuse back across the membrane because of the high molecular weight of this drug–cyclodextrin complex.

It is difficult to apply microdialysis sampling to the *in vivo* analysis of drugs that are either highly protein-bound[14] or hydrophobic in nature.[15] During conditions of high protein binding, free drug concentrations in the extracellular fluid space are low. Determining the concentration of pharmaceuticals in biological matrices with greatly reduced concentrations due to protein binding is a challenging problem in bioanalysis. This problem is further complicated in microdialysis sampling because the RR is often much less than 100%.

Using cyclodextrins in the microdialysis perfusion fluid allows direct analysis of the samples by HPLC methods without additional sample preparation. This is unlike previous work which included albumin in the perfusion fluid to enhance lipid transport through microdialysis membranes.[16] Cyclodextrins are known to be non-toxic and are used extensively in the pharmaceutical industry to improve the formulations and bioavailability of different drugs. The many uses of cyclodextrins in analytical chemistry have been reviewed.[17]

In this work, the effect of the addition β-CD or 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) to the microdialysis perfusion fluid on the RR for amitriptyline, carbamazepine, desipramine, imipramine and promethazine *in vitro* was examined principally with polycarbonate (PC) membranes. The choice of a PC membrane as the principal membrane to study is based on this membrane exhibiting RR greater than 100% for ibuprofen with β-CD included in the perfusion fluid.[9] The inability of other membrane materials to achieve RR much greater than 100% with cyclodextrins in the perfusion fluid is shown. Enhanced RR during microdialysis sampling from a protein solution is demonstrated. The drugs studied are either members of the tricylic antidepressant class (imipramine, desipramine and amitriptyline) or are structurally related to tricyclic antidepressants (carbamazepine and promethazine). Tricyclic antidepressant drugs are generally highly protein bound[18] and form known inclusion complexes with cyclodextrins.[19,20–23]

Experimental

Chemicals

β-CD (pharmaceutical grade) was obtained as a generous gift from Wacker Chemicals (Norwalk, CT, USA). HP-β-CD and promethazine were purchased from Aldrich (Milwaukee, WI, USA). Carbamazepine, imipramine, desipramine and amitriptyline were obtained from Sigma (St. Louis, MO, USA). Acetonitrile and methanol (HPLC grade, Fisher Scientific, Fair Lawn, NJ, USA) were used as received. All other chemicals were of analytical-reagent grade.

Microdialysis

Three different microdialysis probes, each with a 4 mm length membrane, were used in the experiments. The membranes and their manufacturers are as follows: polycarbonate–polyether (PC), CMA-12 (CMA/Microdialysis, Acton, MA, USA), 20 000 molecular weight cutoff (MWCO), 0.5 mm od, 0.4 mm id; Cuprophan (CUP), a regenerated cellulose, CMA-11 (CMA/Microdialysis), 6000 MWCO, 0.24 mm od, 0.19 mm id; and AN-69, a copolymer of polyacrylonitrile and methyl sulfonate, BR-4 (Bioanalytical Systems, Inc., West Lafayette, IN, USA) 29 000 MWCO, 0.34 mm od, 0.24 mm id. Prior to use, the microdialysis probes were prepared according to the manufacture's instructions. A CMA/102 microsyringe pump (CMA/Microdialysis) was used to pump the perfusion fluid through the microdialysis probes. The perfusion fluid consisted of saline (0.9% w/v) buffered with 10 mmol l^{-1} sodium phosphate (pH 7.4) (PBS) with the addition of either β-CD (0.88–17.63 mmol l^{-1}; 0.1–2% w/v) or HP-β-CD (0–32.5 mmol l^{-1}; 0-5% w/v). Sample solutions (10 ml) were also prepared in PBS with a final analyte concentration of 10 μmol l^{-1}. All experiments were performed at ambient room temperature (25 ± 0.5 °C). Analyte solutions were stirred using a Thermolyne (Barnstead, Dubuque, IA, USA) magnetic stirrer at constant stirring rate to reduce solution boundary layer effects and to achieve maximum RR through the membrane.[24] No significant decrease in analyte sample concentrations was observed during extended sampling periods. Unless stated otherwise, perfusion flow rates were varied from high to low.

No-net flux experiments were performed according to the method of Lönnroth *et al*.[25] Carbamazepine was used as the calibration analyte in these experiments. A stirred 10 μmol l^{-1} carbamazepine solution in 10 ml of PBS was used during the no-net flux experiments. Carbamazepine in the concentration range between 0 and 30 μmol l^{-1} was included in the perfusion fluid that contained 4.4 mmol l^{-1} β-CD. The microdialysis perfusion flow rate was 1.0 μl min^{-1}. Inlet concentrations of carbamazepine were randomized

Liquid Chromatography

Dialysate samples were analyzed by HPLC-UV. The chromatographic experiments were performed with Spectra Systems HPLC equipment (Thermo Separation Products, Inc., Riviera Beach, FL, USA) using a column (150 × 2 mm id) packed with Spherex 3 C-8 (Phenomenex, Torrance, CA, USA). The mobile phase of acetonitrile–methanol–water (75 + 8.3 + 16.6 v/v/v) with a final concentration of 0.01 mol l^{-1} sodium phosphate buffer (pH 2.6) was used to assay imipramine, desipramine and amitriptyline at 0.25 ml min^{-1} with detection at 210 nm or promethazine at 0.4 ml min^{-1} and detection at 245 nm. The assay of carbamazepine was performed using a mobile phase of acetonitrile–methanol–water (45 + 5 + 10 v/v/v) with 0.1 mol l^{-1} sodium phosphate buffer (pH 2.6) at 0.18 ml min^{-1} with detection at 210 nm. The injection volume was 5 μl. Analyte concentration was determined by comparing peak area to a calibration curve constructed for each drug. Calibration curves that were prepared with the addition of cyclodextrins to the standards gave linear slopes identical with those of standards injected without cyclodextrin.

β-CD E_d

An 8.8 mmol l^{-1} (1% w/v) β-CD solution was perfused at 0.5 μl min^{-1} through the different microdialysis membrane probes placed in an individual microcentrifuge tube containing 1 ml of stirred 0.9% w/v saline for 12 h at room temperature. The concentration of β-CD was determined by a spectrophotometric method utilizing Methyl Orange as a binding reagent.[26] The E_d of β-CD by delivery was defined as the ratio of cyclodextrin lost from the perfusion fluid to the initial β-CD concentration in the perfusion solution as shown in eqn. (1). Absorbance measurements were performed with a Hitachi (Tokyo, Japan) U-2000 spectrophotometer at 530 nm with a 1 cm cuvet. Standard and

unknown β-CD solutions were prepared with 5.0×10^{-5} mol l^{-1} Methyl Orange, buffered with 10 mmol l^{-1} sodium phosphate buffer (pH 3.3). The concentration of β-CD was calculated by non-linear regression analysis of the absorbance–concentration curve.

Desipramine sampling in a protein solution

A solution containing 4% w/v bovine serum albumin (Sigma) was prepared in phosphate buffered saline. This solution was used to prepare a 10 ml solution containing 10 μmol l^{-1} desipramine. A PC probe with a perfusion fluid consisting of either PBS or PBS with 8.8 mmol l^{-1} (1% w/v) β-CD was used to sample this stirred solution.

Results and discussion

RR enhancement with β-CD

Table 1 shows the percentage enhancement of RR obtained with 4.41 mmol l^{-1} (0.5% w/v) β-CD in the perfusion fluid for the different drugs studied *versus* controls with a PC membrane. The PC membrane material was chosen as the principal membrane to study because only this membrane material previously exhibited greater than 100% RR for ibuprofen with the inclusion of β-CD in the microdialysis perfusion fluid.[9] The inclusion of 4.41 mmol l^{-1} β-CD in the microdialysis perfusion fluid increased RR for all the drugs at perfusion flow rates of 0.5 and 1.0 μl min^{-1} through PC membranes. The percentage increase in the microdialysis RR was dependent upon the perfusion flow rate and the drug studied. A concentration of 4.41 mmol l^{-1} β-CD was chosen because of our experience with ibuprofen RR reaching a maximum at this concentration and then declining with increasing concentration of β-CD in the microdialysis perfusion fluid. The explanation for this unexpected equilibrium behavior has been described previously and could be due to a combination of different parameters such as insolubility of the ibuprofen–cyclodextrin complex or aggregate formation.[9] The RR values obtained for the tricyclic antidepressants and structurally related compounds with 4.41 mmol l^{-1} β-CD included in the perfusion fluid are significantly higher than that obtained for ibuprofen (118%).

Decreasing the microdialysis perfusion fluid flow rate leads to an increase in the RR, which is expected in microdialysis sampling.[27] However, unlike microdialysis sampling without β-CD, which cannot achieve greater than 100% RR, enhanced microdialysis transport can give analyte outlet concentrations that are higher than those in the sample solution surrounding the microdialysis probe.[9] The complexation reaction with cyclodextrin in the perfusion fluid leads to a decreased free analyte concentration at the inner fiber lumen of the microdialysis probe, which causes the RR to become greater than 100%. In these experiments, an increase in the analyte outlet concentration compared to controls (no cyclodextrin) of greater than 97% was observed at 1.0 and 0.5 μl min^{-1} for all the drugs studied. The maximum increase in RR *versus* control was found for promethazine. However, desipramine had the highest enhanced RR value (284) and had an outlet concentration that was 2.8 times higher than the concentration in the sample solution when 4.41 mmol 1^{-1} β-CD was included in the perfusion fluid.

Amitriptyline and promethazine exhibit small changes in RR with the 0.5 and 1.0 μl min^{-1} perfusion flow rates, which is an indication of possible fouling or slow membrane mass transport (approach to steady state) for these compounds through PC membranes. This is partially explained by their hydrophobicity and high octanol–water partition coefficients at pH 7.4 (imipramine 2.52, amitriptyline 2.50, and promethazine 4.73).[28] This type of adsorption phenomenon has been reported previously for other dialysis separation procedures.[29] Promethazine is a particularly difficult drug to work with in microdialysis sampling, as shown in Table 1 with its anomalous behavior with a lower RR (16.4) at 0.5 μl min^{-1} compared to 1.0 μl min^{-1} (21.2). Hydrophobic drugs have been reported to be difficult to work with during microdialysis sampling.[14,15] This behavior was also observed during the assay of imipramine, which gave RR values of 50.1 and 52.1% at 0.5 μl min^{-1} compared to 1.0 μl min^{-1} (shown in Fig. 1). The imipramine experiment was repeated to allow a longer equilibration time between the switching of the flow rate and gave values of 65.9 and 51.5% as shown in Table 1. A slow approach to steady state concentrations was noticed with the table data and replicate samples were not obtained until steady state concentrations were reached. This slow approach to the steady state appears to be related to the probe materials and not to non-specific adsorption on the microdialysis outlet tubing. A slow approach to steady state concentrations at low flow rates was observed despite passing a large volume of 100 μmol l^{-1} imipramine (10 times higher than the sample solution concentration) through only the outlet tubing prior to initiating microdialysis sampling.

The data in Table 2 illustrate the slow approach to steady state concentrations for imipramine with the PC membrane material. In these experiments the perfusion flow rate through the

Table 1 Percentage RR enhancements over control for the drugs studied

Flow rate/ μl min^{-1}	Analyte	Control, RR (%)	RR (%), β-CD (4.4 mmol l^{-1})	Enhancement (%)
0.5	Imipramine	65.9 ± 0.5	242.8 ± 2.8	268.4
1.0		51.5 ± 0.3	144.7 ± 6.1	181.0
0.5	Desipramine	71.4 ± 2.7	284.0 ± 0.7	297.9
1.0		58.5 ± 3.5	188.7 ± 1.1	222.6
0.5	Amitriptyline	30.7 ± 2.9	225.6 ± 8.8	634.3
1.0		27.3 ± 0.2	98.4 ± 4.9	259.9
0.5	Carbamazepine	80.5 ± 0.5	189.5 ± 2.9	135.5
1.0		62.9 ± 0.2	124.1 ± 0.9	97.2
0.5	Promethazine	16.4 ± 0.4	178.3 ± 4.6	987.2
1.0		21.2 ± 0.2	119.1 ± 1.3	461.8

Sample solution, 0.10 μmol l^{-1} analyte, pH 7.4 (10 mmol l^{-1} sodium phosphate buffer), 0.9% saline; β-CD concentration in the perfusion fluid, 4.4 mmol l^{-1}. All results are means ± SD (n = 3).

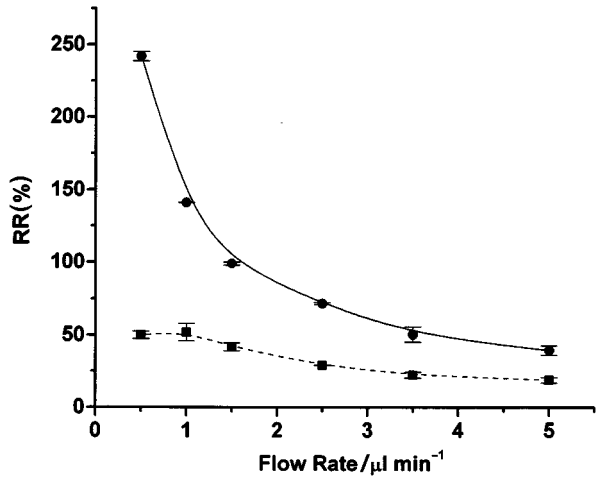

Fig. 1 Plot of imipramine RR *versus* microdialysis perfusion flow rate for PC probe. The perfusion fluid contained either 4.41 (●) or 0 mmol l^{-1} β-CD (■) [0.5, 0% w/v, respectively]. Error bars are means ± SD (n = 3).

microdialysis probe was varied between 1.0 and 2.5 μl min^{-1}. After switching the perfusion fluid flow rate, the system was allowed to come to steady state for about 10 min. In the ideal case, the RR should be identical during such a cycling experiment, which would indicate a rapid approach to steady state concentrations during the microdialysis process. However, in this experiment, the RR steadily increases as the experiment progresses, indicating a slow equilibration process with the dialysis membrane or other probe material. This slow equilibration process may be due to a dual transport mechanism of imipramine through the membrane material *via* the water-filled pores and the polymer. Including β-CD in the microdialysis perfusion fluid leads to the inhibition of the observed drug–membrane interaction, as indicated in Fig. 1. Fig. 1 shows an expected exponentially decaying RR *versus* flow rate curve for imipramine when β-CD is included in the microdialysis perfusion fluid. Since only carbamazepine diffusive transport was found not to be coupled with non-specific chemical interactions with the PC probe, as shown in Table 2, the microdialysis behavior of carbamazepine with different cyclodextrin materials included in the perfusion fluid was examined in more detail.

There are two parameters that could be used to predict the extent of enhanced RR. For the following equilibrium binding process:

$$\begin{aligned} A + CD &\xrightarrow{k_1} A\text{–}CD \quad (a) \\ A\text{–}CD &\xrightarrow{k_{-1}} A + CD \quad (b) \\ A + CD &\rightleftharpoons A\text{–}CD \quad (c) \end{aligned} \qquad (3)$$

where A represents the analyte and CD represents the cyclodextrin, there are two sets of simplifying assumptions that can be used to describe the total flux enhancement of analyte.[30] The first case assumes that the reaction in eqn. (3) is fast, giving equilibrium concentrations of the species immediately in the perfusion fluid [reaction (c)]. The second simplifying case would involve a situation where the forward formation reaction is slow enough and the concentration of CD is in large enough excess that concentrations of free CD and the A–CD complex are essentially unchanged. This second assumption gives an overall flux analysis that is dependent upon the relative value of k_1, the forward formation rate constant. In most cases, neither of these two simplifying assumptions applies and the concentrations and flux analysis within a simplified membrane would need to be solved numerically.[30] Which of these two assumptions applies in the microdialysis case is uncertain since only a few investigators have studied the relative formation rate constants for cyclodextrin inclusion complexes. In one case, a value of k_1 of 3×10^4 mol l^{-1} s^{-1} for dihydroxycholate ions[31] was found with β-CD and 11 s^{-1} for an azo dye with an excess of β-CD.[32] However, extensive thermodynamic data exist for the stability constants between different analytes and different cyclodextrins.[23] Both assumptions could be applied to these data since the concentration of β-CD in the perfusion fluid ranges between approximately 100 and 1700 times greater than the analyte concentration external to the microdialysis probe (second assumption).

Table 2 Effect of varying microdialysis perfusion flow rate on imipramine and carbamazepine RR through a PC membrane

Sequence[a]	Flow rate/μl min^{-1}	RR (%)
Imipramine—		
1	2.5	24.9 ± 1.9
2	1.0	42.2 ± 0.8
3	2.5	33.5 ± 0.6
4	1.0	52.1 ± 0.6
Carbamazepine—		
1	2.5	45.9 ± 0.8
2	1.0	74.4 ± 1.1
3	2.5	46.9 ± 0.4
4	1.0	74.6 ± 1.0
5	2.5	46.4 ± 0.4

β-CD was not included in the perfusion fluid. All results are means ± SD (n = 3). [a] The order in which the experiments were performed.

A trend towards the cyclodextrin binding equilibrium constant affecting the percentage RR enhancement of the antidepressants is noted with these data. Imipramine and amitryptiline have reported log $K_{\beta\text{-CD}}$ of 3.94 and 4.38, respectively,[23] whereas carbamazepine has a reported log $K_{\beta\text{-CD}}$ of 2.6.[22] Table 1 shows that adding β-CD to the perfusion fluid enhanced the RR for imipramine (268%) and amitryptiline (634%) more than for carbamazepine (136%). Fig. 2 shows that as the β-CD concentration is increased the RR enhancement increases for carbamazepine. Whether or not the equilibrium value is more important than the first-order rate constant for predicting the extent of RR enhancement is unknown since few published data exist for first-order binding rate constants of analytes with cyclodextrins.

Influence of cyclodextrin concentration on carbamazepine RR

The effect of β-CD concentration in the perfusion fluid on the carbamazepine RR was examined in the range 0.88–17.63 mmol l^{-1} β-CD. A maximum of 17.63 mmol l^{-1} β-CD was used in these experiments because of the limited solubility of β-CD in aqueous solution.[33] Fig. 2 illustrates the influence of β-CD concentration on the carbamazepine RR through PC at different perfusion flow rates. The outlet concentration of carbamazepine achieved at 0.5 μl min^{-1} with 17.63 mmol l^{-1} β-CD added was three times greater than its concentration external to the microdialysis probe. This enhancement is due either to the cyclodextrin–guest complexation equilibrium being shifted by increasing of cyclodextrin concentration in the perfusion solution or the rate increasing as the concentration of cyclodextrin is increased. As a result of the binding reaction, the difference in the unbound carbamazepine concentration gradient at the inner radius of the membrane causes a higher flux into the microdialysis probe and carbamazepine RR is improved.

β-CD and its derivatives are known to increase the low aqueous solubility of carbamazepine.[19–21] HP-β-CD is highly

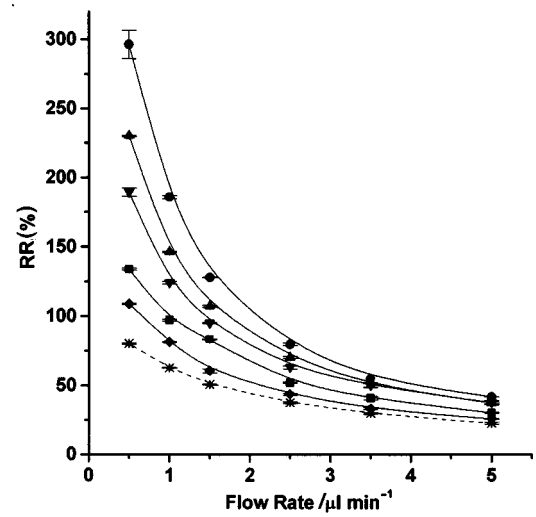

Fig. 2 PC membrane. RR *vs.* flow rate for carbamazepine with different β-CD concentrations (mmol l^{-1}) included in the microdialysis perfusion fluid: 0.88 (◆), 1.76 (■), 4.41 (▼), 8.82 (▲), 17.63 mol l^{-1} (●); dashed line (★), control (0 mmol l^{-1}) [0.1, 0.2, 0.5, 1.0 and 2.0% w/v, respectively]. Error bars are means ± SD (n = 3).

water soluble, unlike β-CD, but retains the binding properties of β-CD.[20] Therefore, HP-β-CD was chosen as an alternative to β-CD to include in the microdialysis perfusion fluid as a complexing additive with a potential capability to increase RR.

The ability of HP-β-CD to increase carbamazepine RR was examined with PC, CUP and AN-69 membranes using HP-β-CD up to a concentration of 32.5 mmol l^{-1} with perfusion flow rates of 1.0 and 0.5 μl min^{-1}. Fig. 3 shows an increase in carbamazepine RR with increasing concentration of HP-β-CD through a PC membrane. This curve has the shape of a rectangular hyperbola, which has been described for other 1 : 1 associations in equilibrium chemistry.[34,35]

Carbamazepine external concentration

The equilibrium association of cyclodextrins with carbamazepine is concentration dependent. This concentration dependence may affect the carbamazepine RR in our experiments. To ensure that the carbamazepine RR remained constant, experiments were performed with different concentrations of carbamazepine external to the microdialysis probe.

The carbamazepine microdialysis RR was found to be independent of the concentration of carbamazepine in the sample solution, as shown in Table 3. The experiments were performed at a fixed concentration of β-CD in the perfusion fluid (1.0% w/v) with various membrane probes. The carbamazepine concentration was varied in the range 5–50 μmol l^{-1}. There is no statistically significant difference between the carbamazepine RR obtained at its different concentrations in the sample solution for the three membranes studied.

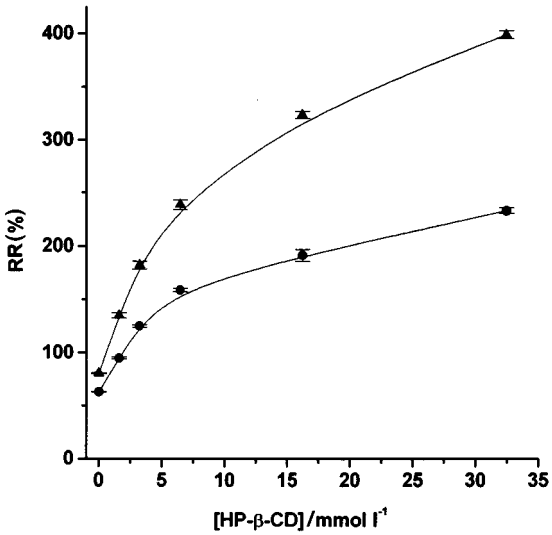

Fig. 3 PC membrane. RR for carbamazepine *vs.* HP-β-CD concentration at 0.5 (▲) and 1.0 μl min^{-1} (●). Error bars are means ± SD (n = 3).

Stirred *versus* unstirred enhanced RR

Table 4 shows the enhancement of RR for carbamazepine with β-CD in stirred and unstirred solutions. For the control experiments, the stirred solution gives a higher RR than the quiescent solution, which is expected in microdialysis sampling.[24] The percentage enhancement in the stirred solution is also more than twice that of the unstirred solution. This observation is explained by the differences in the diffusion processes for the cyclodextrins in these two cases. The stirred solution will remove the cyclodextrin that has diffused out of the inner fiber lumen of the membrane from the region nearest the probe. This whisking away process will allow free carbamazepine to diffuse directly through the probe without being extensively bound by cyclodextrin. In the unstirred case, cyclodextrin that has diffused through the membrane can bind some of the carbamazepine, forming an inclusion complex with a high molecular weight. This high molecular weight inclusion complex would have a lower aqueous diffusion coefficient than unbound carbamazepine. In this situation, the bound complex will have a lower RR because of its lower diffusion coefficient.[6,36]

Enhanced RR through other membranes and with HP-β-CD

Table 5 shows the effect of β-CD concentration on carbamazepine RR through AN-69 and CUP membranes, respectively. The increase in carbamazepine RR approaches a plateau at the highest β-CD concentration through AN-69 and CUP membranes. The increase in carbamazepine RR achieves a maximum with a 2% w/v HP-β-CD concentration through CUP and AN-69 membranes. These effects of either β-CD or HP-β-CD concentration in the perfusion fluid on carbamazepine RR through different membranes cannot be explained only by a difference in the pore sizes of the membrane materials studied. For both the CUP and AN-69 membranes, the enhanced RR is not nearly as great as for PC membrane materials. Since the PC membrane has the largest internal radius of all three membrane materials, it is likely that its geometry significantly affects the enhanced RR. This explanation is shown mathematically in the second term of eqn. (2).

Fig. 2 and 3 illustrate how the two cyclodextrins studied have similar ability to enhance microdialysis RR of carbamazepine through PC membrane. However, including HP-β-CD in the perfusion fluid exhibited a higher carbamazepine RR through CUP and AN-69 membranes than β-CD. This may be explained by either a higher binding constant for carbamazepine with HP-β-CD or a larger forward rate constant for the association reaction between carbamazepine and HP-β-CD as compared to β-CD.

β-CD E_d

In these experiments, the PC membrane exhibits the greatest RR enhancement for carbamazepine when the two different cyclo-

Table 3 Effect of varying carbamazepine (CBZ) concentration on enhanced RR through various microdialysis membranes

Flow rate/ μl min^{-1}	Membrane	1.0% w/v β-CD, 10 μm CBZ	1.0% w/v β-CD, 5 μm CBZ	1.0% w/v β-CD, 10 μm CBZ	1.0% w/v β-CD, 25 μm CBZ	1.0% w/v β-CD, 50 μm CBZ
1.0	PC	62.9 ± 0.2	147.7 ± 3.2	146.4 ± 0.5	145.6 ± 1.0	145.5 ± 1.1
0.5		80.5 ± 0.5	228.1 ± 3.1	230.1 ± 0.5	232.0 ± 2.7	228.0 ± 7.0
1.0	AN-69	45.4 ± 0.1	61.2 ± 0.6	60.8 ± 0.4	64.2 ± 1.8	63.6 ± 0.3
0.5		68.5 ± 0.3	92.4 ± 3.1	95.8 ± 2.2	95.7 ± 0.9	95.6 ± 0.1
1.0	CUP	17.7 ± 0.2	47.9 ± 0.4	46.4 ± 1.2	44.2 ± 0.2	45.4 ± 0.8
0.5		43.4 ± 0.5	81.7 ± 2.7	86.4 ± 0.2	81.9 ± 2.0	84.4 ± 1.0

RR is given as mean ± SD (n = 3) from each probe. 1% w/v = 8.8 mmol l^{-1} β-CD.

dextrins were placed in the microdialysis perfusion fluid. This behavior was observed with the ibuprofen–β-CD system that has been described previously.[9] We had hypothesized that perhaps cyclodextrins diffuse through PC membranes at a higher rate than through AN-69 or CUP membranes. This hypothesis is not completely unfounded because β-CD has a molecular weight of 1135 and has many polar hydroxyl groups. The MWCO values for the microdialysis membranes are 6000 (CUP), 20 000 (PC) and 29 000 (AN-69). Because of its high molecular weight, β-CD would be expected to have a very low RR through CUP membranes. AN-69 membranes possess a negative charge, which would make it possible for β-CD to undergo hindered diffusion through these membranes. The RR values of β-CD delivered (0.5 μl min^{-1}) to a stirred solution at room temperature were 3.4% (CUP), 9.7% (PC) and 11.6% (AN-69). These data are in agreement with the pore dimensions of the membrane materials studied. These data do not describe the observed enhancement of RR through PC membranes compared to AN-69 and CUP membranes. It is suspected that geometry differences between the probes may be affecting the RR enhancement, as shown in the second term of eqn. (2).

Enhanced RR in a protein solution

A principal factor that will need to be addressed with sampling from biological fluids, particularly plasma samples with enhanced microdialysis, will be the ability of the cyclodextrins to enhance analyte RR in protein solutions. Desipramine was chosen as the drug with which to study these interactions since its protein binding has been extensively studied. In human plasma, desipramine is more extensively protein bound (82%) than carbamazepine (74%).[16] Fig. 4 shows the enhancement of desipramine in a stirred 4% solution of bovine serum albumin (BSA) with 8.8 mmol l^{-1} β-CD in the perfusion fluid. The concentration of BSA is similar to that expected for human serum albumin in human plasma.[37] The concentration of desipramine exiting the probe was significantly enhanced with the addition of β-CD, although not as much as in a buffer solution (Table 1). The extent of desipramine protein binding to BSA was found to be greater than 85% by using the method of no-net flux. Although this is higher than the reported human plasma data mentioned above, it has been reported that desipramine and imipramine are more highly protein bound to BSA than to human serum albumin.[38] Fig. 4 indicates that cyclodextrin mediated enhanced RR may be used to sample from complex biological matrices such as blood.

Table 4 Percentage RR enhancements over control for carbamazepine in stirred and unstirred sample solutions

Flow rate/ μl min^{-1}	Control	RR (%)	Enhancement (%)
Stirred solution—			
0.5	80.5 ± 0.5	230.1 ± 0.5	186.0
1.0	62.9 ± 0.2	146.4 ± 0.5	132.7
Unstirred solution—			
0.5	70.8 ± 0.8	127.4 ± 2.1	79.9
1.0	54.5 ± 0.5	76.3 ± 2.4	40.0

Sample solution, 10 μmol l^{-1} carbamazepine, pH 7.4 (10 mmol l^{-1} sodium phosphate buffer), 0.9% saline; β-CD concentration in the perfusion fluid, 8.8 mmol l^{-1}. All results are means ± SD (n = 3).

Calibration of the enhanced RR system

It should be emphasized that calibration may pose difficulties with this system in small constrained sample spaces such as brain tissue. We have attempted to try different forms of calibration to observe the effects on the enhanced RR value. A no-net flux experiment with a sample concentration of 10 μmol l^{-1} carbamazepine was performed to determine the value of the extraction fraction (E_d) as defined in eqn. (1). The results of this experiment are shown in Fig. 5. The slope of the regression line gives an E_d of 42.1% with an intercept of 29 μmol l^{-1}. This data clearly shows that eqn. (1) in the E_d form cannot be used to calibrate microdialysis probes when cyclodextrins are included in the perfusion fluid. A calibration was performed with a delivery experiment; 10 μmol l^{-1} carbamazepine was included in the microdialysis perfusion fluid with 8.8 mmol l^{-1} β-CD and was locally delivered *via* a PC probe to a stirred 10 ml solution of PBS. The E_d for carbamazepine under these conditions was 32.9%, which does not match the RR value with the same conditions for a recovery experiment. These data indicate that alternative means of calibration may be necessary such as comparing microdialysis sample concentrations to serial blood samples.

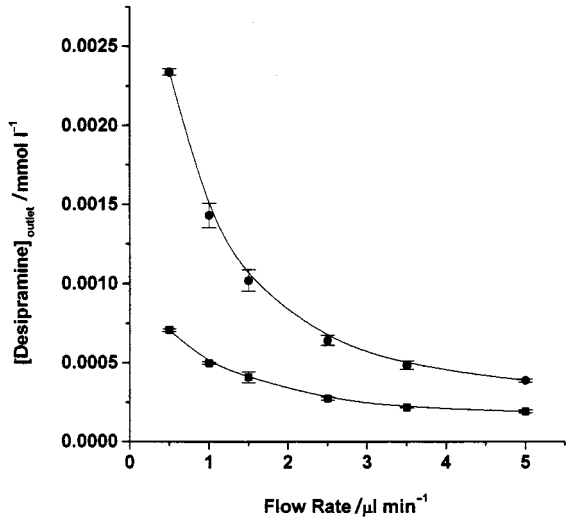

Fig. 4 Enhancement in desipramine concentration in a BSA stirred solution. The solution contained 10 μmol l^{-1} desipramine with 4% BSA in PBS. The microdialysis perfusion fluid contained either PBS, pH 7.4 (■) or PBS with 8.8 mmol l^{-1} β-CD (●). Error bars are means ± SD (n = 3).

Table 5 Enhancement of carbamazepine RR through CUP and AN-69 membranes

Membrane	Flow rate/ μl min^{-1}	Control, RR (%)	β-CD[a]		HP-β-CD[b]		
			1% w/v	2% w/v[c]	1% w/v	2% w/v	5% w/v
CUP	0.5	43.4 ± 0.5	86.4 ± 0.2	75.9 ± 1.9	91.2 ± 0.3	124.2 ± 0.6	120.8 ± 0.4
	1.0	17.7 ± 0.2	46.4 ± 1.2	33.9 ± 0.4	51.3 ± 0.7	67.2 ± 0.7	63.2 ± 0.2
AN-69	0.5	68.5 ± 0.3	95.8 ± 2.2	96.7 ± 1.0	100.4 ± 2.4	110.8 ± 0.7	102.7 ± 2.3
	1.0	45.4 ± 0.1	60.8 ± 0.4	61.6 ± 1.0	66.1 ± 1.8	67.6 ± 0.8	54.7 ± 0.9

[a] 1% w/v = 8.8 mmol l^{-1}, 2% w/v = 17.6 mmol l^{-1}. [b] 1% w/v = 6.5 mmol l^{-1}, 2% w/v = 13.0 mmol l^{-1}, and 5% w/v = 32.5 mmol l^{-1}. [c] 2% w/v is the maximum solubility of β-CD under the conditions.

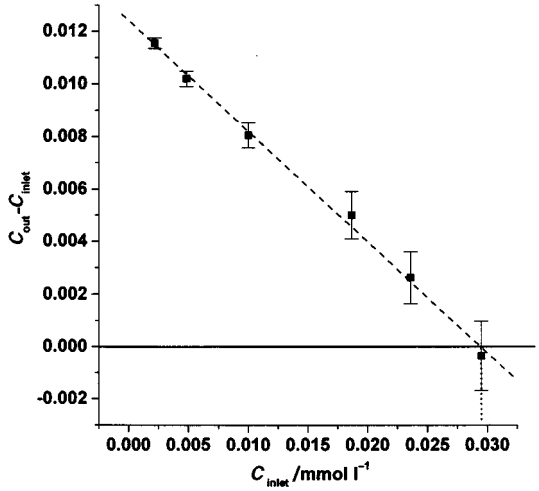

Fig. 5 No-net flux plot with a PC probe. A 10 μmol l^{-1} carbamazepine external solution (10 ml) was used with 4.4 mmol l^{-1} β-CD included in the microdialysis perfusion fluid at 1.0 μl min^{-1}. Error bars are means ± SD (n = 3).

Conclusions

The use of cyclodextrins as a complexing agent included in the microdialysis perfusion fluid leads to enhanced analyte mass transport into the microdialysis probe. We have shown enhancement in RR for a series of drugs with similar tricyclic molecular structures. However, this approach is applicable to enhance the microdialysis RR for numerous organic compounds (pharmaceuticals, metabolites, xenobiotics, *etc*.) capable of forming strong complexes with cyclodextrin. This approach may be used for sampling from large sample spaces such as blood, but may pose difficulties in smaller tissue spaces. The extraction fraction (E_d) equation cannot be used for calibration of this microdialysis system.

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