

SUPPORTING INFORMATION

A new approach to measure protein binding based on a parallel artificial membrane assay and human serum albumin

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I. Berkeley Madonna script used to solve equations 8-10

```
;donor plate
d/dt(Dd[1..N]) = -kon*Da[i]*Dd[i] +koff*Dda[i] +Pe*A*(Md[i]/Vm) -Pe*A*(Dd[i]/Vd)
d/dt(Da[1..N]) = -kon*Da[i]*Dd[i] +koff*Dda[i]
d/dt(Dda[1..N])= kon*Da[i]*Dd[i] -koff*Dda[i]

;membrane
d/dt(Md[1..N]) = -Pe*A*(Md[i]/Vm) +Pe*A*(Dd[i]/Vd) -Pe*A*(Md[i]/Vm) +Pe*A*(Ad[i]/Vd)

;acceptor plate
d/dt(Ad[1..N]) = +Pe*A*(Md[i]/Vm) -Pe*A*(Ad[i]/Vd)

init(Dd[1..N]) = 50*10^-6 ;free drug in donor plate, M
init(Da[1]) = 0 ;protein in Donor plate, M
init(Da[2]) = 100*10^-6 ;protein in Donor plate, M
init(Dda[1..N]) = 0 ;complex drug-albumin in Donor, M
init(Ad[1..N]) = 0 ;drug in acceptor plate, M
init(Md[1..N]) = 0 ;drug in membrane, M

DTD[1..N]=Dd[i]+Dda[i] ;total drug in Donor, M
ATD[1..N]=Ad[i] ;total drug in Acceptor, M
ATD1=Ad[1]
ATD2=Ad[2]

N=2 ;number of experiments in the array
Pe = 1*10^-3 ;permeability, cm/s
A = 0.048 ;area(cm2)*porosity
Vr = 0.3 ;acceptor volume, mL
Vd = 0.3 ;donor volume, mL
Vm = 7.5e-4*Kh ;volume membrane, mL, multiplied by partition coefficient to give apparent volume
Kh = 100 ;hexadecane partition coefficient
kon = koff/Kd
koff = 1 ;s-1
Kd = 1.37*10^-4 ;M

METHOD STIFF ;integration method
STARTTIME = 0
STOPTIME = 7200 ;seconds
TOLERANCE = 0.001 ;relative accuracy for the auto and stiff methods
DT = 0.001 ;Integration time step (Euler, RK2, RK4 methods)
DTMIN = 0.001
DTMAX = 1
DOUT = 0 ;output time interval (0 = store every step)
```

II. Details of the PAMPA construct:

Acceptor plate

Filterplate reference used with **hexadecane** membrane:

Millipore, Multiscreen plate, ref MPC4NTR10

Filterplate reference used with **octanol** membrane:

Millipore, Multiscreen plate, ref S2EJ014L01

Donor plate (Teflon, non-disposable):

Millipore reference: MSSACCEPTOR