Improved Method for Predicting Dermis Permeability *In Vitro*

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Introduction

Introduction

- Our skin comes into contact with chemicals on a daily basis in both occupational and leisure settings.
- The exposure of hazardous chemicals to the skin may lead to dermatologic disease and/or systemic illness.

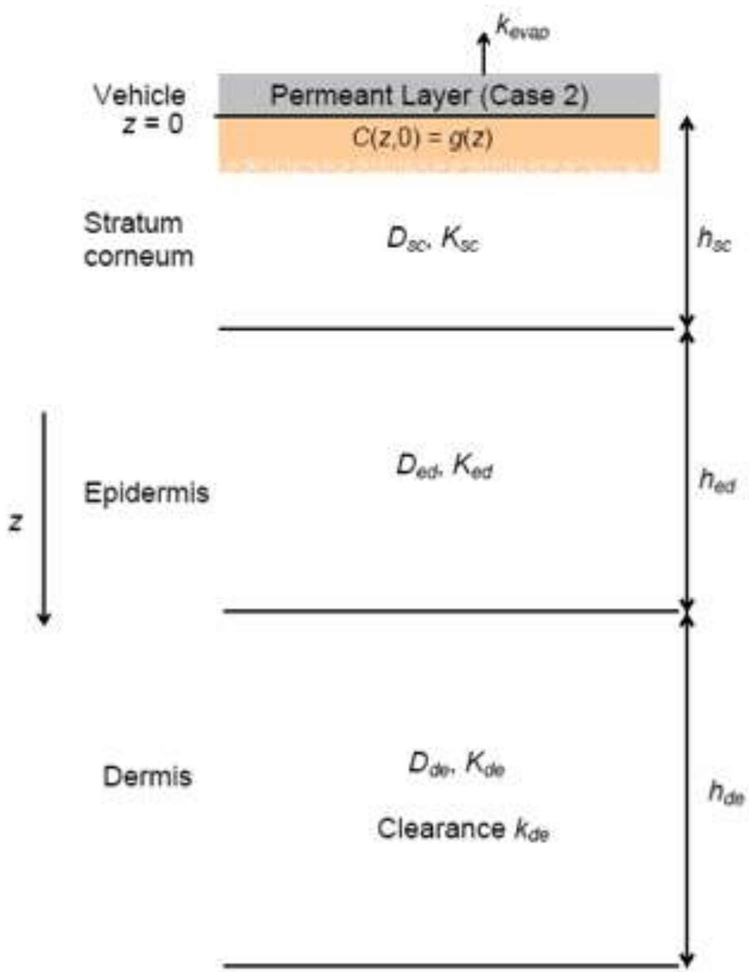
Introduction

- Knowledge of the rate and extent of dermal absorption is important in several fields:
  - Cosmetics
  - Topical Drug Delivery
  - Development of Barriers Creams and Insect Repellents
  - Occupational and Environmental Health Risk Assessment

Introduction

- To study each penetrant experimentally would be highly impractical and costly.
- Animal studies are expensive and pose ethical issues.
- As a result, the use of predictive computational models in the field of risk assessment has become of increasing interest.

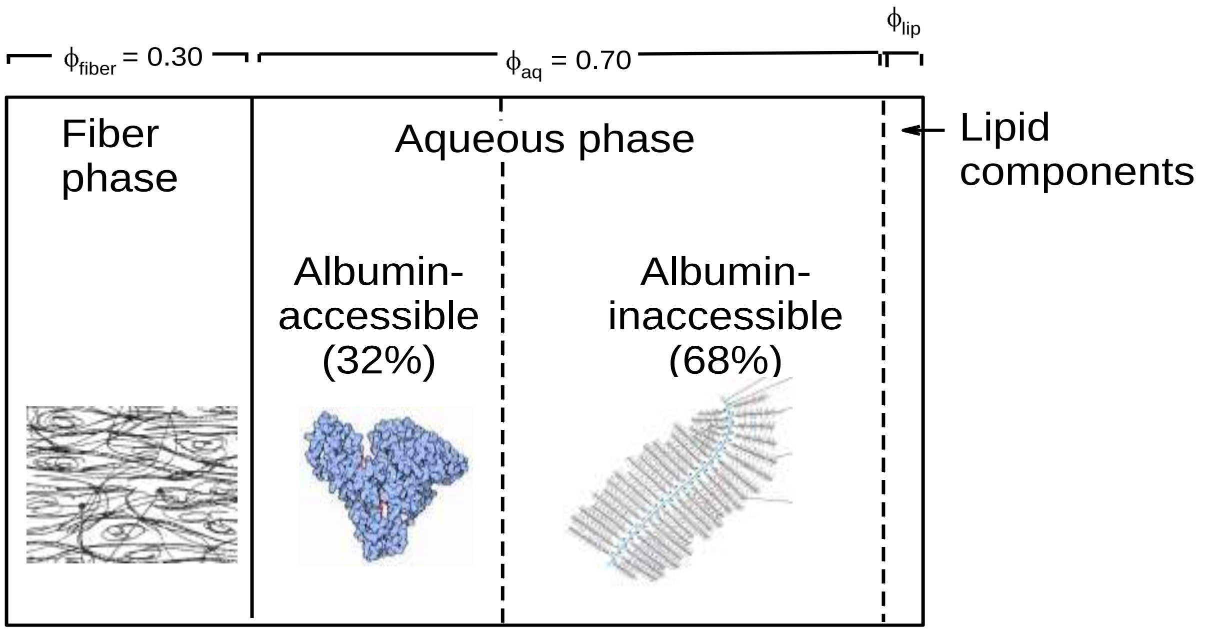
Kasting Laboratory Model



- 3 layer diffusion model.
- SC- Brick and Mortar model
- Dermis- homogenous layer with capillary clearance
- Viable epidermis- unperfused dermis

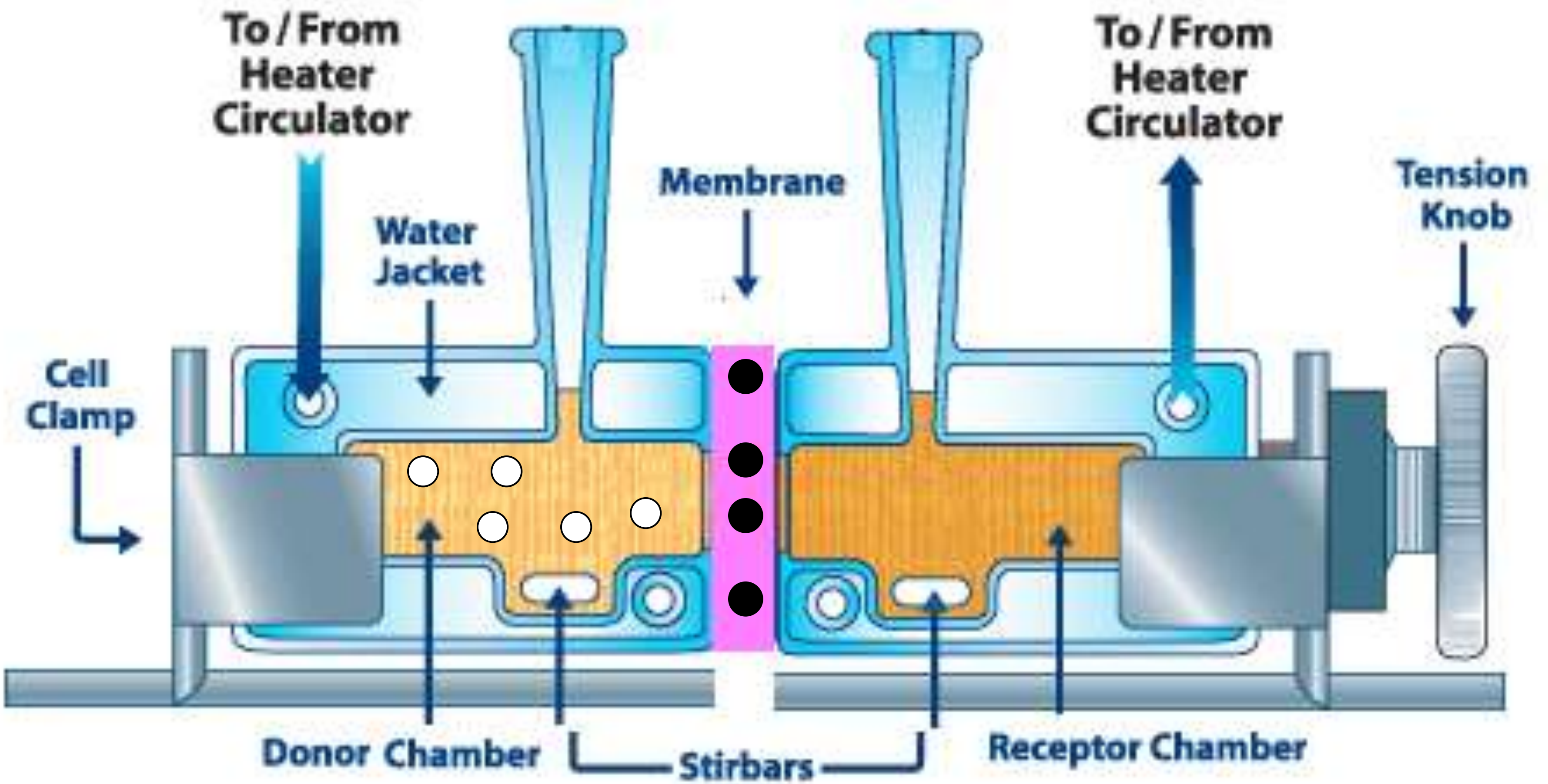
Kasting GB, Miller MA. 2008. *J Occ Env Hyg.* **5**:1-12

Kasting Laboratory Dermis Model



Kretsos et al. 2008. *Int J Pharm.* **346**: 64-79

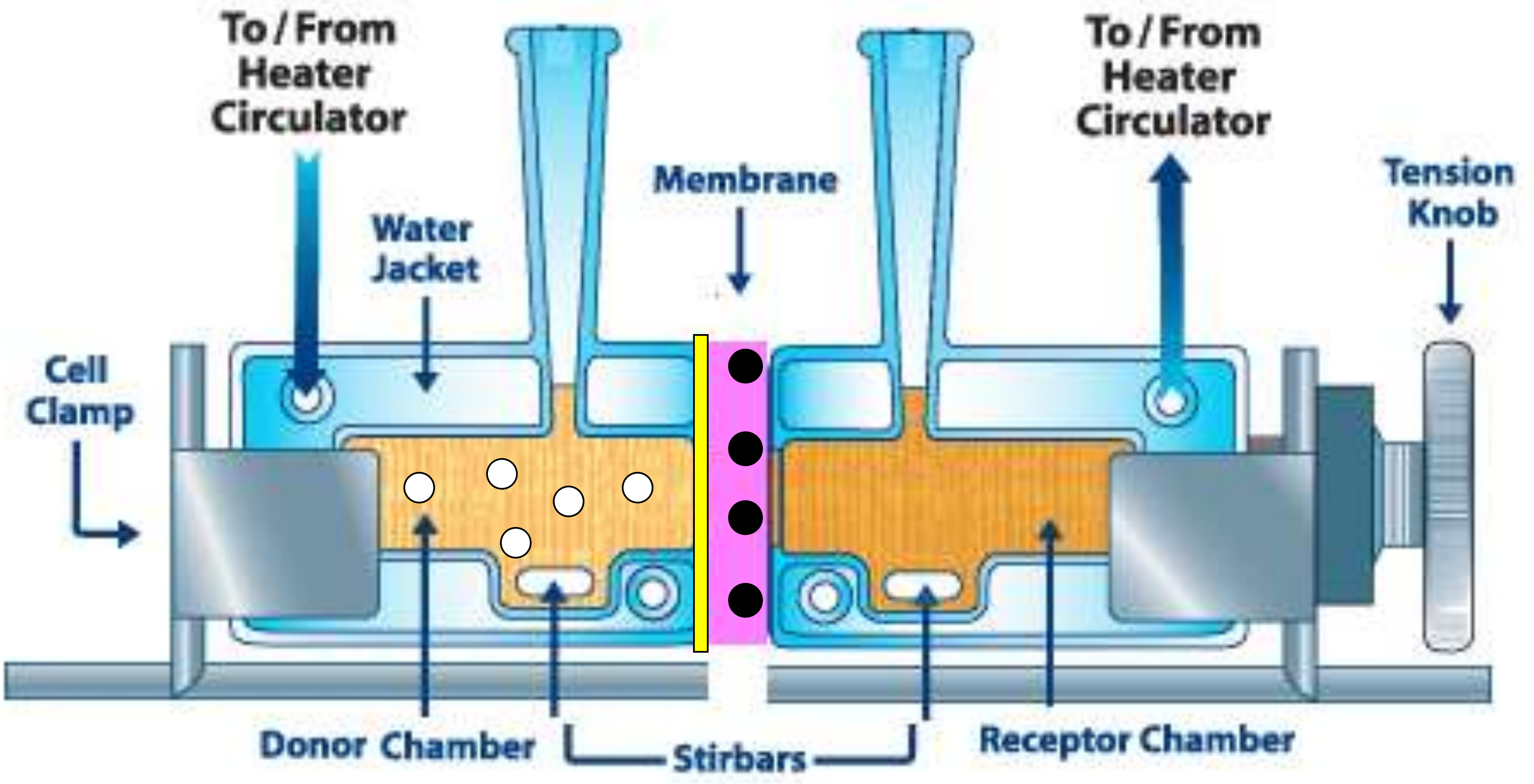
Dermis Permeability Studies



[1]Dermis has been stated to contain 2.7 % w/v of albumin.

[1] Bert et al. 1982. *Biochem J*. **201**: 395-403
Bert et al. 1986. *Microvasc Res*. **32**: 211-23
Kretsos et al. 2008. *Int J Pharm*. **346**: 64-79

www.permegear.com


To / From Heater Circulator
Water Jacket
Membrane
To / From Heater Circulator
Tension Knob
Cell Clamp
Donor Chamber
Stirbars
Receptor Chamber

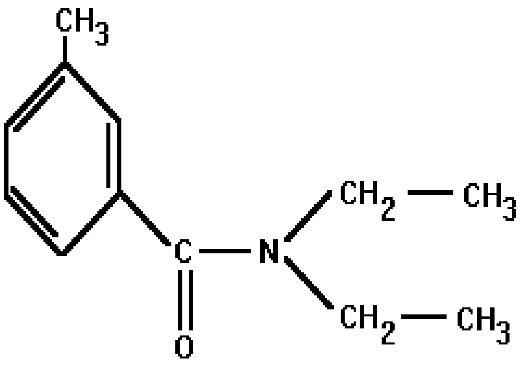
Objectives

- To develop improved methodology for determining transport parameters of lipophilic compounds in the dermis.
- To utilize the developed methodology to determine the transport parameters of selected permeants in excised human dermis.

Materials

- Dialysis Membrane (5000 Da cutoff)
- Human abdominal skin from reduction surgeries

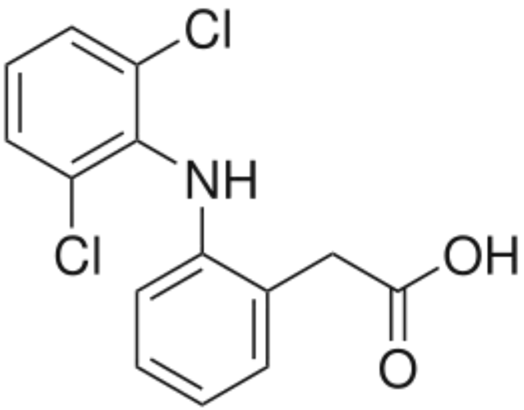
Chemicals



DEET

Log K_{oct}= 2.18
MW= 191.3 Da

f_b= 82% in BSA



Diclofenac

Log K_{oct}= 4.51
pK_a= 4.00
MW= 296.2 Da

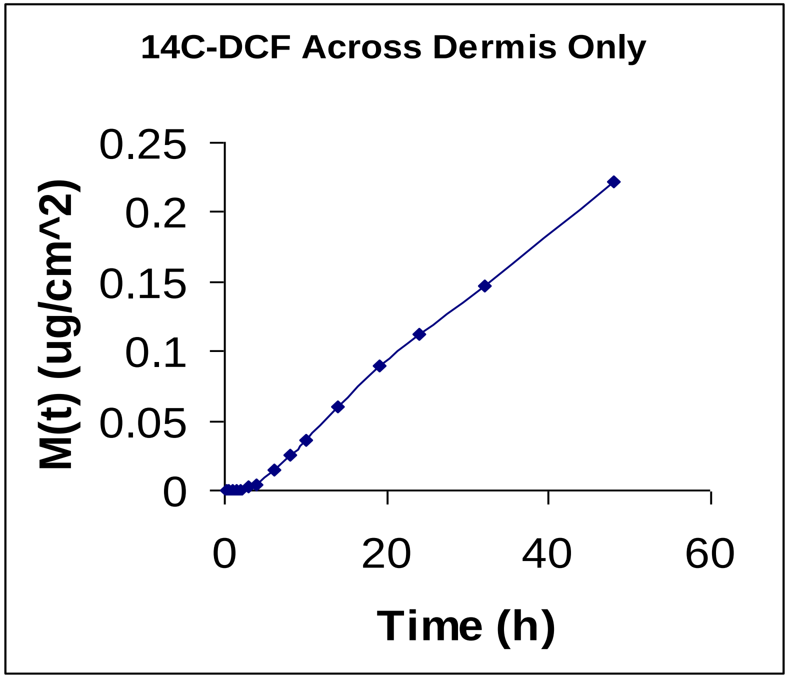
f_b= 97% in BSA
[1]f_b> 99.5% in Plasma

[1] Goodman and Gillman. 10th Edition. 2001

Mathematical Analysis

- Thickness, h_{de} of the tissue is estimated from its weight and surface area.

- Steady-state permeation rates are used to calculate the permeability coefficient k_p^{tot}



$$k_p^{tot} = \frac{J_{ss}}{\Delta C}$$

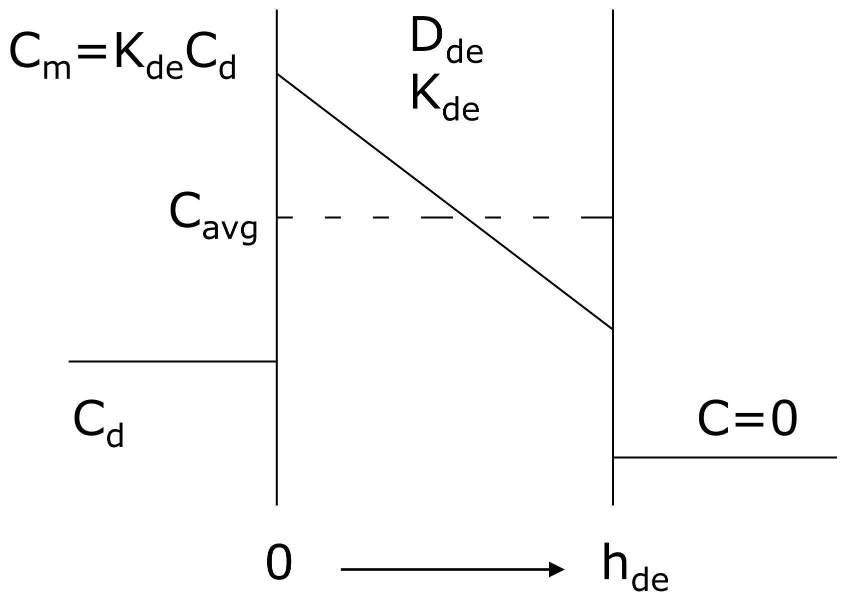
J_{ss} → Steady-state flux

ΔC → Conc. difference across membrane

Mathematical Analysis

- The average concentration in the tissue is used to calculate the partition coefficient K_{de}.

$$K_{de} = 2\frac{C_{avg}}{C_d}$$



C_d = Initial donor concentration
D_{de} = Diffusivity
K_{de} = Partition coefficient
h_{de} = thickness
C_m = Concentration in membrane
C_{avg} = average concentration

Mathematical Analysis

- The diffusivity can be calculated from the thickness and time-lag.

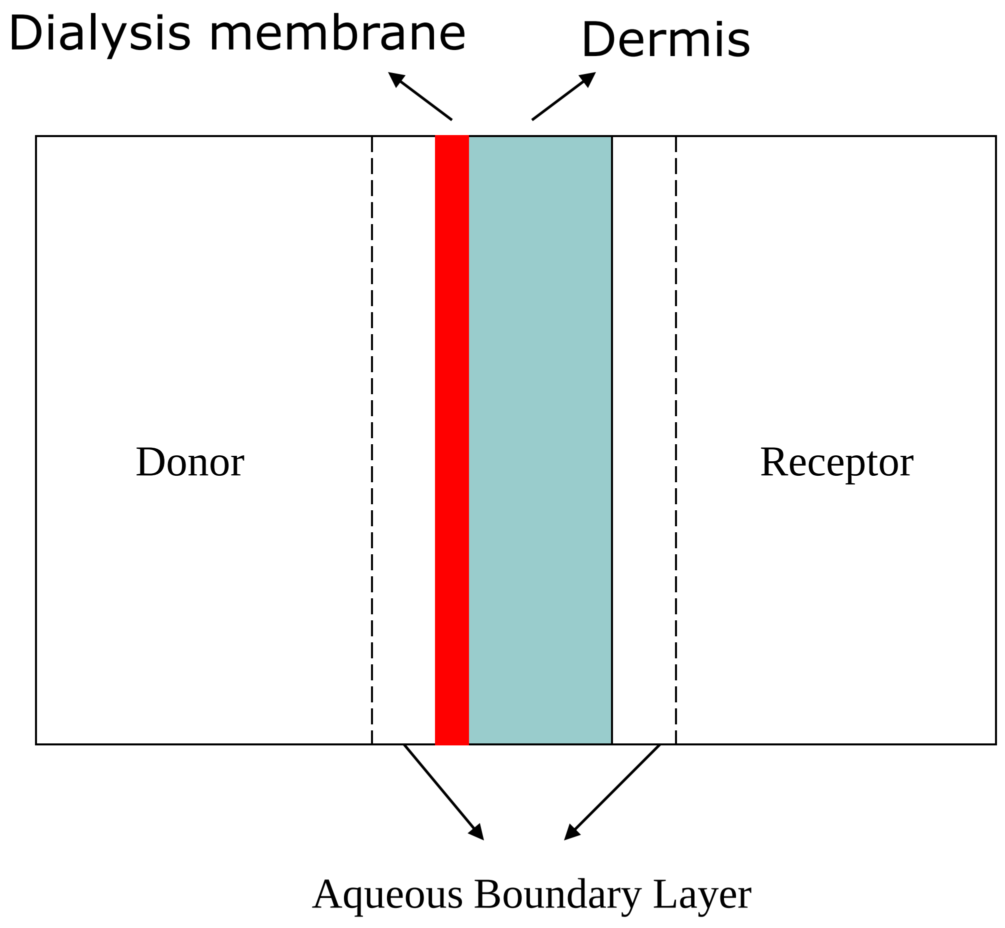
$$D_{de} = \frac{h_{de}{}^{2}}{6t_L}$$

OR

- From the thickness, permeability coefficient and partition coefficient.

$$D_{de} = \frac{k_p^{de} h_{de}}{K_{de}}$$

Mathematical Analysis



Mathematical Analysis

- The permeability coefficient k_p and resistance are corrected for the contributions of the aqueous boundary layers and the dialysis membrane.

$$\frac{1}{k_p^{tot}} = \frac{1}{k_p^{de}} + \frac{1}{k_p^{Dial}} + \frac{1}{k_p^{ABL}}$$

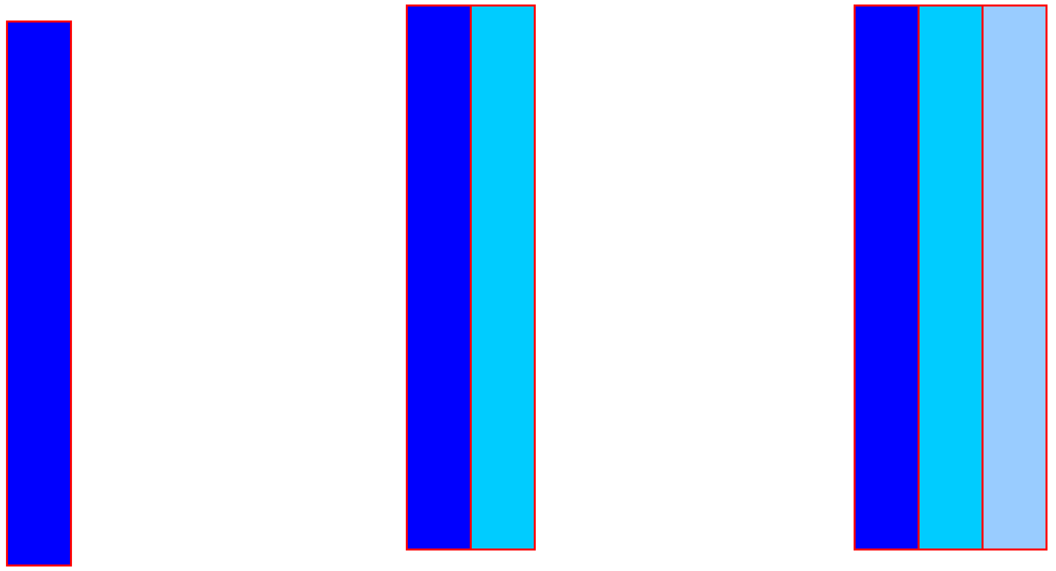
$$k_p^{de} = \frac{1}{R_{de}}$$

$$R_{de} = R_{Tot} - R_{ABL} - R_{Dial}$$

R_{de} = Resistance of dermis
R_{Dial} = Resistance of dialysis membrane
R_{ABL} = Resistance of ABL
R_{Tot} = Total resistance

The Contribution of
The Aqueous Boundary Layers
(ABL)

Experimental Setup

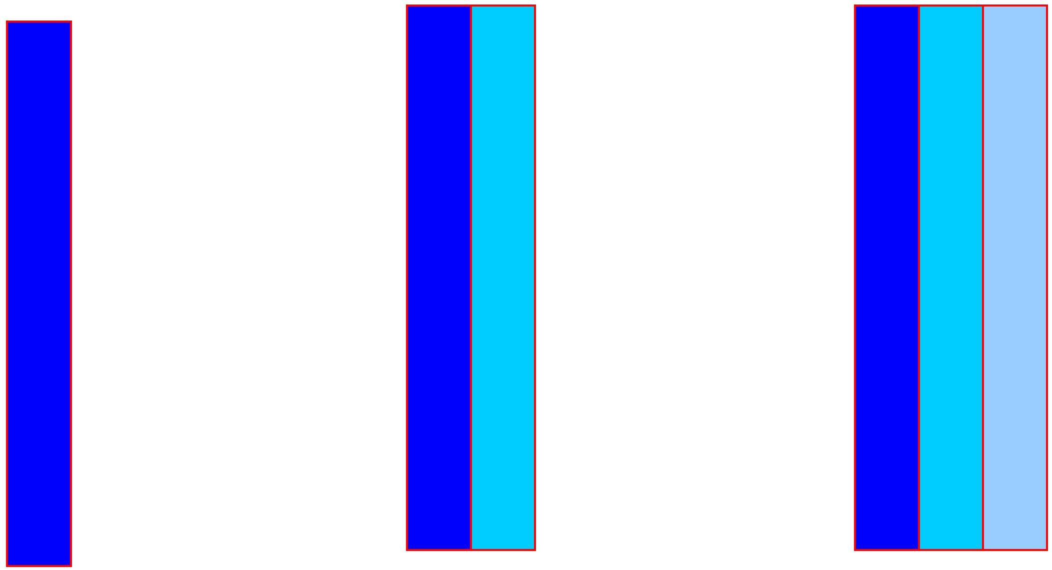


1 Dialysis Membrane 2 Dialysis Membranes 3 Dialysis Membranes

Donor and Receptor Compartments : PBS solution
Donor Compartment: Spiked with radio-labeled compound

ABL Experimental Setup

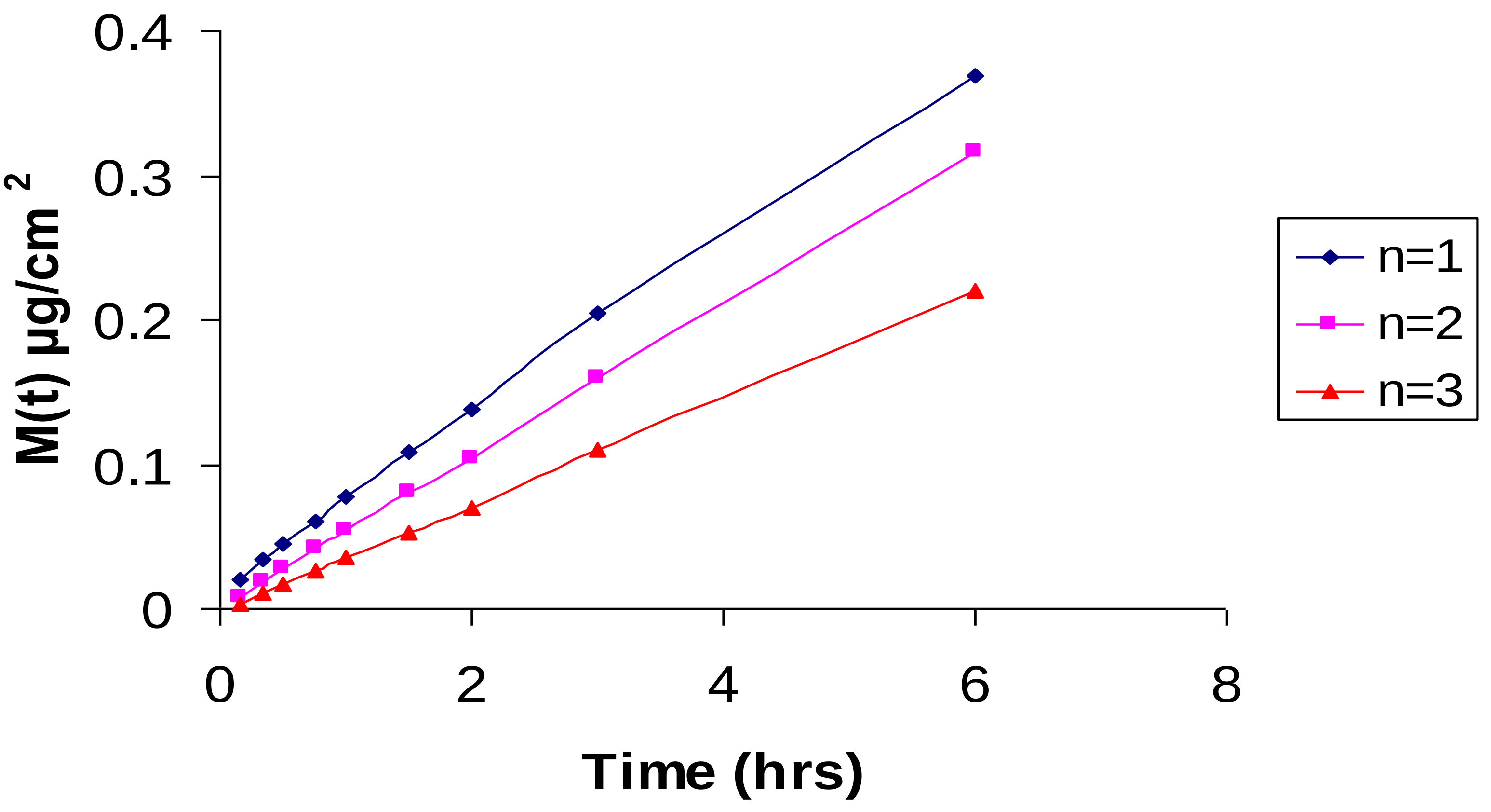
$$R_{tot} = nR_{mem} + R_{ABL}$$



1 Dialysis Membrane   2 Dialysis Membranes   3 Dialysis Membranes

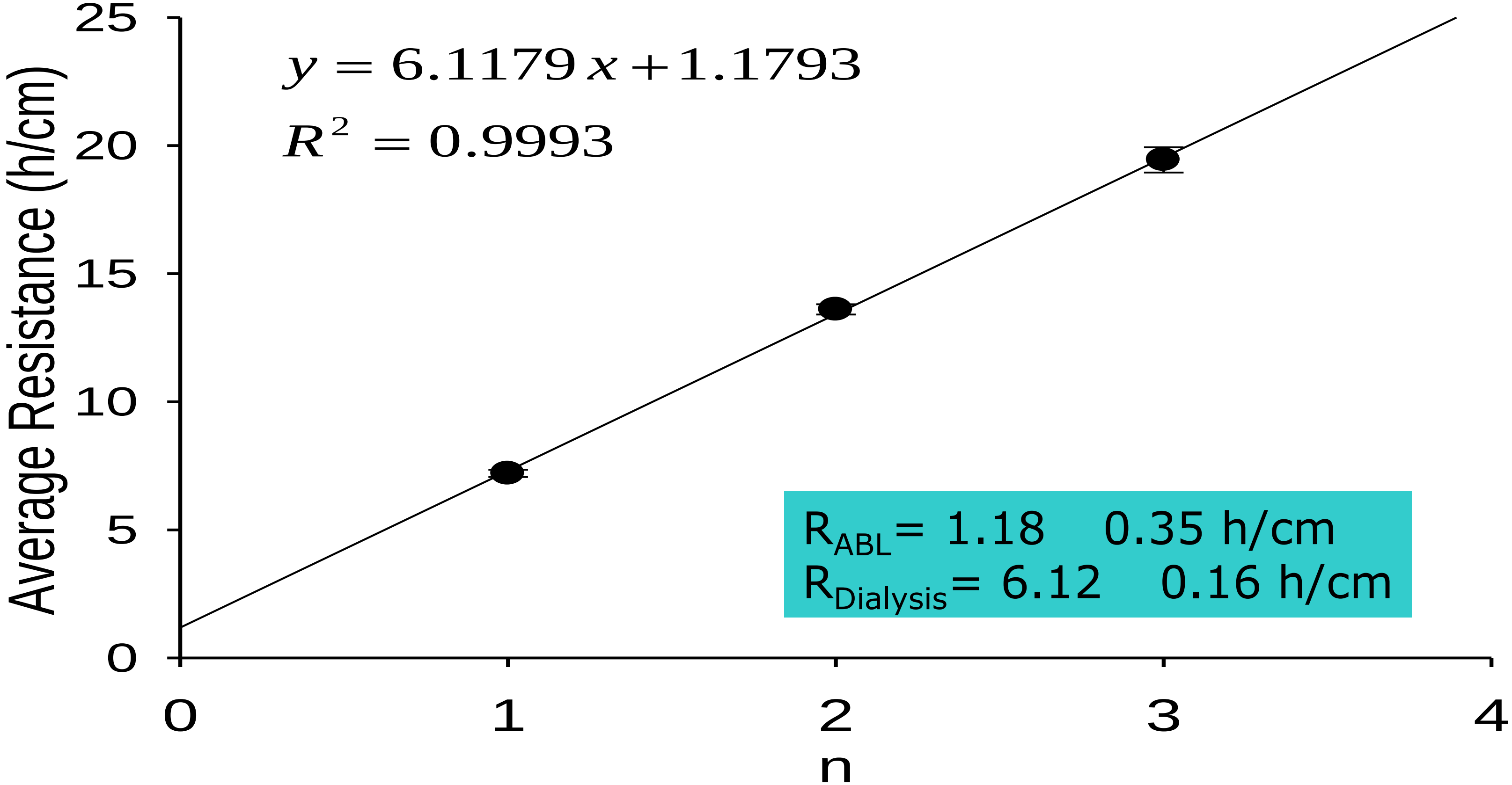
Donor and Receptor Compartments : PBS solution
Donor Compartment: Spiked with radio-labeled compound

Results



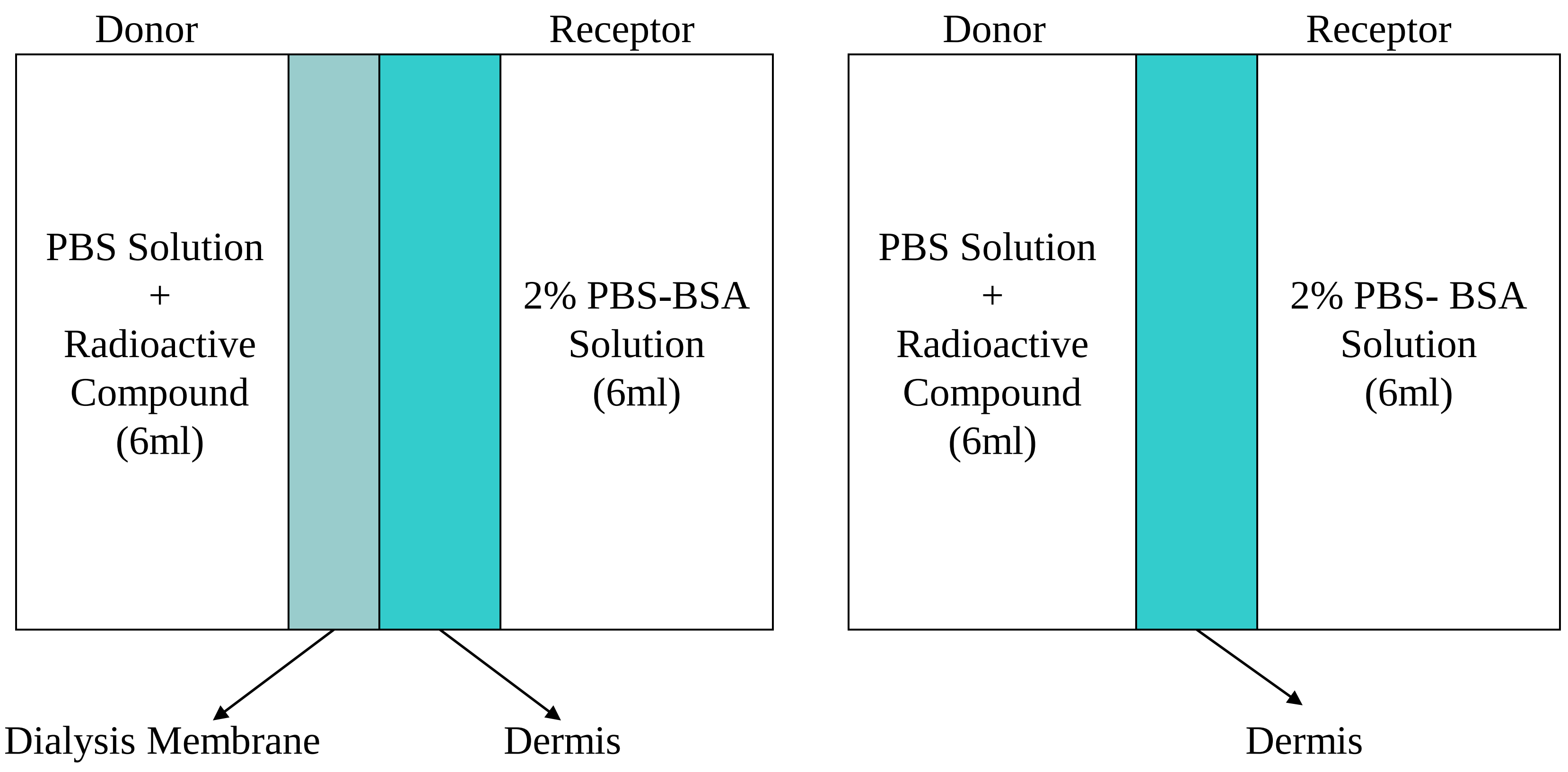
Results

$$R_{tot} = nR_{mem} + R_{ABL}$$



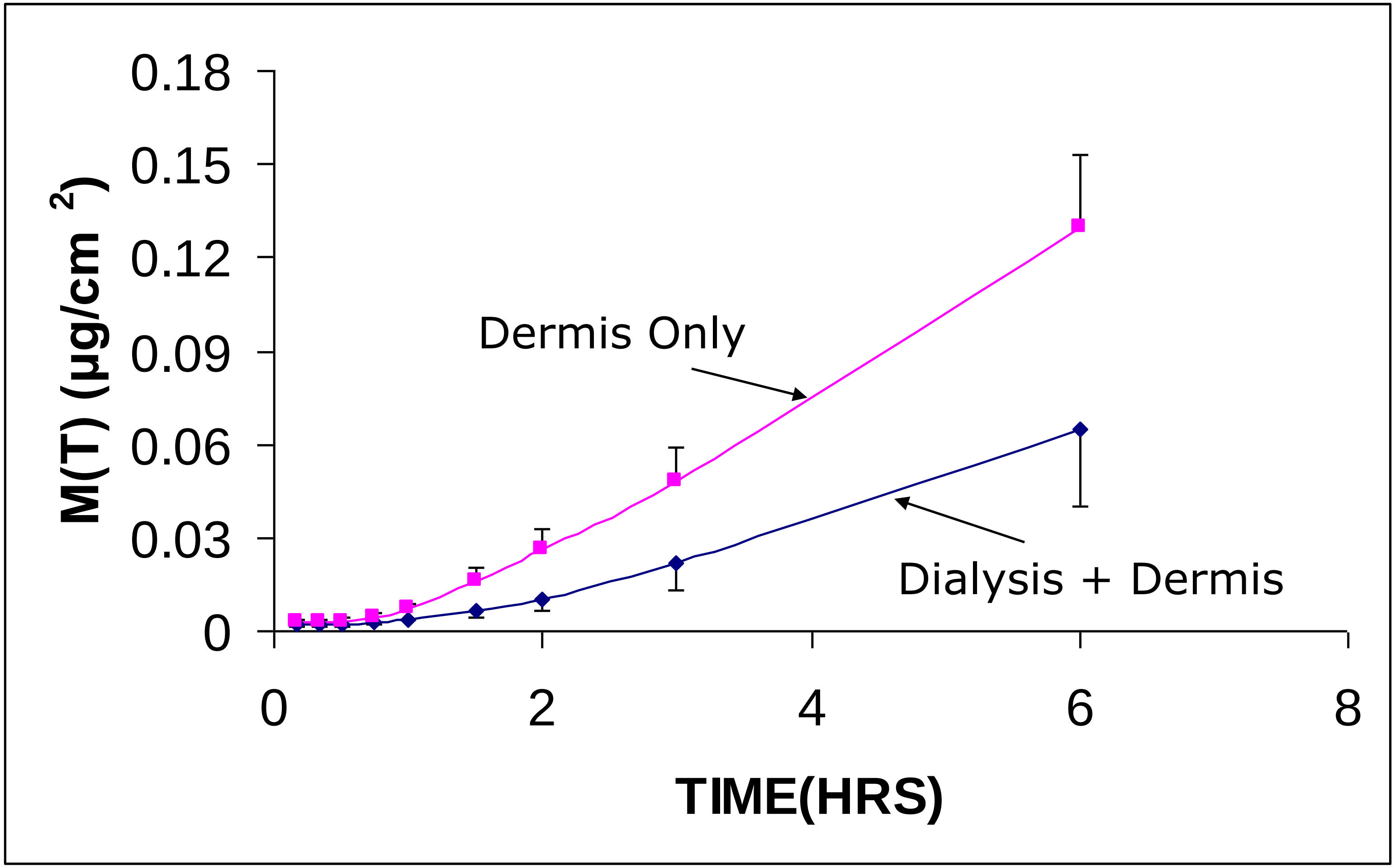
Test The Influence of Diffusable Protein On Permeant Transport.

Dermis Study Experimental Setup



DEET Results

^{14}C DEET PERMEATION



Graph reflects presence of dialysis membrane and ABL

^{14}C DEET PERMEATION

Treatment	R_{de} 10^3 (s/cm)	$D_{de}K_{de}$ 10^6 (cm^2/s)	D_{de} 10^6 (cm^2/s)	K_{de}
Dermis Only CONTROL N=6	8.56 6.68	5.51 0.90	9.63 2.32	0.69 0.10
Dermis + Dialysis N=3	12.5 10.1	3.50 0.84	7.51 1.28	0.46 0.05

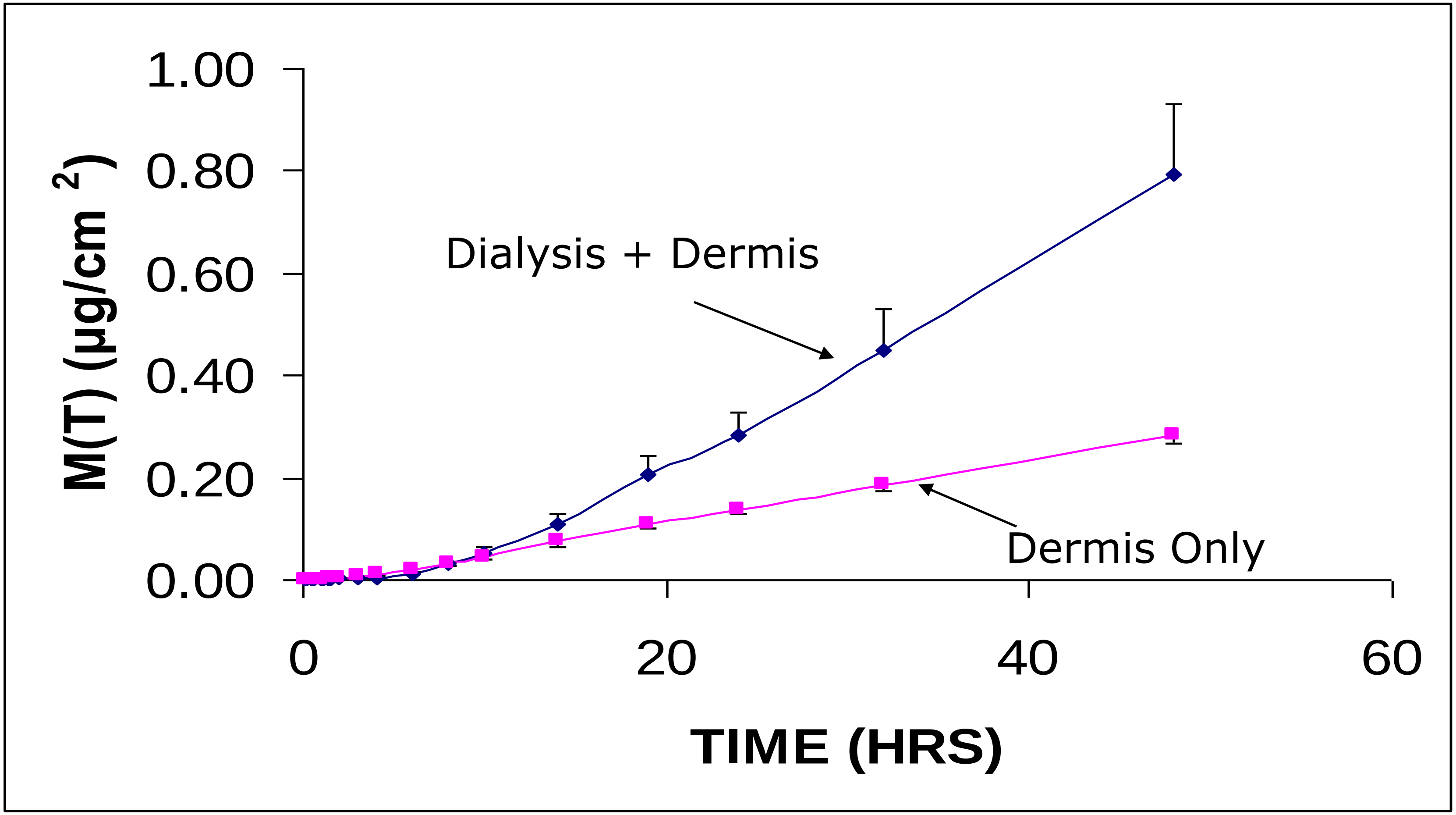
- No significant differences were observed between the treatment and control.

Conclusions for DEET Studies

- The presence of the dialysis membrane had no significant effect on the transport parameters.

Diclofenac (DCF) Results

^{14}C DCF PERMEATION



Graph reflects presence of dialysis membrane and ABL

^{14}C DCF PERMEATION

Treatment	R_{de} 10^{3} (s/cm)	$D_{de}K_{de}$ 10^{6} (cm^{2}/s)	D_{de} 10^{6} (cm^{2}/s)	K_{de}
Dermis Only CONTROL N=11	25.6 13.2	1.30 0.09	1.04 0 .08	1.38 0.17
Dermis + Dialysis N=7	7.78 4.33 *	3.94 0.31 *	1.33 0 .20	3.35 0.37 *

* Denotes: significantly different from control

SKIN CONCENTRATION DATA FOR DEET AND DCF

Permeant	Skin Concentration (µg/ml) per Treatment	
	Dialysis + Dermis	**Dermis Only**
DEET	2.06 0.19 (n=3)	3.15 0.18 * (n=6)
DCF	9.13 1.62 (n=7)	3.42 0.37 * (n=11)

Significant differences were observed between both treatments for both DEET and DCF

Conclusions for DCF Studies

- The presence of dialysis membrane in series with dermis significantly affects the measured tissue concentrations and transport parameters.

- Presence of dialysis membrane is essential for highly protein bound permeants.

Summary

- The presence of a barrier membrane has a significant effect on the transport parameters of protein bound materials which includes many lipophilic chemicals.
- Our data support the hypothesis of albumin diffusion through the tissue sample.
- Our results highlight the need for absolute caution in the use of old data sets of highly lipophilic compounds.

Acknowledgments

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Thank you!