



# Utilization of Reconstructed Cultured Human Skin models as an Alternative Skin for Permeation Studies of Chemical Compounds

城西大学 薬学部 薬粧品動態制御学研究室

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# 皮膚透過性および皮膚中濃度の把握



## ■ 曝露量と反応

安全性(リスク)と有効性(効果)の確認のため、ハザード物質や薬剤の曝露量の把握が重要

皮膚適用製剤の場合、  
皮膚透過量や皮膚中濃度の把握が必要

$$\log P \text{ (cm/s)} = 1.17 \times 10^{-7} K_{o/w}^{0.751} + 2.73 \times 10^{-8}$$

Morimoto Y *et al.*, J Pharm Pharmacol., 44, 634-639 (1992)

$$\log P \text{ (cm/s)} = -6.3 + 0.71 \times \log K_{o/w} - 0.0061 \times MW$$

Potts RO, Guy RH, Pharm. Res., 9, 663-669 (1992)

$$\log P \text{ (cm/s)} = -5.1 + 0.44R_2 - 0.49\pi_2^H - 1.48\Sigma\alpha_2^H - 3.44\Sigma\beta_2^H + 1.94V_x$$

Abraham *et al.*, Pestic. Sci., 55, 78-88 (1999)

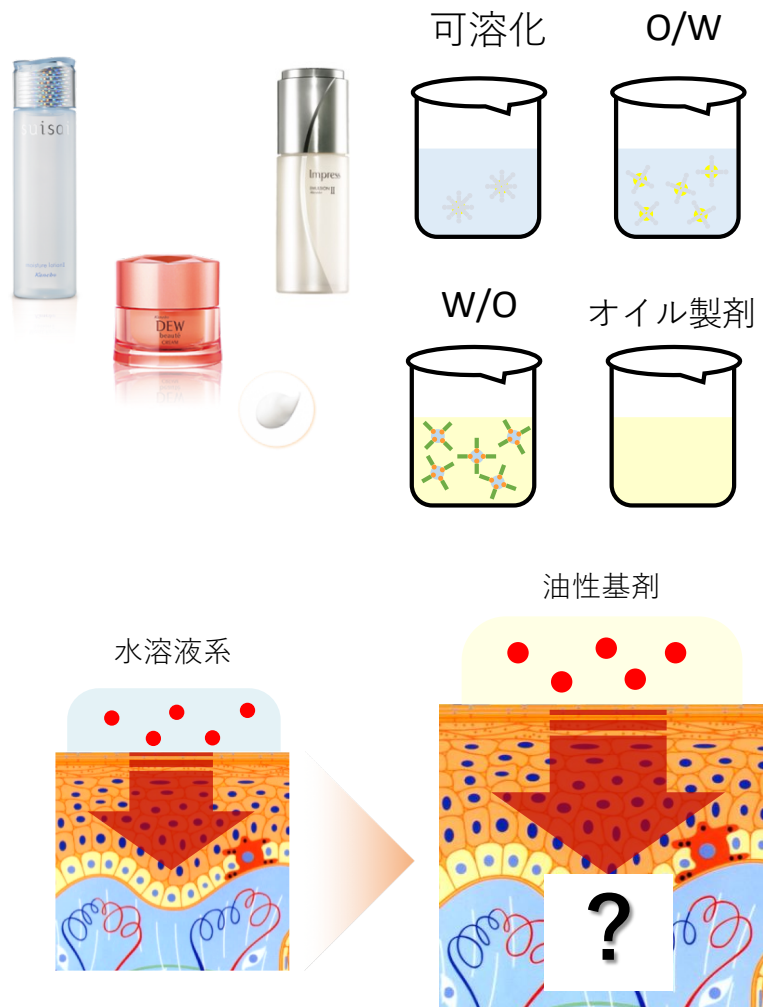
## ■ 透過性 (透過係数: $P$ ) の予測

化合物の透過性の予測は数多く実施

- $K_{o/w}$ : 水/オクタノール分配係数
- MW: 分子量
- $R_2, \pi_2^H, \Sigma\alpha_2^H, \Sigma\beta_2^H, V_x$ :  
Abraham Solvation Parameter

水基剤からの透過予測式

# 皮膚適用製剤の課題



## ■ 皮膚適用製剤の剤形

化粧水、乳液、クリーム、美容液、オイルなど  
様々な剤型が存在

水性基剤ベースの可溶化やO/Wエマルジョン、  
油性基剤ベースのW/Oエマルジョンやオイルなどがある

## ■ 油剤の配合

皮膚への伸ばしやすさなど使用感の向上、  
皮膚の閉塞によるエモリエント効果等  
のため多くの皮膚適用製剤に配合される

## ■ 油剤からの透過

油剤が配合されることによる  
溶解度の変化や皮膚への作用などによって、  
水基剤からの予測値とは異なる

油剤からの透過と油剤の物理化学的特性値との関係を  
網羅的に検討した研究はほとんどない

# 欧州(EU)における動物実験禁止規制

## ■ EU化粧品指令公布（1976年）

## ■ EU化粧品指令第6次改正公布（1993年）

動物試験を用いて化粧品の安全性評価を行なうことを規制した条項、いわゆる動物試験禁止に関する記述がはじめて明示

## ■ EU化粧品指令第7次改正公布（2003年）

### Testing ban（2009年）

EU域内での化粧品成分・製品の動物試験の実施を禁じる

### Marketing ban（2013年）

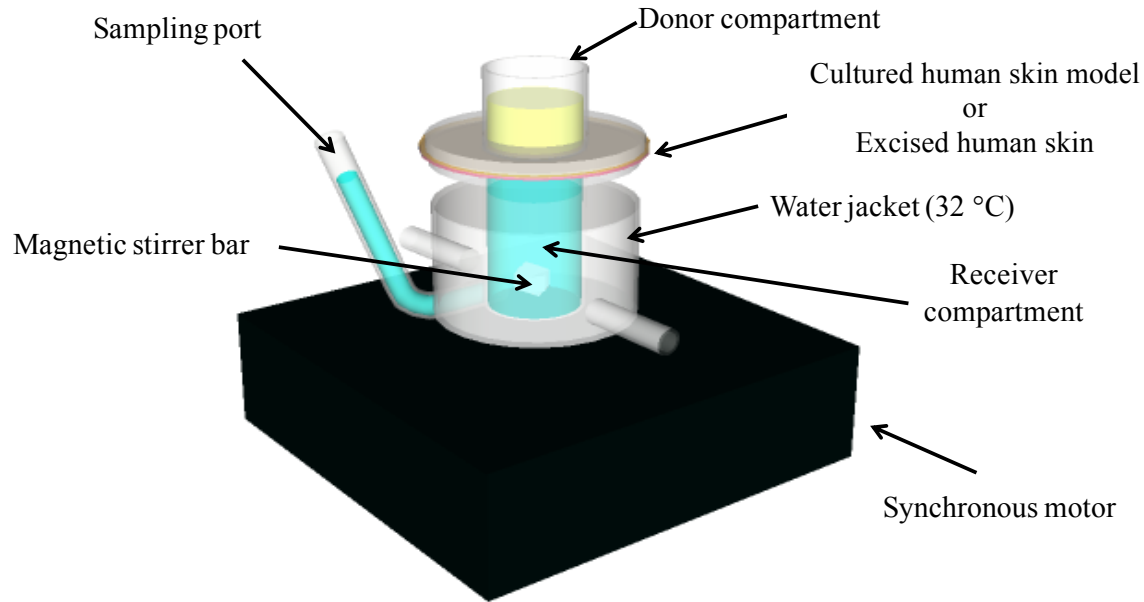
動物試験を行なった化粧品成分を含む製品の販売を禁じる

**2013年3月11日以降**に化粧品およびその原料について動物実験を行った場合、その化粧品は**EU市場で販売できない**

# Agenda

- 3次元培養ヒト皮膚モデルを用いた物質の皮膚透過性評価の有用性
- 皮膚適用物質の皮膚中濃度予測法の構築
- O/Wエマルションからの薬物の*in silico*皮膚透過性予測モデルの構築

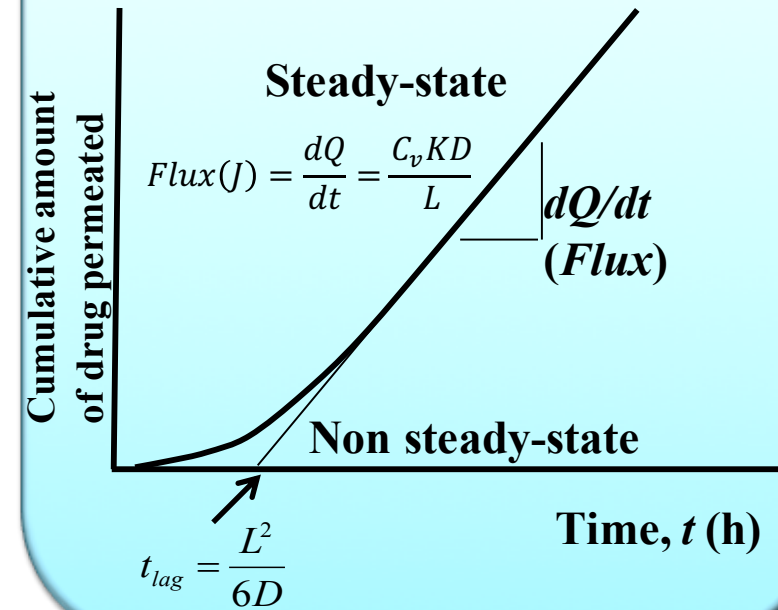
# In vitro skin permeation experiments



**Vertical typed diffusion cell**

- **Permeation profile and parameters**
- **Drug concentration in the membrane**

**Typical skin permeation profile**

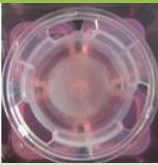


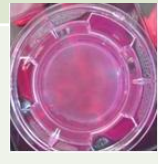

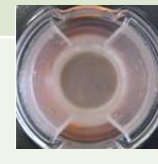


**Typical skin permeation profile**

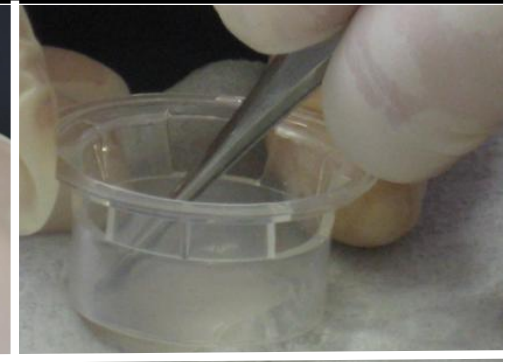
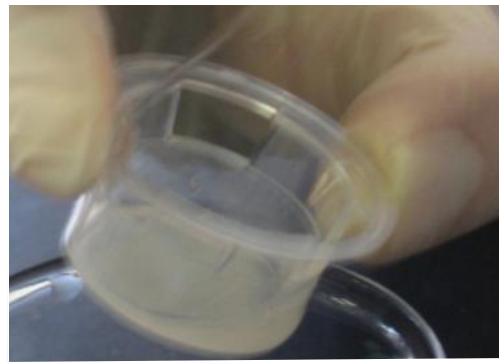
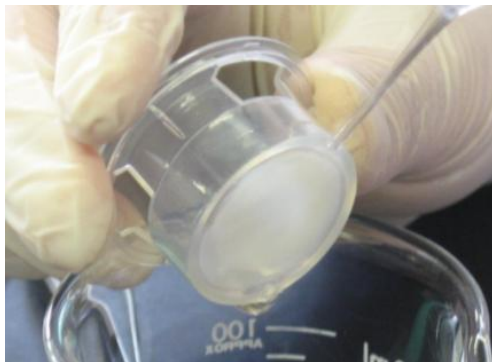
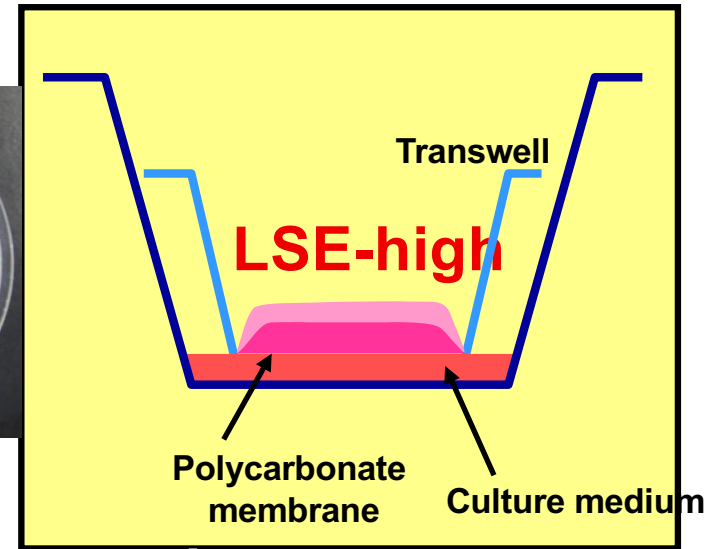
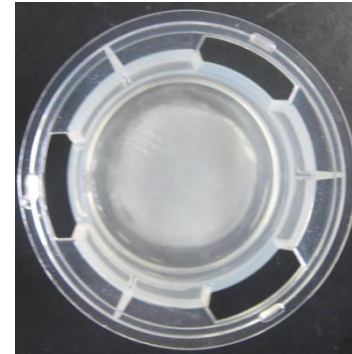
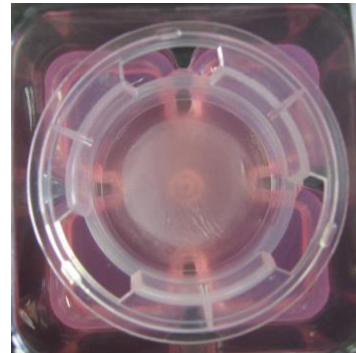
Skin permeation parameters

- Permeation rate (*flux*)
- Permeability coefficient
- Partition coefficient
- Diffusion coefficient

## Reconstructed cultured human skin models used in this experiment

| Bland name<br>(company name)     |                                                                                     | Site of<br>skin for<br>the model | Well | Cell type                           | Effective<br>permeation area<br>(cm <sup>2</sup> ) | Dose<br>condition |
|----------------------------------|-------------------------------------------------------------------------------------|----------------------------------|------|-------------------------------------|----------------------------------------------------|-------------------|
| TESTSKIN™ LSE-high<br>(Toyobo)   |    | Epidermis/<br>dermis             | 6    | Side by side                        | 0.95                                               | infinite          |
| EpiDerm™ 606X<br>(MatTek/Kurabo) |    | Epidermis                        | 6    | Side by side                        | 0.95                                               | infinite          |
| LabCyte Epi-model<br>(J-Tec)     |    | Epidermis                        | 12   | Franz                               | 0.5                                                | finite            |
| Neoderm® -E<br>(TegoScience)     |   | Epidermis                        | 6    | Side by side                        | 0.95                                               | infinite          |
| Vitrolife-skin<br>(Gunze)        |  | Epidermis/<br>dermis             | 6    | Side by side<br>(Ussing<br>chamber) | 0.5                                                | Infinite          |
| Episkin®<br>(SkinEthic)          |  | Epidermis                        | 12   | Franz                               | 0.5                                                | finite            |

# Set-up of 3-dimensional cultured skin model for in vitro permeation experiment (in case of LSE-high)

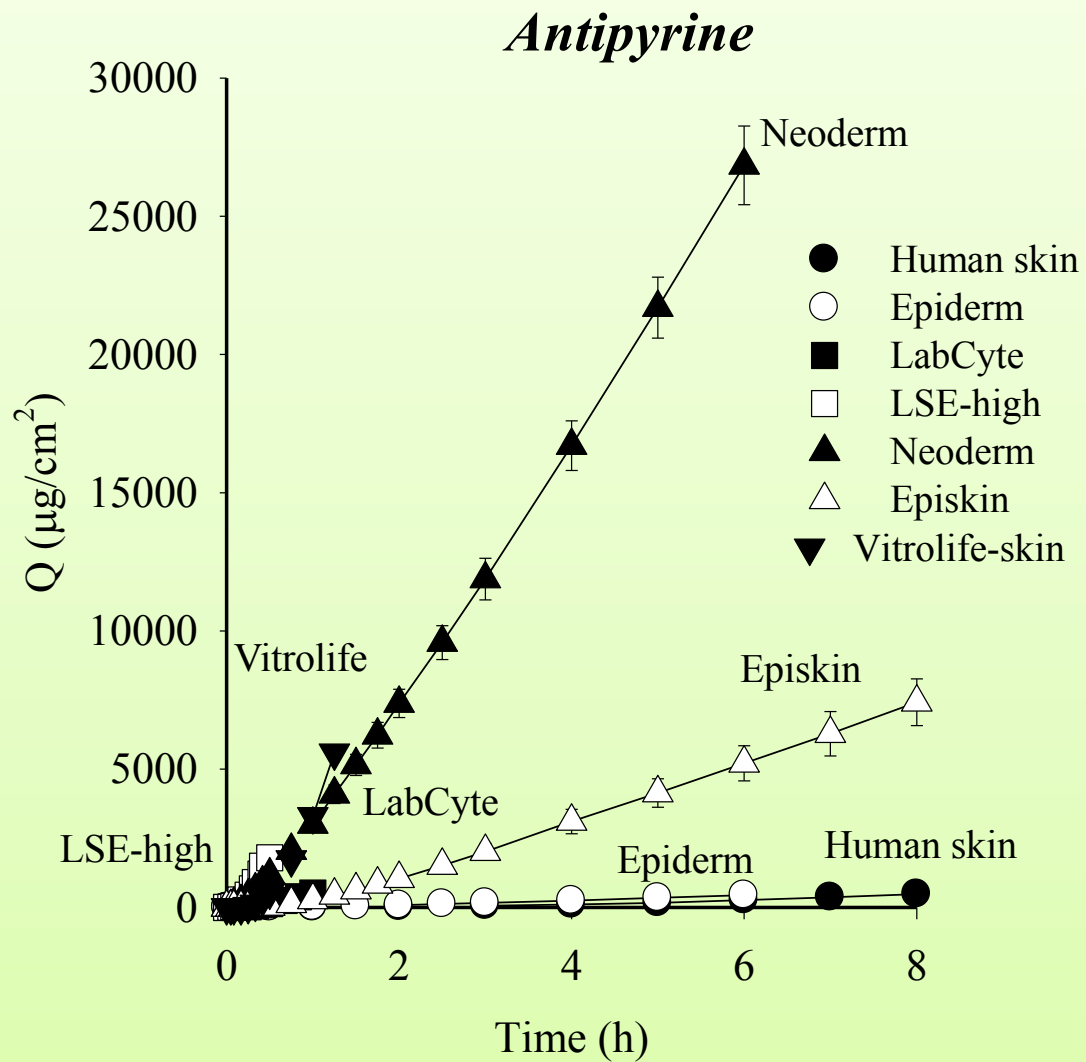




# Materials

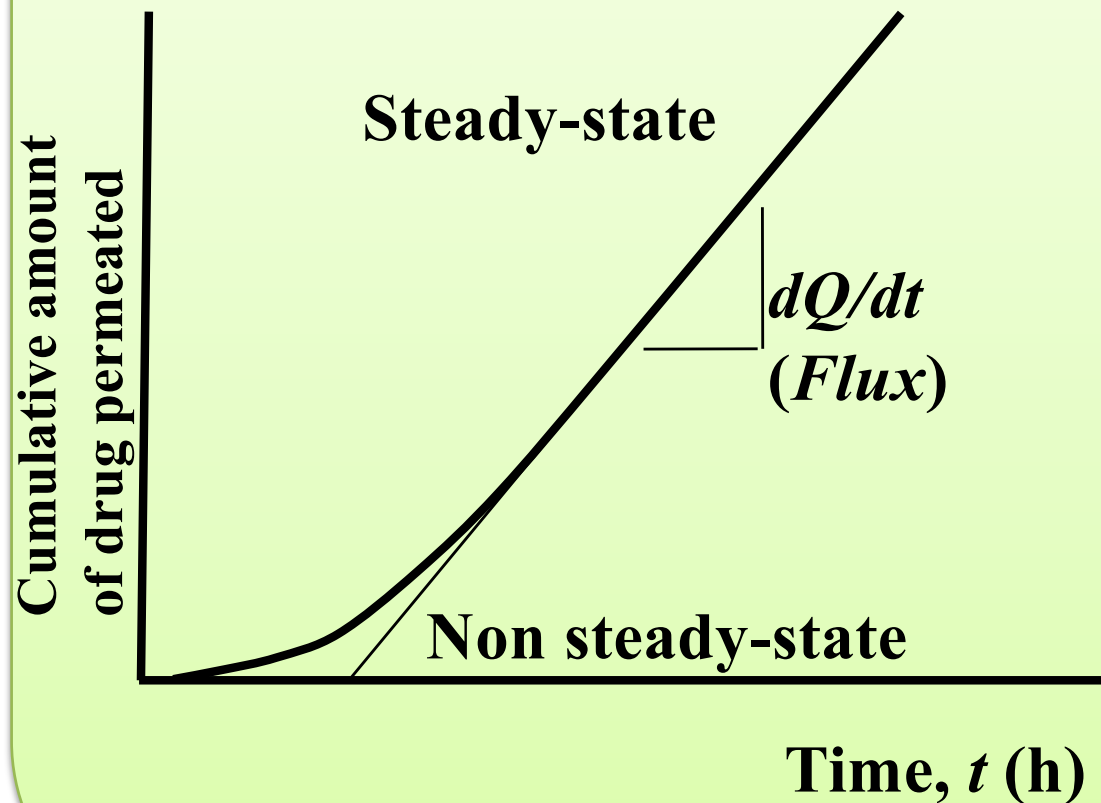
| Drug                     | Abbreviation | Log Ko/w | M.W.   |
|--------------------------|--------------|----------|--------|
| Antipyrine               | ANP          | -1.507   | 188.23 |
| Isosorbide-5-mononitrate | ISMN         | -0.151   | 191.14 |
| Caffeine                 | CAF          | -0.123   | 194.19 |
| Aminopyrine              | AMP          | 1.065    | 231.29 |
| Isosorbide dinitrate     | ISDN         | 1.225    | 236.14 |
| Benzoic acid             | BA           | 1.410    | 122.12 |
| Flurbiprofen             | FP           | 2.179    | 224.27 |

# Comparison of skin permeation through 3-dimensional cultured human skin models



# Calculation of permeation coefficient ( $P$ ) from skin permeation profile

## Typical skin permeation profile



$$\begin{aligned} \text{Flux} &= \frac{dQ}{dt} = \frac{C_v K D}{L} \\ &= P C_v \end{aligned}$$

$C_v$ : Drug conc.

$K$ : Partition coefficient

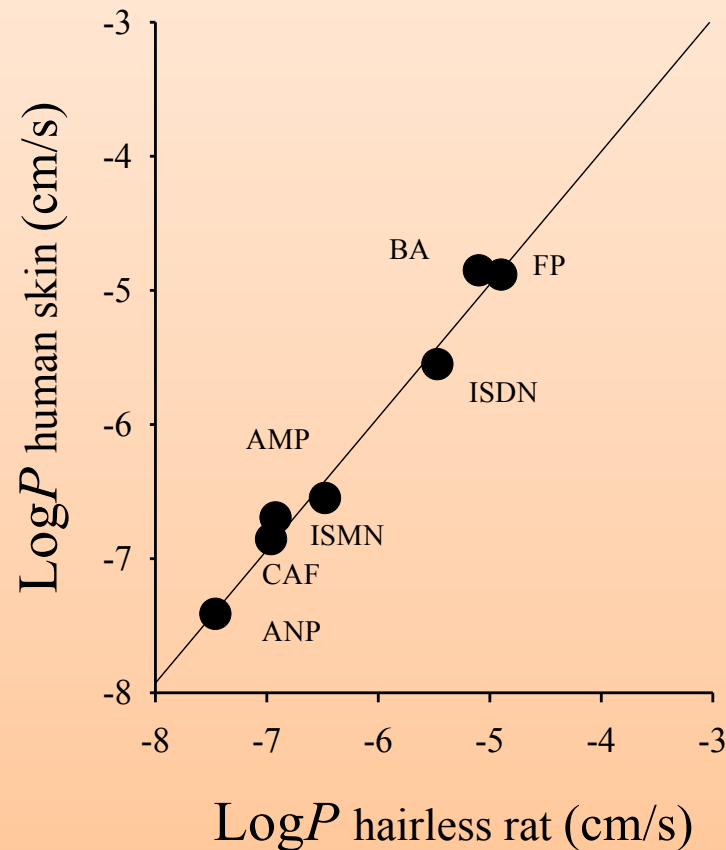
$D$ : Diffusivity

$L$ : Membran thickness

$P$ : Permeation coefficient

# Relationships of membrane permeability coefficients ( $P$ ) of drugs

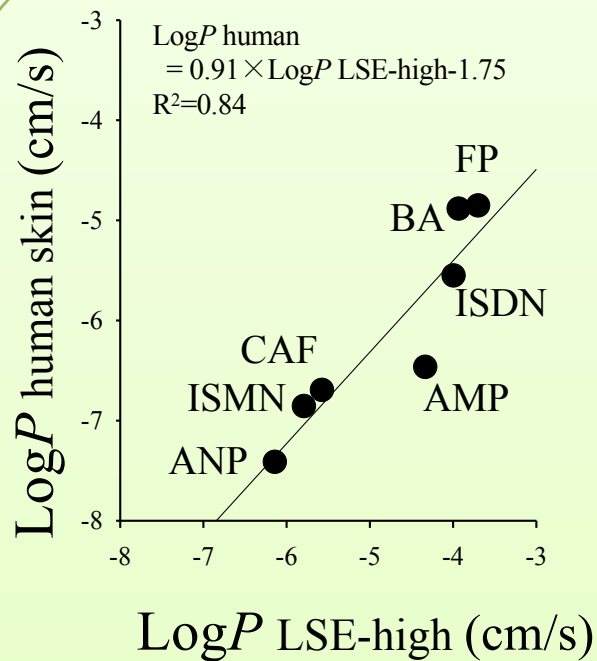
## Human skin/Hairless rat skin



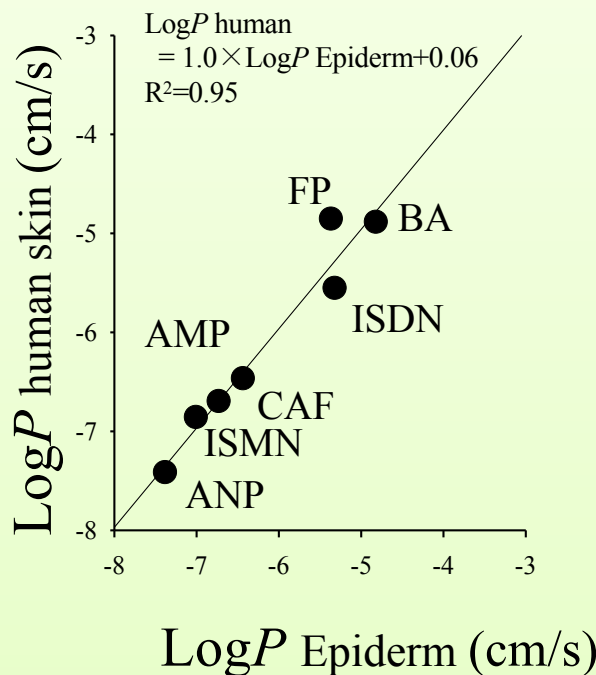
$$\begin{aligned} \text{Log}P_{\text{human}} &= 0.98 \times \text{Log}P_{\text{hairless rat}} - 0.0089 \\ R^2 &= 0.98 \end{aligned}$$

Watanabe, T., Sugibayashi, K. et al., *Altern. Animal Test. Experiment.*, 8, 1-14 (2001)

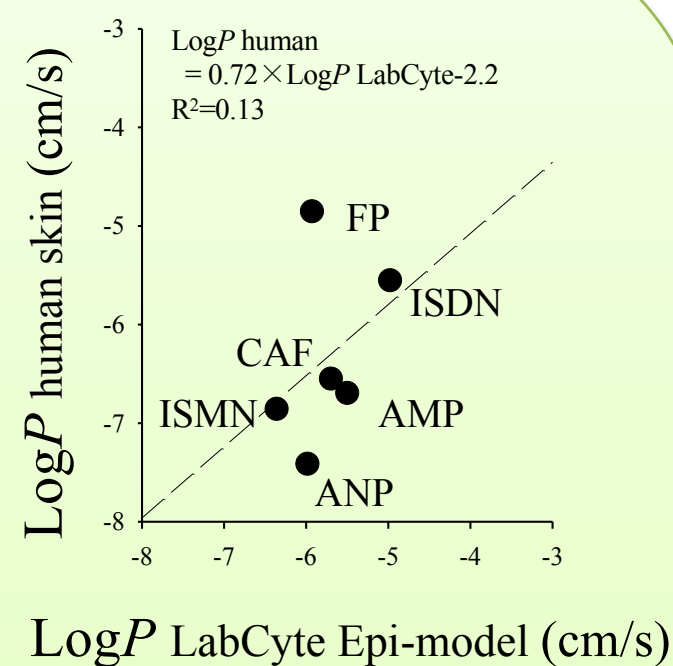
### a) LSE-high



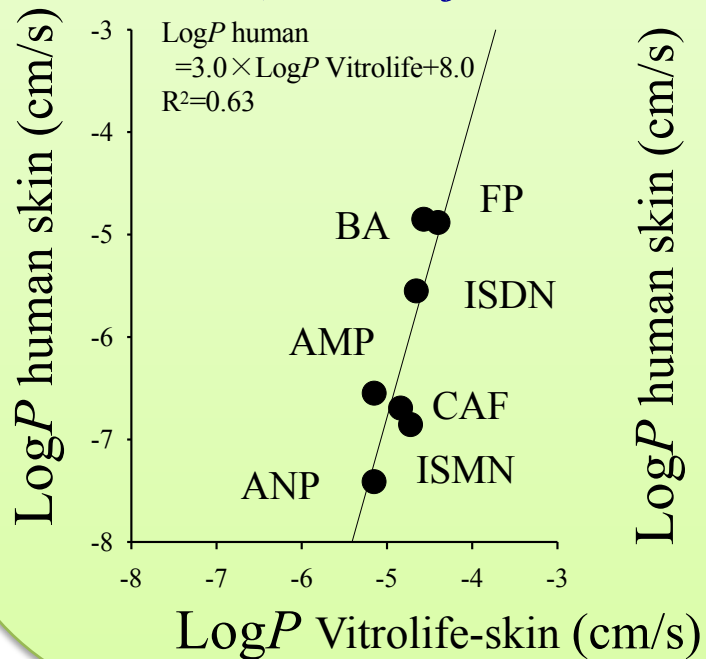
### b) Epiderm



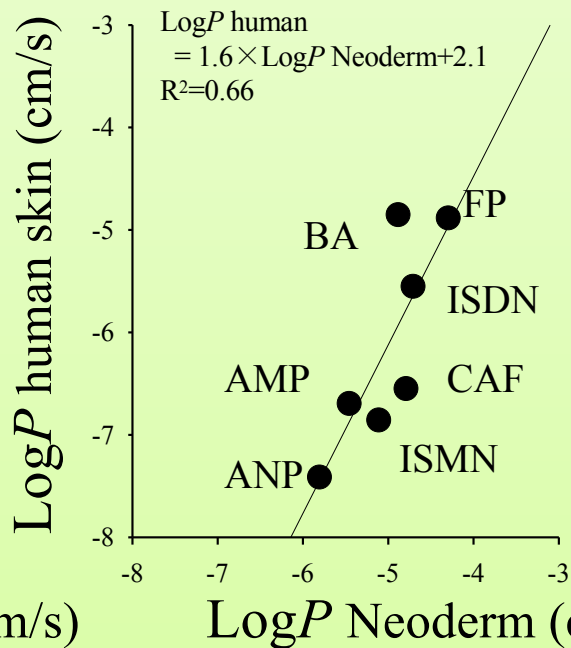
### c) LabCyte-epimodel



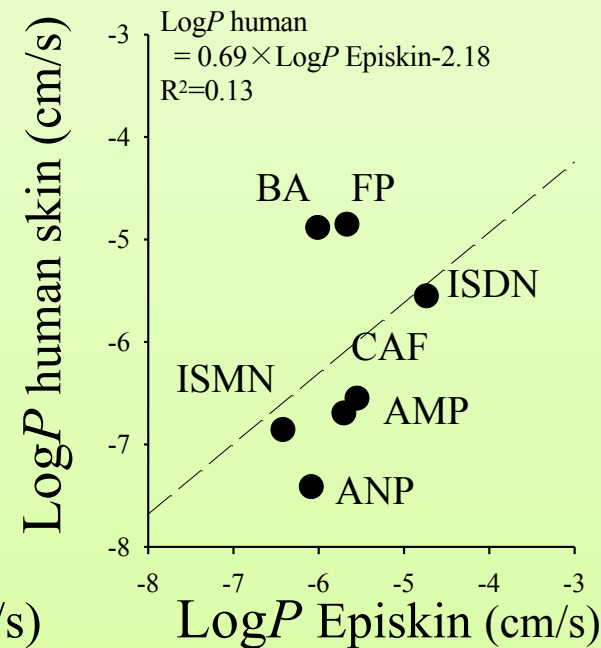
### d) Vitrolife



### e) Neoderm

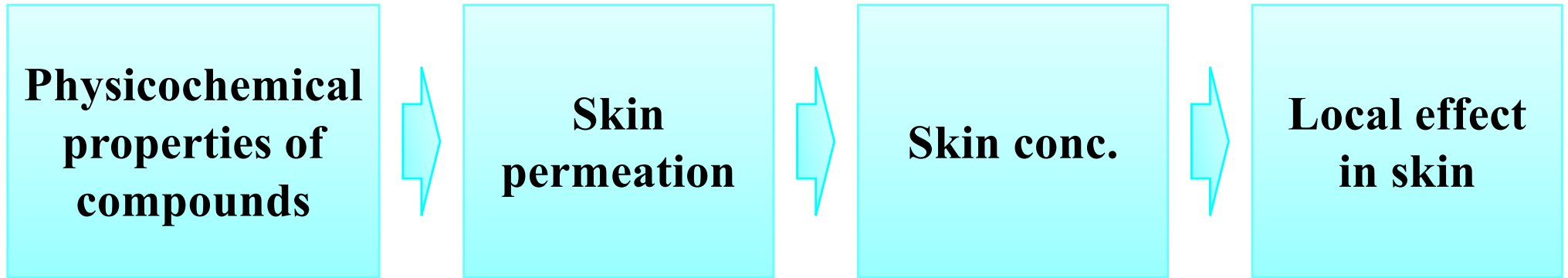


### f) Episkin



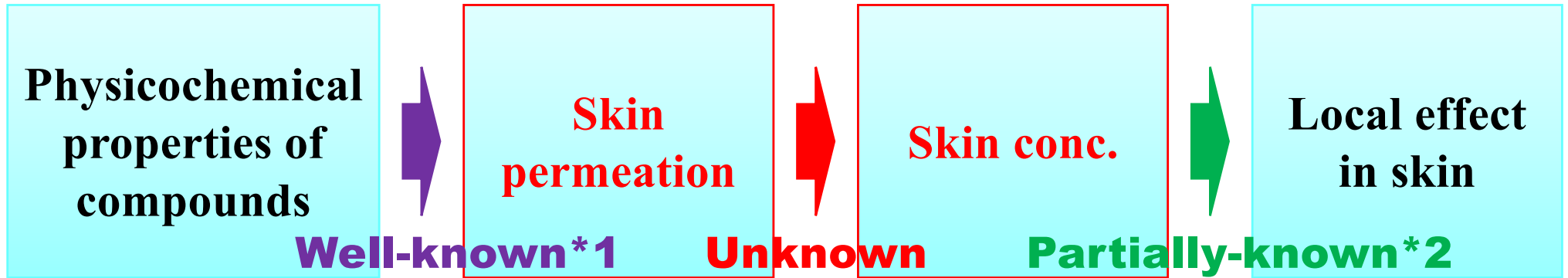
# 皮膚適用する化学物質の有効性・安全性評価

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# これまでの研究

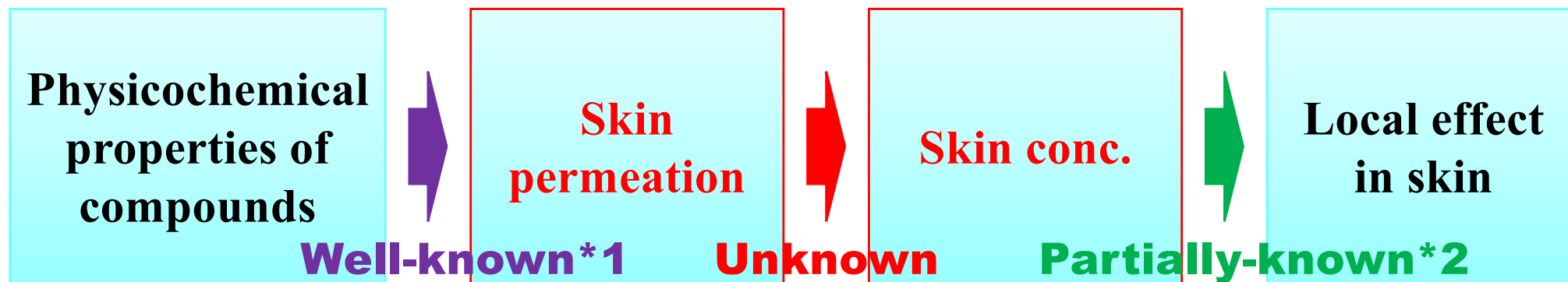
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\*1 Potts R.O., Guy R.H., *Pharm. Res.*, **9**, 663-669 (1992).

\*2 Kano S., Sugibayashi K., *Pharm. Res.*, **23**, 329-335 (2006).

# 本研究の目的



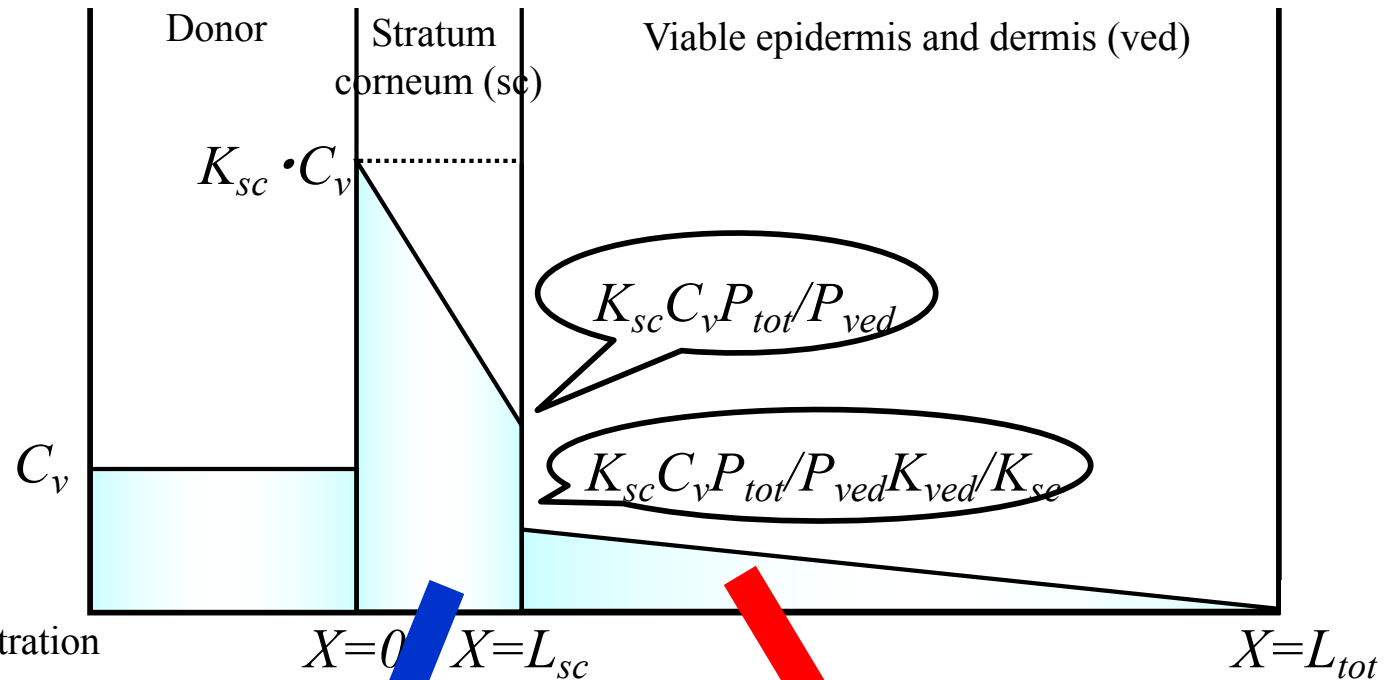
\*1 Potts R.O., Guy R.H., *Pharm. Res.*, **9**, 663-669 (1992).

\*2 Kano S., Sugibayashi K., *Pharm. Res.*, **23**, 329-335 (2006).

皮膚透過性から皮膚中濃度が予測可能であれば、化学物質の物理化学的パラメータから安全性・有効性を評価することができる。すなわち、製剤開発の時間の短縮や実験動物の削減が可能となる。



# Concentration-distance profile at steady state in two-layered skin model



$D$  : Diffusion coefficient  
 $K$  : Partition coefficient  
 $C_v$  : Donor concentration  
 $L_s$  : Thickness of membrane  
 $C$  : Average membrane concentration  
 $P$  : Permeability coefficient

SC concentration

$$C_{ss,sc} = \frac{K_{sc} \cdot C_v + K_{sc} \cdot C_v \cdot P_{tot} / P_{ved}}{2}$$

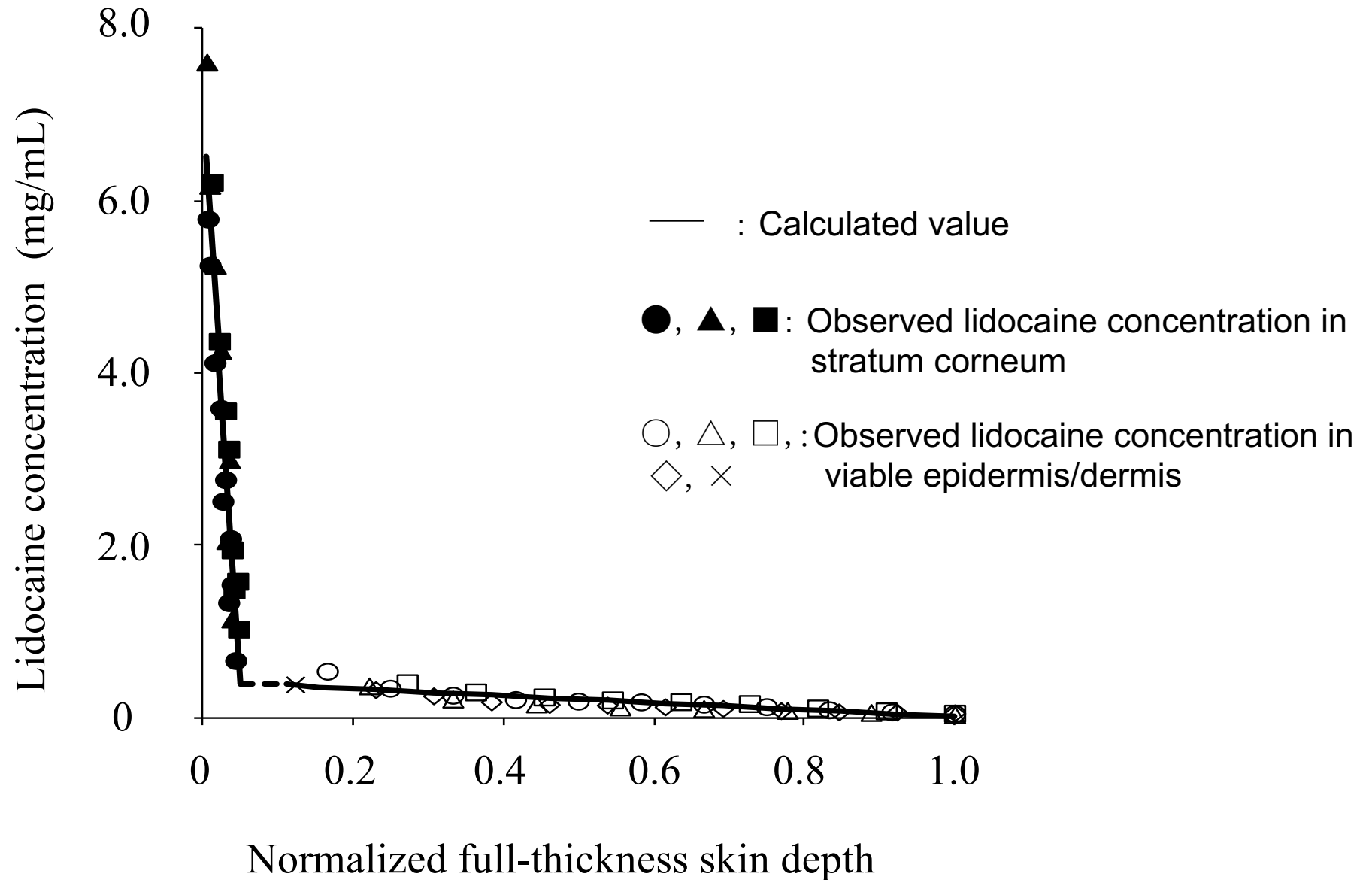
VED concentration

$$C_{ss,ved} = \frac{K_{ved} \cdot C_v \cdot P_{tot} / P_{ved}}{2}$$

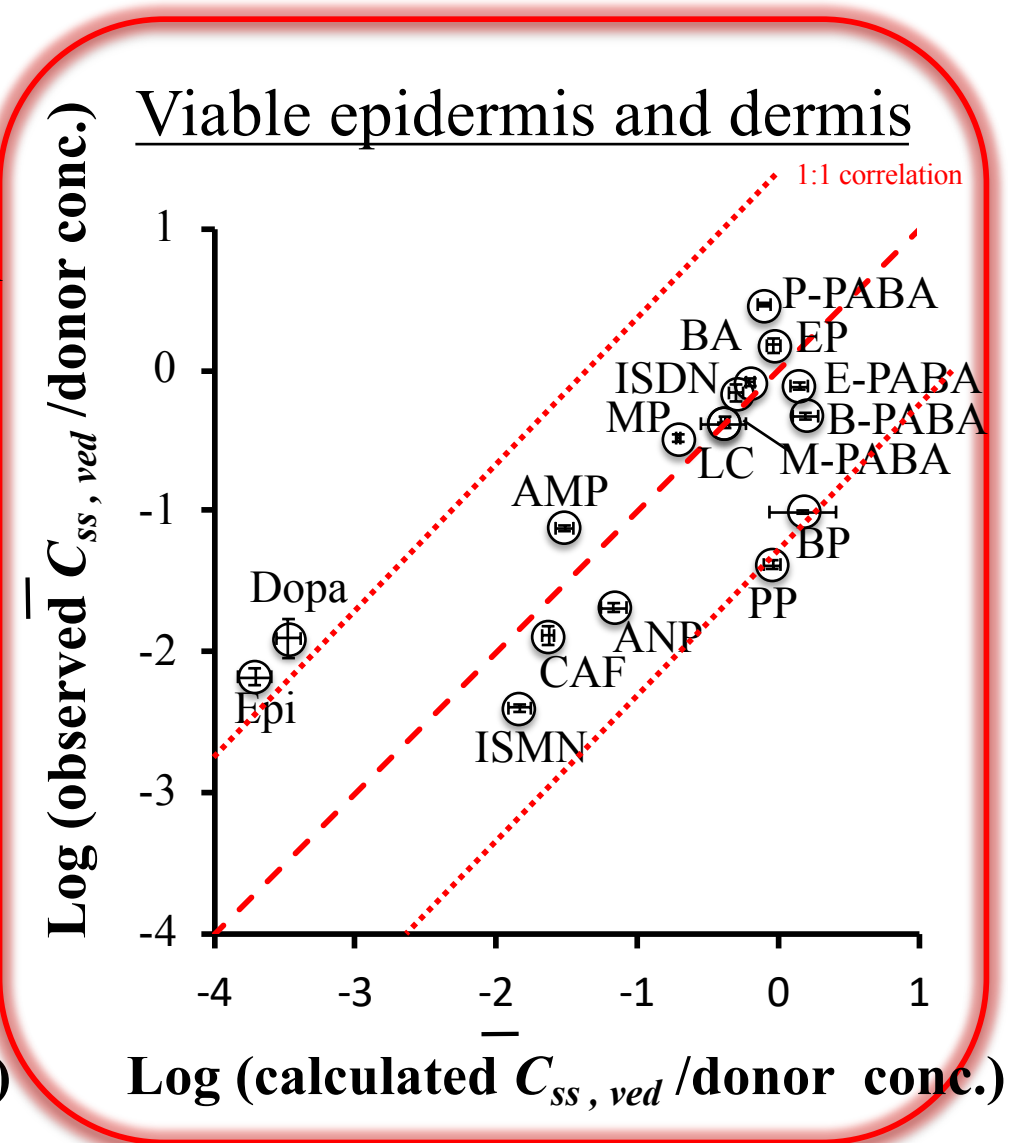
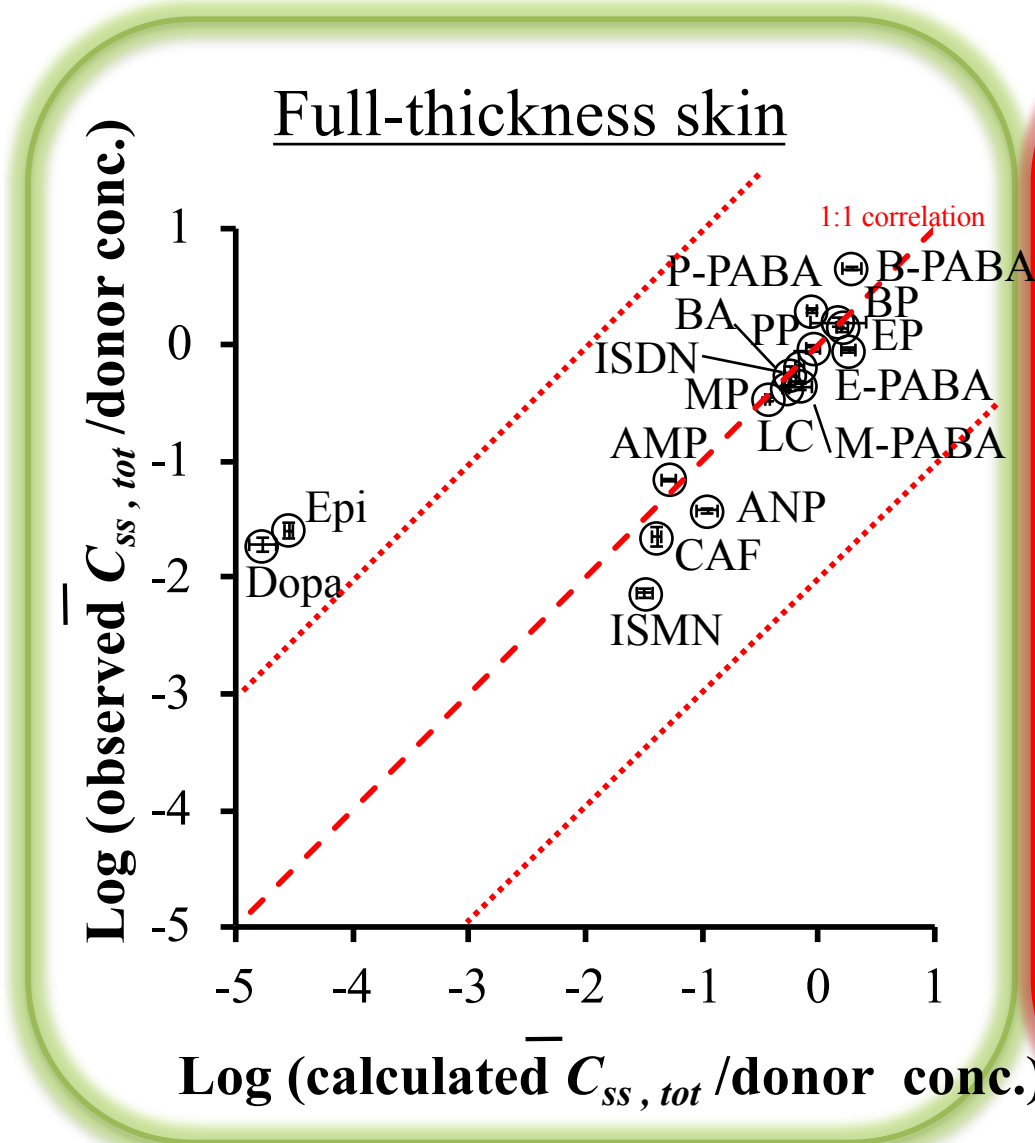
Full-thickness skin concentration

$$\bar{C}_{ss} = \frac{C_v}{2 \cdot L_{tot}} \left\{ K_{sc} \cdot L_{sc} \cdot (1 + P_{tot} / P_{ved}) + K_{ved} \cdot L_{ved} \cdot P_{tot} / P_{ved} \right\}$$

# Steady state drug concentration-depth profile for full-thickness skin



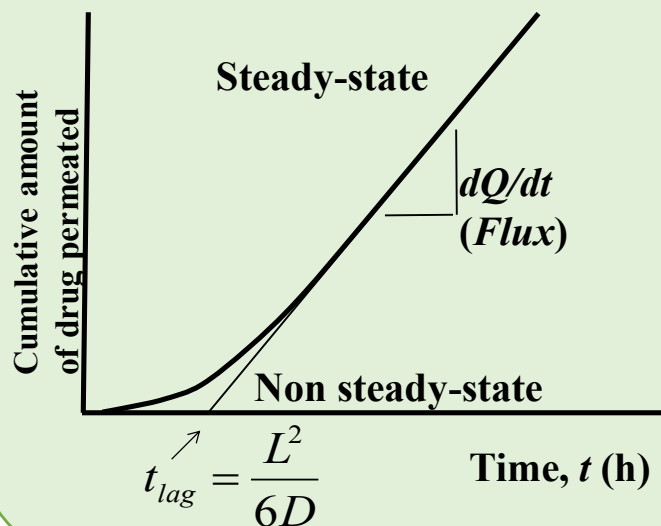
*Relationship between calculated skin conc. and observed skin conc.*



Data range:  $122.12 < M.W. < 234.30$ ,  $-5.54 < ClogP < 3.57$  Mean  $\pm$  S.E. (n = 4)

# In vitro 皮膚透過に及ぼす要因

Typical skin permeation profile



$$Flux(J) = \frac{dQ}{dt} = \frac{C_v K D}{L}$$

$$= \frac{A_v D}{\gamma_s L}$$

$$A_v = \gamma_v \times C_v$$

$$K = \gamma_v / \gamma_s$$

薬物の皮膚への分配係数 ( $K$ )

薬物の溶媒中濃度 ( $C_v$ )

薬物の皮膚中拡散係数 ( $D$ )

皮膚 (バリア層) の厚み ( $L$ )

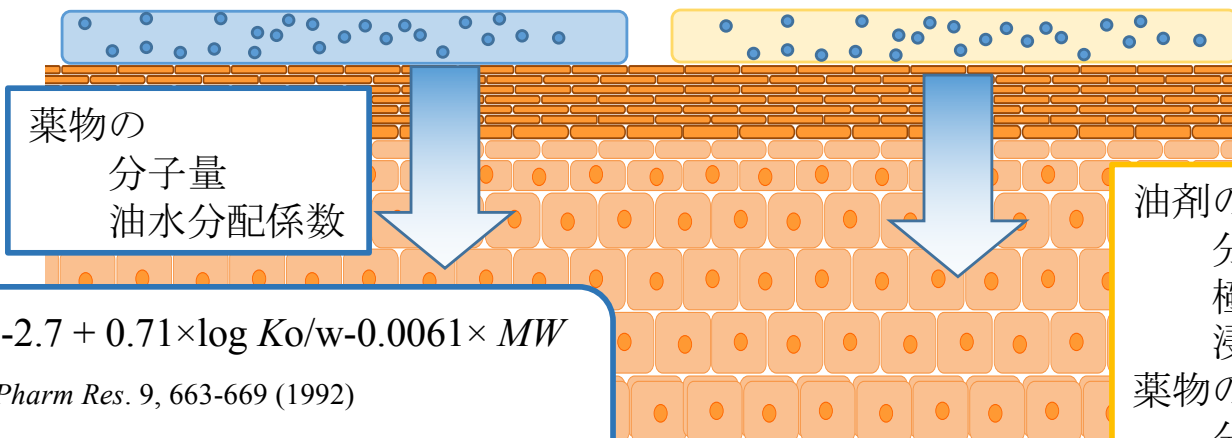
薬物の基剤中熱力学的活動度 ( $A_v$ )

薬物の基剤中活量係数 ( $\gamma_v$ )

薬物の皮膚中活量係数 ( $\gamma_s$ )

水性基剤

油性基剤

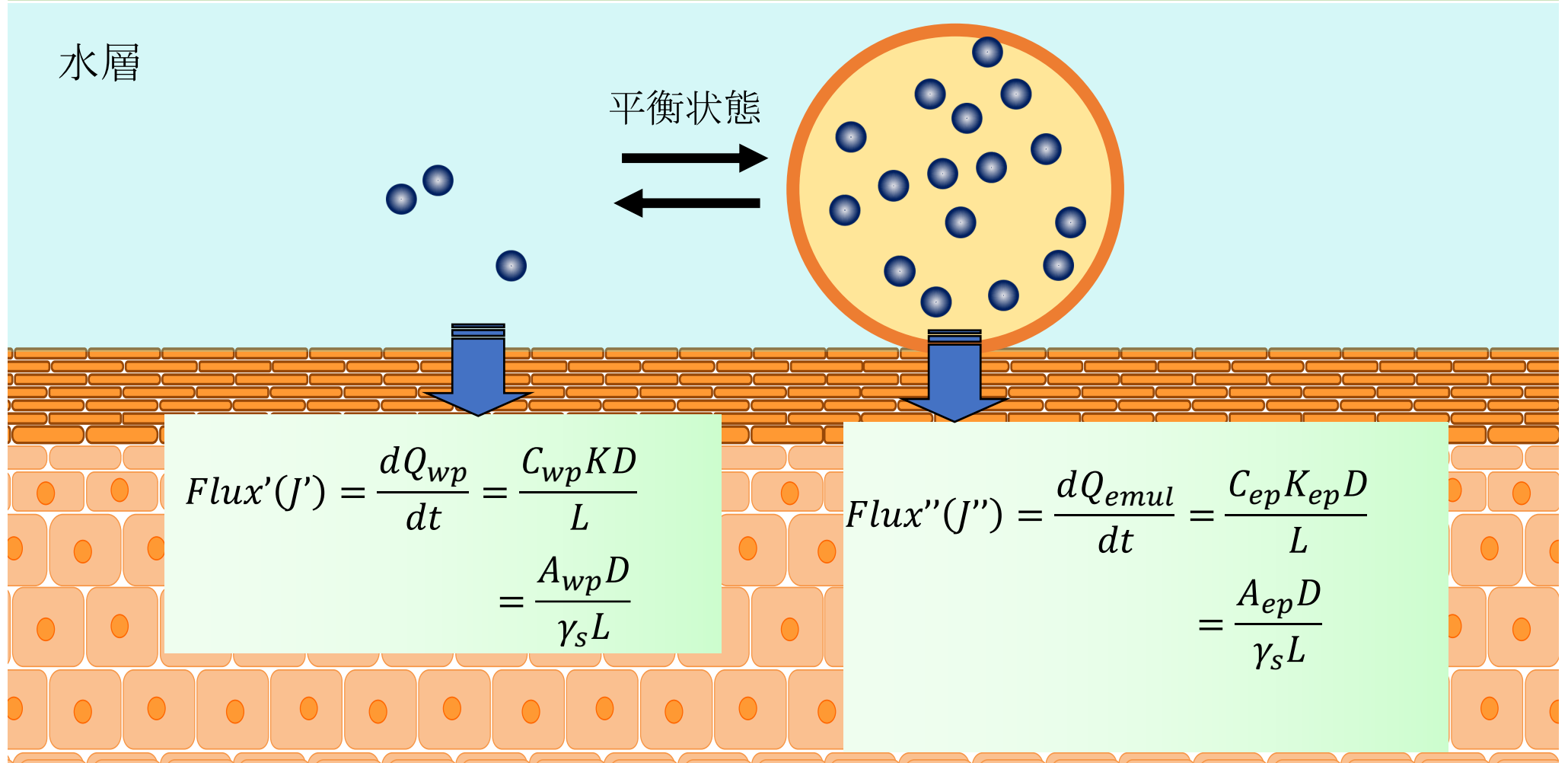


$$\log P \text{ (cm/h)} = -2.7 + 0.71 \times \log Ko/w - 0.0061 \times MW$$

Potts RO, Guy RH., *Pharm Res.* 9, 663-669 (1992)

$r^2=0.67$  (93 compounds)

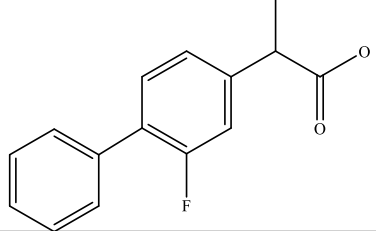
# エマルションからの物質の皮膚透過性



薬物の水層/皮膚分配係数 ( $K$ )  
薬物の水層中濃度 ( $C_{wp}$ )  
薬物の皮膚中拡散係数 ( $D$ )  
薬物の水層中活量 ( $A_{wp}$ )  
薬物の水層からの皮膚透過量 ( $Q_{wp}$ )

薬物のエマルション/皮膚の薬物分配係数 ( $K_{ep}$ )  
薬物のエマルション中濃度 ( $C_{ep}$ )  
薬物の皮膚中拡散係数 ( $D$ )  
薬物のエマルション中活量 ( $A_{ep}$ )  
薬物の皮膚中活量係数 ( $\gamma_s$ )

# Material

| Compound             | Chemical structure                                                                 | $\log K_{o/w}$ | <i>M.W.</i> | Solubility in water<br>( $S_{water}$ , mg/mL) |
|----------------------|------------------------------------------------------------------------------------|----------------|-------------|-----------------------------------------------|
| Flurbiprofen<br>(FP) |  | 2.2            | 224.3       | 0.048                                         |

## Saturated solubility of FP in the ester oils

| Oil name (Abbre.)                              | Saturated solubility<br>( $S_{oil}$ , mg/mL) |
|------------------------------------------------|----------------------------------------------|
| 2-Ethylhexyl isononanoate (EHIN)               | 38.6                                         |
| 2-Ethylhexyl palmitate (EHP)                   | 23.9                                         |
| Diisopropyl adipate (DIPA)                     | 135                                          |
| Di-2-ethylhexyl sebacate (DEHS)                | 58.5                                         |
| Propylene glycol isostearate (PGIS)            | 77.7                                         |
| Diethyl sebacate (DES)                         | 161                                          |
| 2-Octyldodecyl ricinoleate (ODR)               | 65.0                                         |
| 2-Hexyldecyl isostearate (HDIS)                | 11.3                                         |
| Isononyl isononanoate (ININ)                   | 31.9                                         |
| Neopentyl glycol di(2-ethylhexanoate) (NPGDEH) | 52.3                                         |
| Isotridecyl isononanoate (ITDIN)               | 23.3                                         |
| Dioctyl carbonate (DOC)                        | 37.4                                         |

# Preparation of applied formulations

## Oil

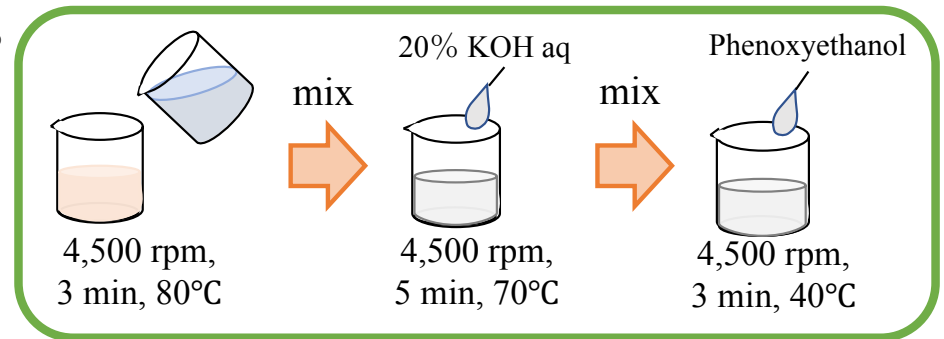
FP solution (10 mg/g) was prepared by completely dissolving FP with ester oil at 80°C followed by stirring at 32°C for 48 h.

## Emulsion

**Total : 20 g**



LABOLUTION®  
(PRIMIX Corp.)



### Composition of o/w emulsions

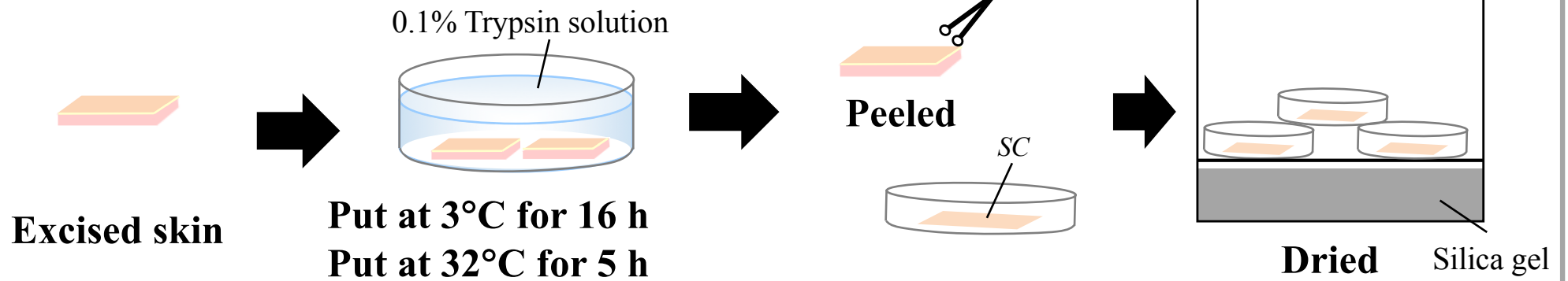
| Component                                             | Ester oil conc. |       |       |       |
|-------------------------------------------------------|-----------------|-------|-------|-------|
|                                                       | 5%              | 10%   | 20%   | 30%   |
| FP                                                    | 0.2             |       |       |       |
| Ester oil                                             | 1               | 2     | 4     | 6     |
| Purified water                                        | 17.13           | 16.13 | 14.13 | 12.13 |
| 2.0% acrylates/C10-30 alkyl acrylate crosspolymer gel | 1.6             |       |       |       |
| 20% KOH                                               | 0.05            |       |       |       |
| Phenoxy ethanol                                       | 0.02            |       |       |       |
| <b>Total</b>                                          | <b>20</b>       |       |       |       |



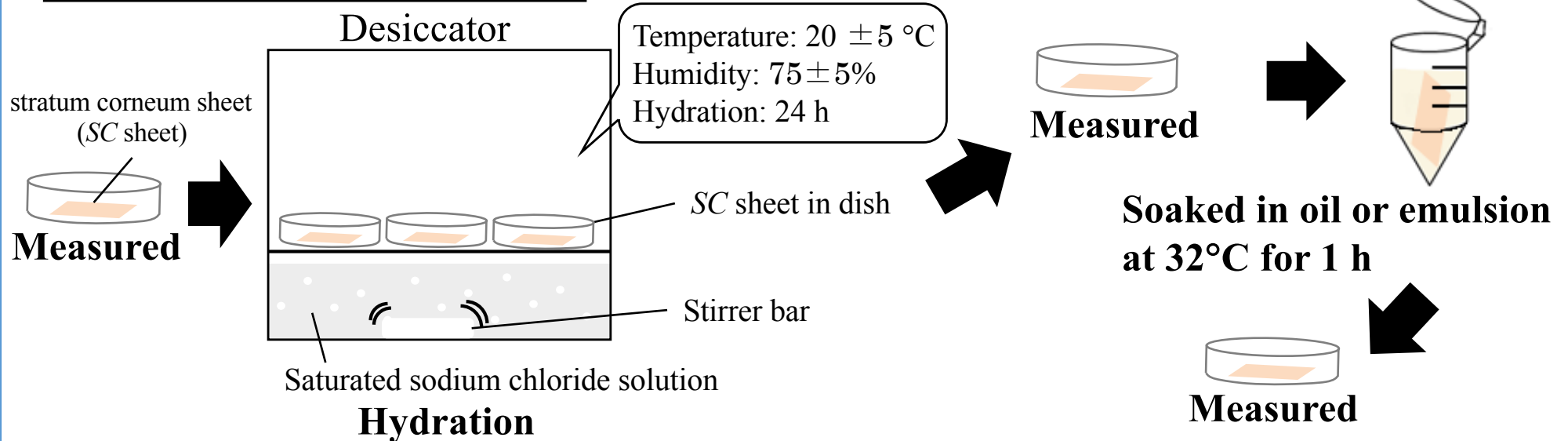
Stored at 32°C

# Oil solvent-uptake experiment

## 角層 (SC) シート作成



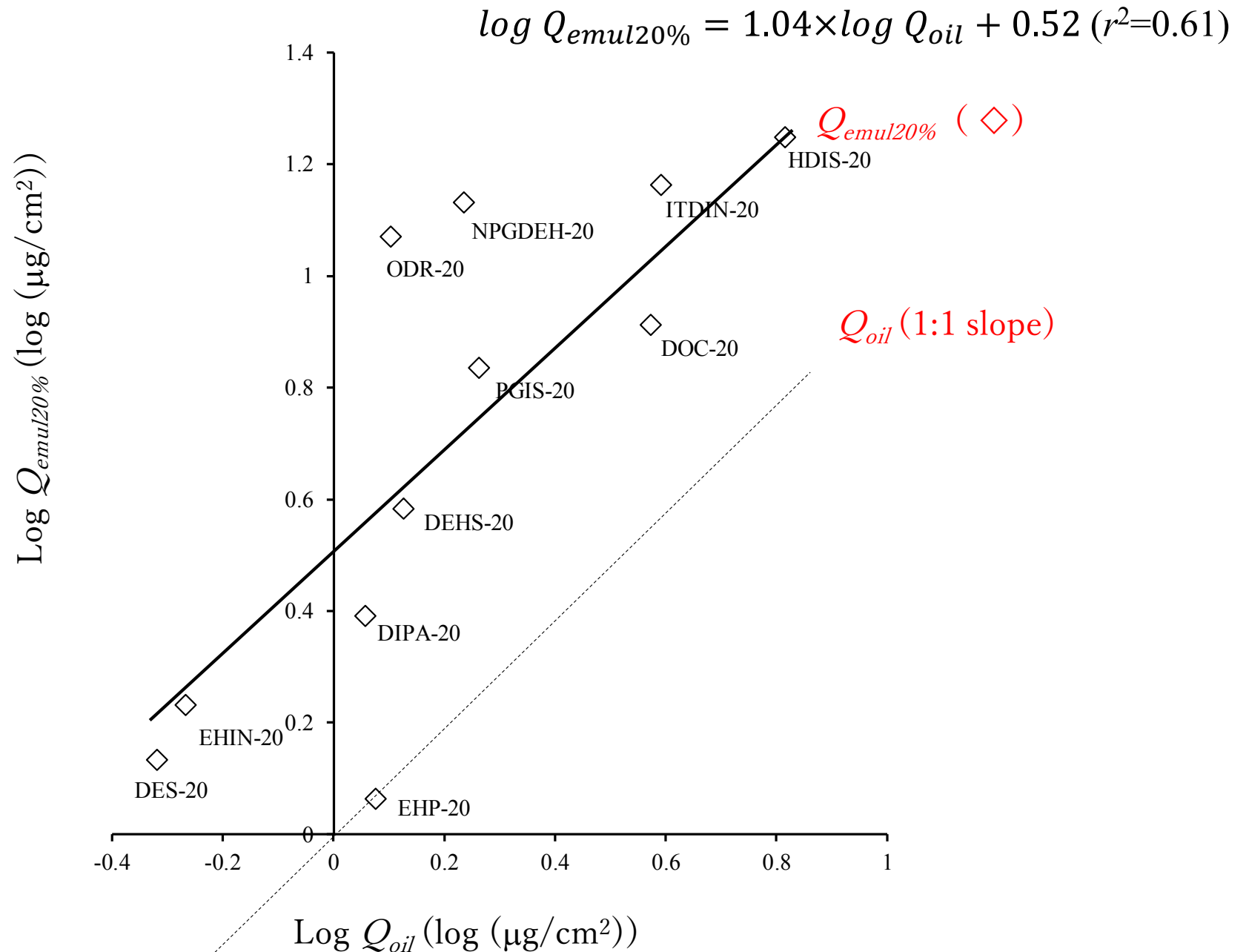
## SCシート取り込み試験



$$\text{SCシートへの油剤取り込み量 (mg/mg)} = \frac{\text{製剤適用前後SCシート重量差 (mg)}}{\text{製剤適用前SCシート重量 (mg)}}$$



# Prediction of skin permeation of FP from *o/w* emulsions



# *In silico* modeling approach

Multi-regression analysis: JMP<sup>®</sup> Pro (ver. 13.1.0, SAS Institute, Cary, NC, USA).

モデルの最適化：Stepwise regression analysis

Cross-validation: 75% training and 25% validation

## Molecular descriptors of oils and measured parameters used in this experiment

| Molecular descriptors of oils and measured parameters used in this experiment |                                        |                                                         |                                                           |
|-------------------------------------------------------------------------------|----------------------------------------|---------------------------------------------------------|-----------------------------------------------------------|
|                                                                               | Abbre.                                 | Descriptors and measured parameters                     |                                                           |
| Measured parameters                                                           | $Q_{oil}$                              | The cumulative amount of FP permeated from the oil base |                                                           |
|                                                                               | $SC_{oil}$                             | Amount of oil uptake into SC sheet                      |                                                           |
|                                                                               | $S_{water}$ and $S_{oil}$              | Solubility of FP in purified water and ester oil        |                                                           |
|                                                                               | $ST$                                   | Surface tension                                         |                                                           |
| Molecular descriptors                                                         | Abraham descriptor                     | $\pi_2^H$                                               | Dipolarity/polarizability                                 |
|                                                                               |                                        | $V_x$                                                   | McGowan characteristic volume                             |
|                                                                               |                                        | $R_2$                                                   | Excess molar refractivity                                 |
|                                                                               |                                        | $\sum \alpha_2^H$                                       | Hydrogen bond donor acidity                               |
|                                                                               |                                        | $\sum \beta_2^H$                                        | Hydrogen bond acceptor basicity                           |
|                                                                               |                                        | $K_{gas/hex}$                                           | Partitioning coefficient between gas phase and hexadecane |
|                                                                               |                                        | $M.W.$                                                  | Molecular weight of oil                                   |
|                                                                               | $d$                                    | Molecular density                                       |                                                           |
|                                                                               | $M_{ref}$                              | Excessive molar refraction                              |                                                           |
|                                                                               | $HA$                                   | Number of hydrogen bond acceptors                       |                                                           |
|                                                                               | $tPSA$                                 | Topological polar surface area                          |                                                           |
|                                                                               | $FRB$                                  | Freely rotatable bonds                                  |                                                           |
|                                                                               | $IR$                                   | Index of refraction                                     |                                                           |
|                                                                               |                                        | Hetero ratio                                            |                                                           |
|                                                                               |                                        | C ratio                                                 |                                                           |
|                                                                               |                                        | N ratio                                                 |                                                           |
|                                                                               | NO ratio                               |                                                         |                                                           |
| $Pa$                                                                          | Parachor, $Pa = ST^{1/4} \cdot M.W./d$ |                                                         |                                                           |

# Multi-regression analysis and cross-validation

$$Q_{oil} = -10.3(\pm 2.7)\pi_2^H + 81.8(\pm 20.9)d + 22.9(\pm 2.5)SC_{oil} - 0.07(\pm 0.02)S_{oil} - 63.5(\pm 17.0)$$

$\pi_2^H$  Dipolarity/polarizability

$d$  Molecular density

$SC_{oil}$  Amount of oil uptake into SC sheet

$S_{oil}$  Solubility of FP in ester oil

$$n = 69, R^2 = 0.68, F_0(4,64) = 30.1, (p < 0.001)$$

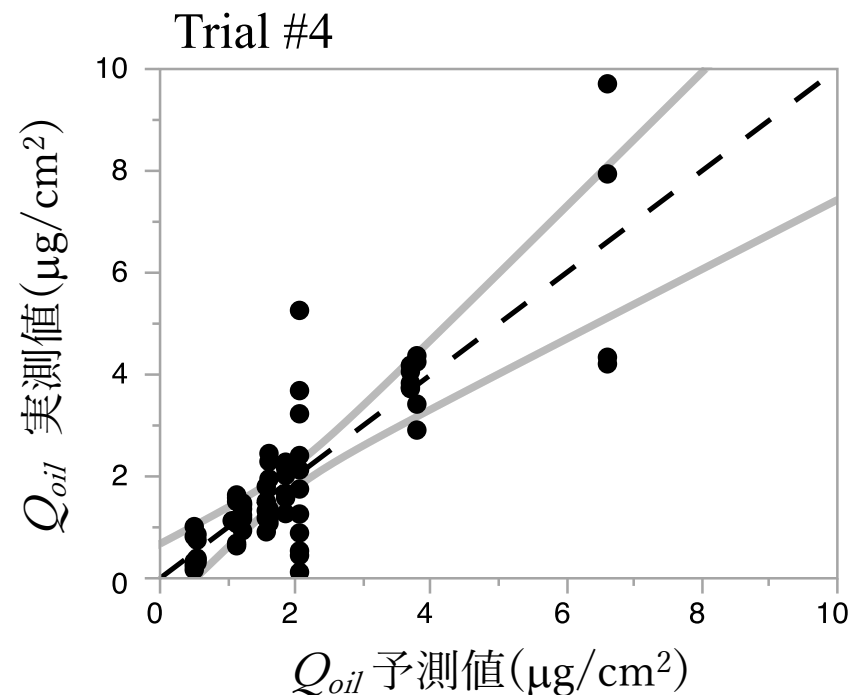
## Cross-validation result

75% training and 25% validation

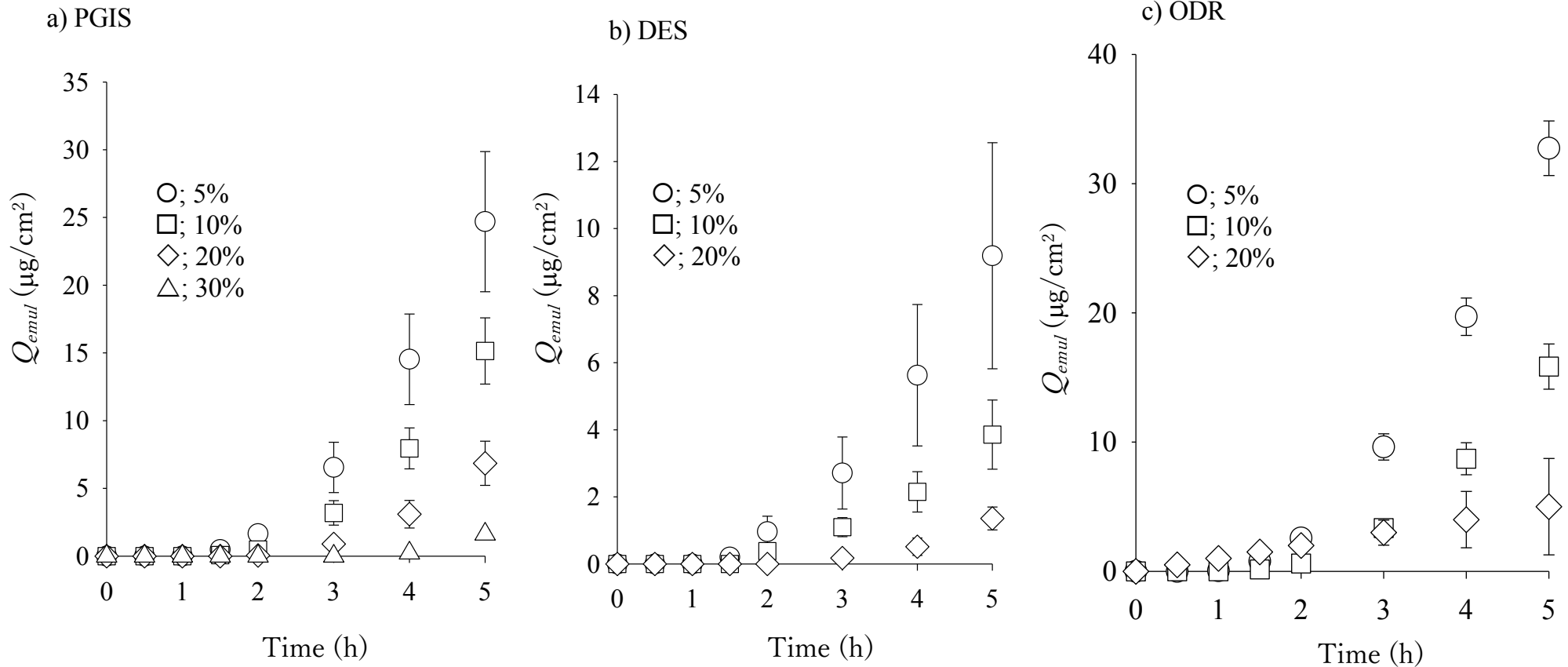
$$R_{CV}^2 = 0.66 \pm 0.05$$

(the mean  $\pm$  S.D.)

$R_{CV}^2$ :  $R^2$  for each cross-validation result

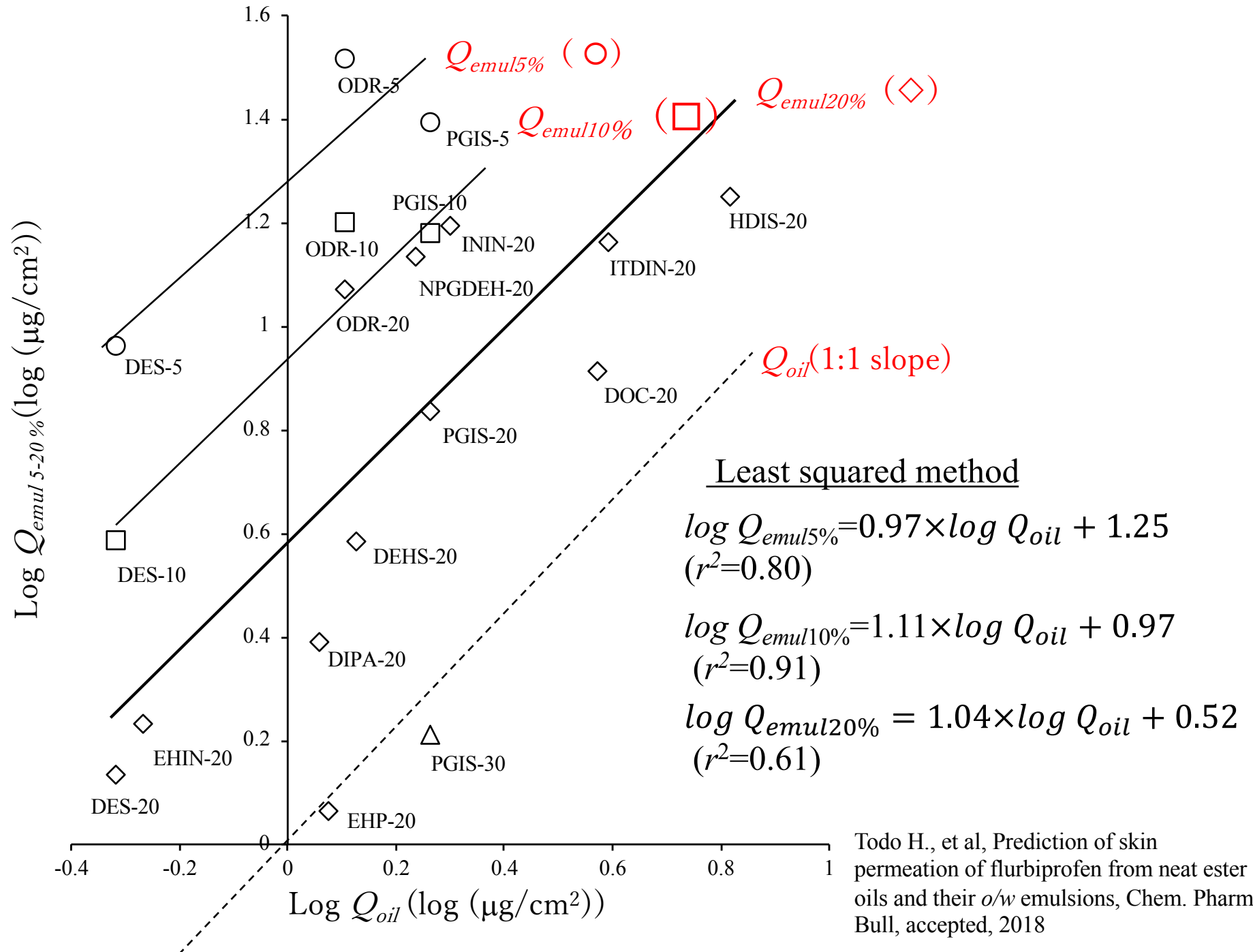


# FP permeation from *o/w* emulsions



PGIS: Propylene glycol isostearate  
DES: Diethyl sebacate  
ODR: 2-Octyldodecyl ricinoleate

# Prediction of skin permeation of FP from o/w emulsions containing 5%, 10% and 20% ester oil



## Conclusion

3次元培養ヒト皮膚モデルが*in vitro*透過試験におけるヒト皮膚および動物皮膚代替膜として利用できる可能性を示した。

皮膚透過パラメータより皮膚中濃度を予測できる*in silico*手法を開発した。

油剤やエマルジョンからの物質の皮膚透過性を予測できる*in silico*手法を開発した。

皮膚適用する医薬品や化粧品製剤の開発や、化学物質の皮膚暴露後の安全性評価にも利用可能となるよう、今後も検討を進めていく。