A Two-Phase Analysis of Solute Partitioning into the Stratum Corneum

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ABSTRACT: An analysis is presented of partition coefficients $K_{SC/w}$ describing solute distribution into fully hydrated stratum corneum (SC) from dilute aqueous solution (w). A comprehensive database is compiled from the experimental literature covering more than eight decades in the octanol/water partition coefficient $K_{o/w}$. It is analyzed according to a two-phase model following that of Anderson, Raykar, and coworkers (1988, 1989), which accounts for uptake by intercellular lipid and corneocyte (keratin plus water) phases having inherently different lipophilicities, as characterized by an SC lipid/water partition coefficient $K_{lip/w}$ and a partition coefficient $PC_{pro/w}$ quantifying cornoeocyte-phase binding. Regression of 72 data points yields useful best-fit recalibrations of power laws (or linear free energy relationships) giving $K_{lip/w}$ and $PC_{pro/w}$ as functions of $K_{o/w}$. The specific conclusions of the analysis are as follows: (i) The two-phase model offers substantial improvements over previously proposed analytical representations of $K_{SC/w}$, yielding an rms error in $\log_{10}K_{SC/w}$ of 0.30 limited by the scatter in the data. (ii) The best-fit description of the lipid phase is given by the power law $K_{lip/w} = 0.43\ (K_{o/w})^{0.81}$, suggesting about half the absolute value of $K_{lip/w}$ relative to previous estimates. (iii) The best-fit description of corneocyte-phase binding differs negligibly from the correlation found by Anderson, Raykar, and coworkers for the more limited set of compounds studied by them. Explicit consideration of the two-phase nature of the SC also furnishes a rational basis for predicting the effects of varying hydration state upon $K_{SC/w}$. © 2006 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 95:649–666, 2006

Keywords: stratum corneum (SC); partition coefficient; microstructure; intercellular lipid; corneocyte; hydration; keratin protein; binding

INTRODUCTION

The outermost layer of the epidermis, the stratum corneum (SC), serves as the primary barrier to and regulator of molecular passage through the skin.[1,2] It has a heterogeneous two-phase structure comprising cornoecytes embedded in a lipid matrix.[1,3,4] Quantitative aspects of diffusion through this layer have attracted considerable experimental and theoretical study in connection with topical and transdermal drug delivery[2,5–7] as well as risk assessment of chemical exposure.[8–10] At steady state, such passage is characterized by a permeability coefficient $P_{SC/w}$, measurable *in vitro*, representing the constant of proportionality between permeant flux J (mol/cm^2·s) through an SC sample and the driving difference in permeant concentrations between two aqueous solutions separated by the sample. (The subscript "w" represents shorthand designating the aqueous solution environment at a prescribed pH.) Many authors have developed useful empirical

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correlations[11,12] and microscopic "brick-and-mortar" models[13–21] giving the dependence of $P_{SC/w}$ (or a related maximum-flux parameter[22]) upon key physicochemical properties of the solute, such as its octanol/water partition coefficient $K_{o/w}$ and molecular weight MW. Although the aggregate parameter $P_{SC/w}$ suffices to quantify steady flux, calculation of lag times and other aspects of transient percutaneous absorption requires its decomposition into the product of solubility and mobility parameters as[1,7,11,22–25]

$$P_{SC/w} = K_{SC/w} D_{SC} / h_{SC}, \quad (1)$$

in which $K_{SC/w}$ is the partition coefficient of the permeating solute in the SC (relative to an aqueous solution "w" at a prescribed pH), D_{SC} is its effective diffusivity, and h_{SC} is the total thickness of the SC. The multiphase complexity of SC microstructure does not imply that steady state transport of solute at low concentrations through this tissue layer cannot be described by a Fick's-law effective diffusivity; indeed, it certainly can.[20,26] The only caveat is that the value of D_{SC} (as well as $K_{SC/w}$) must reflect possible occupancy and flux associated with all elements of the microstructure. Given a measured or theoretically predicted value of $P_{SC/w}$ and a measured thickness h_{SC}, the effective diffusivity can be obtained by solving Eq. 1 for D_{SC}. This determination requires a reliable value of the partition coefficient $K_{SC/w}$.

The purpose of this study is to consolidate existing literature to date on hydrated SC/water partition coefficients[1,27–41] to produce a comprehensive database on measured values of $K_{SC/w}$. In the process, different types of commonly used units are unified. As discussed below, $K_{SC/w}$ represents a volume average of lipid- and corneocyte-phase partition coefficients, $K_{lip/w}$ and $K_{cor/w}$, respectively. Our analysis treats the corneocyte interior phase as an aqueous medium with excluded volume deriving from keratin microfibrils, as well as solute-keratin binding.[31–33,40] Based on this view, we produce useful best-fit correlations describing both $K_{lip/w}$ and solute-keratin binding as functions of $K_{o/w}$, based partly on Anderson, Raykar, and coworkers'[31–33] phase-specific characterization of partitioning into the SC. Our interest in $K_{lip/w}$ is largely connected with practical application of a new microscopic diffusion model of SC permeability,[21] for which requisite inputs are phase-specific partition coefficients (as well as other physicochemical parameters). We also develop a framework for estimating SC partition coefficients for partitioning from vehicles that do not hydrate or otherwise perturb the skin.

PHYSICOCHEMICAL BASIS FOR $K_{SC/w}$, $K_{lip/w}$, AND $K_{cor/w}$

Figure 1 gives a schematic representation of SC microstructure at the level of detail needed to analyze all the partitioning data considered. It comprises total masses m_{lip} of SC lipids, m_{pro} of keratin protein, and m_{water} of hydrating water. The dry SC mass is $m_{SC,dry} = m_{lip} + m_{pro}$. Dry SC typically contains 5–15 mass percent lipids.[31,42–49] Unless the composition is explicitly measured and reported,[31] we take $w_{lip} \equiv m_{lip}/m_{SC,dry} = 0.1$ (equivalently, $m_{lip}/m_{pro} = 1/9$) as a reasonable representative value.[34] (The symbol "w," used here to denote mass ratios based on dry SC mass, corresponds to Anderson, Raykar, and coworkers'[31,32] "W_f"). Considerable data exist on water content of stratum corneum.[40,50,51] Partitioning experiments correspond to fully hydrated tissue, because the sample becomes fully saturated with water in the process of solute uptake. The SC in this state contains about $v \equiv m_{water}/m_{SC,dry} = 2.75$ g water per g of dry tissue.[40,52] (The symbol "v" is used here to denote the ratio $m_{water}/m_{SC,dry}$ for consistency with previous accepted notation.[40,52]) In the Discussion, we also consider a "partially hydrated" state that refers to a typical *in vivo* water content of 30 wt%.[50–52] This latter value represents the ratio $m_{water}/(m_{SC,dry} + m_{water})$, and it is equivalent to $v \equiv m_{water}/m_{SC,dry} = 0.43$ g water per g of dry tissue. The preceding figures define our average model of the SC, used to interpret most of the partitioning data. A slightly different breakdown among lipid, protein and water constituents reported by Raykar et al.[31] is used specifically to analyze data from this group of investigators.[31–33] (see below). Table 1 summarizes all the compositional parameters, which are worked out in Appendix A.

As proposed by Hansen and Yellin,[53] about 0.30 g water per g dry SC exists in a less mobile bound state, and the remainder of the water in a state that is freer but nevertheless has restricted mobility relative to bulk water.[40,52] Notwithstanding the more or less diminished mobility, Raykar et al.[31] concluded from an observed close correspondence between water and ^{14}C-labeled sucrose uptake that bound water within the SC has solvent properties essentially identical to those of bulk water. It is therefore valid to take solute

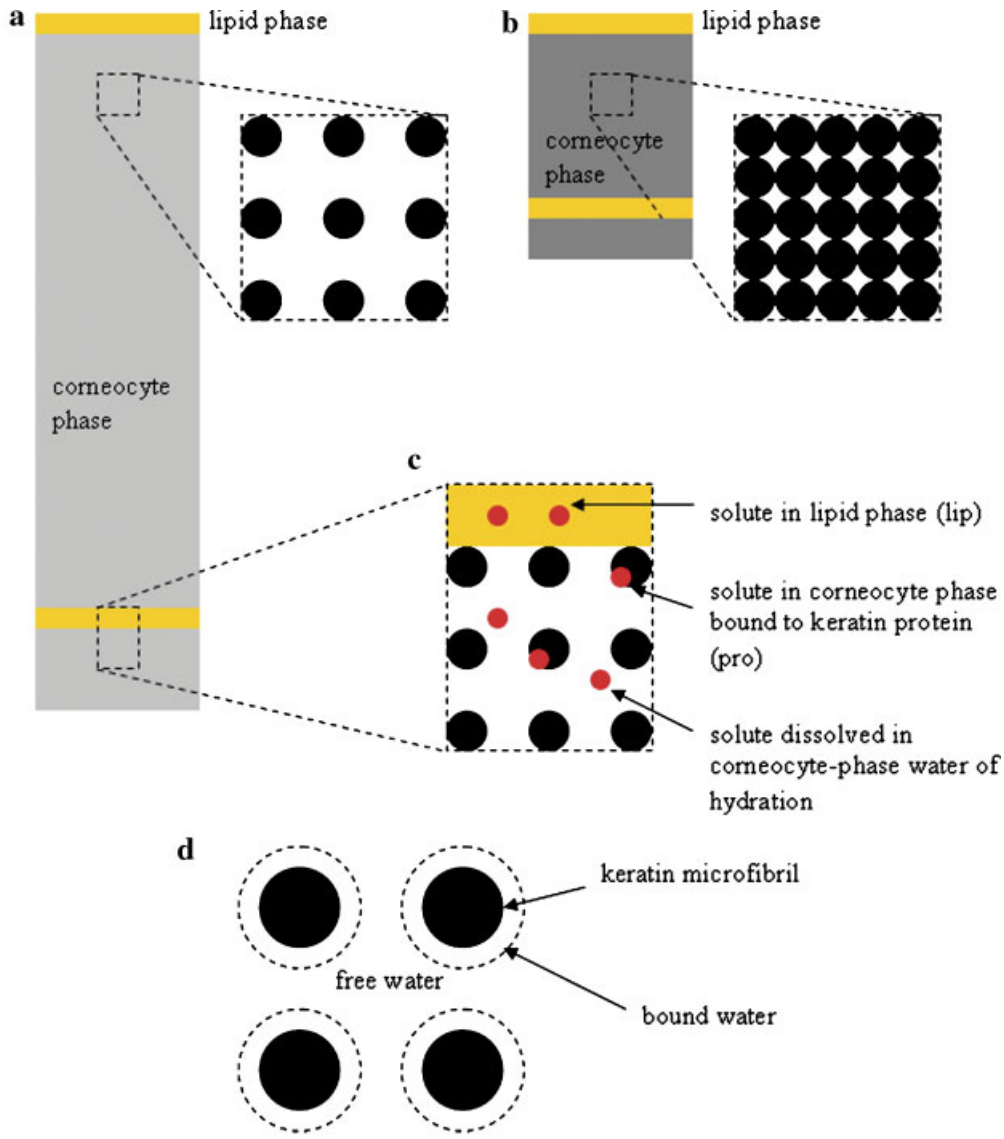

Figure 1. Schematic representation of SC microstructure. (a) Fully hydrated state. (b) Partially hydrated state. (c) Solute distribution among the lipid phase "lip," the keratin-bound state "pro," and the aqueous part of the corneocyte phase "water." (d) Representation of free and bound states within the corneocyte phase for the special case of water as solute. [Color figure can be seen in the online version of this article, available on the website, www.interscience.wiley.com.]

concentrations to be identical in the aqueous part (mass m_{water}) of the SC and in the adjacent bulk aqueous solution at equilibrium.

The analysis below is phrased in terms of a two-phase breakdown of the SC into lipid and corneocye (keratin plus water) phases, respectively distinguished by subscripts "lip" and "cor." We idealize the corneocyte phase as a composite medium comprising keratin microfibrils of radius $a_f = 35$ Å[54] immersed within the surrounding water at a volume fraction φ_f determined by the degree of hydration (see Fig. 1 and Tab. 1). As discussed later, experimental assessment of the corneocyte phase using delipidized SC[31–33] implicitly includes the cornified cell envelopes and associated covalently bound lipid in this phase.

Phase-Specific Breakdown of the SC/Water Partition Coefficient

Essentially all authors discussing SC permeability[1,7,11,22–25] decompose $P_{SC/w}$ into partitioning and diffusivity factors (Eq. 1) using $K_{SC/w}$ tacitly defined as

Table 1. Compositional Parameters Characterizing SC Microstructure

Symbol	Definition	Value for Average Model of Fully Hydrated SC	Value Based on Measurements by Raykar et al.[31] for Fully Hydrated SC	Value for Average Model of Partially Hydrated SC
w_{lip}	$m_{lip}/m_{SC,dry}$	0.10	0.16	0.10
w_{pro}	$m_{pro}/m_{SC,dry}$	0.90	0.84	0.90
v	$m_{water}/m_{SC,dry}$	2.75	2.91	0.43
ϕ_{lip}	Lipid volume fraction of SC	0.0316	0.0480	0.0927
ϕ_{pro}	Protein volume fraction of SC	0.1867	0.1657	0.5483
ϕ_{water}	Water volume fraction of SC	0.7817	0.7863	0.3589
ϕ_{cor}	Corneocyte-phase volume fraction of SC ($=\phi_{pro}+\phi_{water}$)	0.9684	0.9520	0.9073
φ_f	Keratin microfibril volume fraction of corneocyte phase ($=\phi_{pro}/\phi_{cor}$)	0.1928	0.1740	0.6044
	Volume of hydrated SC per unit mass of original dry SC	3.518 cm^3/g	3.701 cm^3/g	1.198 cm^3/g
	Density of hydrated SC	1.066 g/cm^3	1.056 g/cm^3	1.194 g/cm^3

$K_{SC/w}$ = (moles of solute absorbed in the hydrated SC per unit volume of the hydrated SC)/(moles of solute per unit volume of the adjacent solution), (2)

because this is the most natural definition for a description of diffusion. $K_{SC/w}$ is given by the volume-average formula[13,21]

$$K_{SC/w} = \phi_{lip} K_{lip/w} + \phi_{cor} K_{cor/w}, \quad (3)$$

where ϕ_{lip} and ϕ_{cor} denote the volume fractions of the lipid and corneocyte phases, and

$K_{lip/w}$ = (moles of solute absorbed by the lipid phase per unit volume of the lipid phase)/(moles of solute per unit volume of the adjacent solution), (4)

$K_{cor/w}$ = (moles of solute absorbed by the corneocyte phase per unit volume of the corneocyte phase)/(moles of solute per unit volume of the adjacent solution). (5)

These formulas are based on a view of the corneocyte phase as a composite continuum comprising the keratin microfibrils and the water of hydration. Aside from being dissolved in the water of hydration (which has solvent properties essentially identical to those of bulk water), solute generally also adsorbs to the keratin (as well as other constituents of the corneocyte phase, for example, cornified cell envelope proteins and the lipids covalently bonded to them[31,35]). This binding process, discussed below, is quantified by an appropriate isotherm (cf. Eqs. 7 and 11). Equation B8 in Appendix B shows explicitly the manner in which $K_{cor/w}$ reflects both contributions to solute holdup within the corneocyte phase.

SC/w partition coefficients are usually measured and reported in terms of several mass based (as opposed to volume based) units.[27,28,30,31,33,35,36,38,39] Relations among the various types of units, which are important to distinguish, are developed in Appendix B (see Eqs. B9 and B10).

Lipid/Water Partition Coefficient

In the percutaneous transport literature, the lipid-phase partition coefficient is usually correlated with $K_{o/w}$ according to the power-law (linear free energy relationship)[17,19,22,32]

$$K_{lip/w} = c\,(K_{o/w})^{\beta} \quad \text{or} \quad \log_{10} K_{lip/w} = \alpha + \beta \log_{10} K_{o/w} \quad (6)$$

(where $\alpha = \log_{10} c$). Some authors take $c = 1$ ($\alpha = 0$) and suggest values of β ranging from 0.70 to 0.76.[17,19] However, considering both α and β as fitted parameters, other authors suggest values of β closer to unity (e.g., 0.91[31,32]), implying that octanol may be more similar to SC lipids. Estimation of the exponent β is sometimes based on analysis of permeability data[11,22] (as opposed to partitioning data). However, permeability is generally the outcome of several intertwined partitioning and transport processes playing themselves out on the stage of heterogeneous SC microstructure, as is becoming increasingly understood with brick-and-mortar models.[13–21] $K_{o/w}$ could enter such models as a correlating variable for several microscopic parameters, which themselves enter the predicted permeability nonlinearly if there is more than one phase or pathway. Thus, it is difficult to guess *a priori* the precise manner in which the scaling of $K_{lip/w}$ with $K_{o/w}$ might be reflected in $P_{SC/w}$. For this reason, we here adopt the view that $K_{lip/w}$ should be assessed directly on the basis of partitioning data alone, uncolored by any considerations of permeability.

Corneocyte-Phase Binding

Any adsorption or binding of the solute to the surfaces of the keratin microfibrils (described by some appropriate isotherm[40,52,56]), or any other elements associated with the corneocyte phase, could increase holdup of solute in this phase. The studies of Anderson, Raykar, and coworkers[31–33] have effectively quantified this phenomenon in terms of a partition coefficient $PC_{pro/w}$ defined as the ratio of the mass of solute adsorbed on protein per unit mass of protein to the mass of solute per unit mass of water in the adjacent solution (see Eq. B4 in Appendix B). Their subscript "pro" is understood here in the generalized sense of representing keratin protein as well as other constituents of the corneocyte phase, for example, cornified cell envelope proteins and the lipids covalently bonded to them.[31,42,55] The coefficient $PC_{pro/w}$ was determined as the SC/water partition coefficient $PC_{intrinsic}$ measured using delipidized SC (cf. Eq. B5). This equivalence assumes that chloroform-methanol extraction does not alter the solute binding properties of the keratin. Close agreement between SC/water partition coefficients measured with untreated and delipidized skin for (the less lipophilic) solutes that partition primarily into the protein domain supports this assumption.[31] It is worth noting that use of a partition coefficient to describe keratin binding tacitly implies a linear isotherm and is valid in the limit of small solute concentrations.

Anderson and Raykar[33] found substantial consistency among data for $PC_{pro/w}$ measured for hydrocortisone esters[31,32] and methyl-substituted *p*-cresols,[33] described by the correlation

$$\log_{10} PC_{pro/w} = [0.75] + \mathbf{0.27} \log_{10} K_{o/w} \quad \text{or}$$
$$PC_{pro/w} = [5.6]\,(K_{o/w})^{\mathbf{0.27}} \qquad (7)$$

(see Eq. 1 and Fig. 1 of Ref. 33). (For later discussion we write the numerical value of the slope parameter (or exponent)–to be retained–in boldface type, to distinguish it from the constant term (or prefactor)–to be adjusted below–in square brackets.) We regard this result as the best available general descriptor to date of corneocyte-phase binding, used here in computing $K_{cor/w}$ via Eq. B8.

METHODS

Analysis of SC partition coefficients was based on the compositional parameters listed in Table 1 for our average model of fully hydrated SC. The only exceptions to this procedure were made for the data sets from Anderson, Raykar, and coworkers,[31,33] which were analyzed using the average SC composition reported by them.[31]

Compilation and Conversion of SC Partitioning Data

The SC/water partitioning data considered in this study are presented in Table 2. Some ambiguities exist in the data reported and compiled by Scheuplein and coworkers (1965)[27], (1969)[28], and (1971)[1] regarding variations among values reported for water and the shorter-chain alkanols,[1,27] as well as the precise meaning of the reported partition coefficient "K_m,"[1,27,28] although it is evidently equivalent to $PC_{intrinsic}$ and not $K_{SC/w}$.[57] Anderson, Raykar, and coworkers (1988),[31] (1989)[33] and Wagner et al. (2002)[38] also reported $PC_{intrinsic}$, which gives PC upon addition of v (see Eq. B5 in Appendix B). Surber et al. (1990a),[35] (1990b)[36] effectively reported PC, although they noted in passing that it differed negligibly from $PC_{intrinsic}$. Anderson et al. (1976)[30] and Cross et al. (2003)[39] reported PC'. The

Table 2. Reported Data on Fully Hydrated SC/Water Partition Coefficients, Together with Molecular Parameters and Derived Estimates of $K_{lip/w}$

Compound	MW	$\log_{10} K_{o/w}$ See Note[a]	Source; Type of Reported SC/w Partition coeff.; Temperature	Value of Reported SC/w Partition Coeff.	$K_{SC/w}$	$\log_{10} K_{lip/w}$ (Estimated from $K_{SC/w}$)
Water[b]	18.02	−1.38	Ref. 27; $PC_{intrinsic}$;	0.3	0.87	0.43
Methanol	32.04	−0.77	25°C	0.6	0.95	
Ethanol	46.07	−0.31		0.6	0.95	
Propanol	60.10	0.25		2.0	1.35	
Butanol	74.12	0.88		2.5	1.49	
Pentanol	88.15	1.56		5.0	2.20	
Hexanol	102.18	2.03		10	3.62	
Heptanol	116.20	2.72		30	9.31	0.95
Octanol	130.23	3.00		50	15.0	1.02
Progesterone	314.47	3.87	Ref. 28; $PC_{intrinsic}$	104	30.3	2.63
Pregnenolone	316.49	4.22		50	15.0	
Hydroxypregnenolone	332.49	3.40[c]		43	13.0	0.99
Hydroxyprogesterone	330.47	3.17		40	12.2	1.52
Cortexone (desoxycorticosterone)	330.47	2.88		37	11.3	1.78
Testosterone	288.43	3.32		23	7.32	
Cortexolone	346.47	2.52		23	7.32	
Corticosterone	346.47	1.94		17	5.61	−0.07
Cortisone	360.45	1.47		8.5	3.20	
Hydrocortisone	362.47	1.61		7	2.77	
Aldosterone	360.45	1.02[c]		6.8	2.71	
Estrone	270.37	3.13		46	13.9	1.98
Estradiol	272.39	4.01		46	13.9	
Estriol	288.39	2.45		23	7.32	
Resorcinol	110.11	0.80	Ref. 30; PC'; 25°C	1.8	1.92	
Phenol	94.11	1.47		5.4	5.76	1.64
p-cresol	108.14	1.94		10.6	11.3	2.26
o-cresol	108.14	1.95		10.6	11.3	2.25
m-cresol	108.14	1.96		10.6	11.3	2.25
m-nitrophenol	139.11	2.00		12.1	12.9	2.35
p-nitrophenol	139.11	1.91		12.8	13.6	2.41
o-chlorophenol	128.56	2.15		13.8	14.7	2.43
3,4-xylenol	122.17	2.23		16.9	18.0	2.56
p-ethylphenol	122.17	2.47		18.3	19.5	2.58
p-chlorophenol	128.56	2.39		20.4	21.7	2.66
p-bromophenol	173.01	2.59		27.2	29.0	2.82
2-napthol	144.17	2.70		33.4	35.6	2.93
2,4-dichlorophenol	163.00	3.06		45.4	48.4	3.08
Chlorocresol	142.59	3.10		50.4	53.7	3.13
Chloroxylenol	156.61	3.27		60.8	64.8	3.22
Thymol	150.22	3.30		72.7	77.5	3.32
2,4,6-trichlorophenol	197.45	3.69		89.0	94.9	3.40
1a	461.56	1.43	Ref. 31; $PC_{intrinsic}$;	9	3.22	
1b	489.61	2.03	37°C	12	4.03	
1c	476.57	2.58		22	6.73	
1d hy-hemisuccinate (pH 5.5)[d]	462.54	2.11[e]		11	3.76	

Table 2. (*Continued*)

Compound	MW	$\log_{10} K_{o/w}$ See Note[a]	Source; Type of Reported SC/w Partition Coeff.; Temperature	Value of Reported SC/w Partition Coeff.	$K_{SC/w}$	$\log_{10} K_{lip/w}$ (Estimated from $K_{SC/w}$)
1e (pH4)	504.62	3.26[e]		68	19.2	2.26
1f	503.64	2.30		25	7.54	1.47
1g hy-6-OH-hexanoate	476.61	2.79		20	6.19	
1h hy-propionate	418.53	3.00		30	8.89	
1i	518.65	3.70		133[f]	36.7	2.68
1j hy-hexanoate	460.61	4.48		208[f]	57.0	2.87
1k hy-octanoate[g]	488.67	5.49		3640[f]	984	4.29
1a 4-hydroxyphenyl-acetamide	151.17	−0.09	Ref 33; $PC_{intrinsic}$; 37°C	5	2.14	0.47
1b 4-hydroxybenzyl alcohol	124.14	0.32		9	3.22	1.26
1c 4-hydroxyphenylacetic acid (pH4)	152.15	0.93		14	4.57	1.50
1d methyl 4-hydroxyphenylacetate	166.18	1.63		13	4.30	
1e *p*-cresol	108.14	1.95		22	6.73	1.54
Water[b]	18.02	−1.38	Ref. 34; See Note[h];		0.82	0.08
Ethanol	46.07	−0.31	32°C		0.82	
Acitretin[g]	326.44	6.07[c]	Ref. 35; $\log_{10} PC$;	2.4	71.4	2.40
Progesterone	314.47	3.87	25°C	2.3	56.7	3.10
Testosterone	288.43	3.32		1.6	11.3	
Diazepam	284.75	2.99		1.8	17.9	2.40
Estradiol	272.39	4.01		2.1	35.8	2.75
Hydrocortisone	362.47	1.61		0.98	2.71	
Caffeine	194.19	−0.07		0.96	2.59	1.14
4-acetamidophenol	151.17	0.51	Ref. 36; $\log_{10} PC$;	0.7	1.42	
4-cyanophenol	119.12	1.60	25°C	0.9	2.26	
4-iodophenol	220.01	2.91		1.8	17.9	2.42
4-pentyloxyphenol	180.25	3.50		1.9	22.6	2.46
PCB (polychlorinated biphenyls)[g]		6.40[i]		2.3	56.7	
DDT (1,1,1-trichloro-2,2-bis(*p*-chloro-phenyl)ethane)[g]	354.49	6.91		2.5	89.9	
Flufenamic acid[g]	281.24	5.25	Ref. 38; $PC_{intrinsic}$; 32°C	139[j]	40.3	1.78
Ethanol	46.07	−0.31	Ref. 39; PC'	0.5	0.53	
Butanol	74.12	0.88		0.8	0.85	
Hexanol	102.18	2.03		2.3	2.45	
Octanol	130.23	3.00		16.0	17.1	2.34
Decanol[k]	158.28	4.57		2392.7	2550	4.90
Water[b]	18.02	−1.38	Ref. 40; See Note[l]	0.78	0.78	
Nicotinamide	122.13	−0.37	Ref. 41; $K_{SC/w}$;	1.16	1.16	

(*Continued*)

Table 2. (*Continued*)

Compound	MW	$\log_{10} K_{o/w}$ See Note[a]	Source; Type of Reported SC/w Partition Coeff.; Temperature	Value of Reported SC/w Partition Coeff.	$K_{SC/w}$	$\log_{10} K_{lip/w}$ (Estimated from $K_{SC/w}$)
Testosterone	288.43	3.32	30°C	25	25.0	2.61
Testosterone	288.43	3.32		6.8	6.80	

[a]Unless noted otherwise, values of $\log_{10}K_{o/w}$ listed here come from the MEDCHEM database and (in the absence of a measured value therein) the CLOGP Program Vers. 2.0.0.[58] However, for the hydrocortisone esters[31,32] and methyl-substituted *p*-cresols[33] studied by Anderson, Raykar, and coworkers, their measured values of $K_{o/w}$ were used.

[b]As discussed in the main text, water represents an exceptional case for which $K_{SC/w}$ is essentially completely insensitive to the coefficients appearing in Eqs. 7 and 9. It is excluded from the final regression of $\log_{10}K_{SC/w}$ values.

[c]Value of $\log_{10}K_{o/w}$ obtained from CLOGP Program Vers. 2.0.0 in the absence of measured values in MEDCHEM database.[58]

[d]Ionization states of solute in SC lipid and adjacent buffer likely differed; this compound excluded from final regression of $\log_{10}K_{SC/w}$ values.

[e]Value of $\log_{10}K_{o/w}$ refers aqueous phase at low pH, and characterizes unionized form of acid.

[f]Arithmetic mean of all values reported corresponding to varying amounts of SC lipid content.

[g]As discussed in the main text, $K_{SC/w}$ for highly lipophilic compounds is very sensitive to the lipid content of SC samples, and therefore subject to high variability and uncertainty; this compound excluded from final regression of $\log_{10}K_{SC/w}$ values based on the lipophilicity criterion $\log_{10} K_{o/w} > 5$.

[h]Partition coefficients for water and ethanol derived from Ref. 34 represent values calculated from their Figure 2 and inferred from their Figure 4, respectively.

[i]Listed value of $\log_{10}K_{o/w}$ is that given in Ref. 36.

[j]Arithmetic mean of three values reported for three different skin flaps with varying SC lipid compositions.

[k]Clear outlier from trend established by all the other data; this datum excluded from final regression of $\log_{10}K_{SC/w}$ values.

[l]Authors give estimate of ϕ_{water} in hydrated SC, which is equivalent to $K_{SC/w}$ for water.

reported data were converted to $K_{SC/w}$ using Eq. B9 (for *PC*) and Eq. B10 (for *PC′*). The first "a" version of each of these equations was used in all cases excepting the data sets of Anderson, Raykar, and coworkers,[31,33] for which the second "b" version of Eq. B9 (based on their average measured SC composition[31]) was used. The values of $K_{SC/w}$ for ethanol listed for Berner et al. (1989)[34] represent values respectively calculated from their Figure 2 (at the limit of zero ethanol volume fraction), and inferred from their Figure 4 (which shows a lack of solvent selectivity at high water volume fractions).

An existing compilation of SC partitioning data[37] did not distinguish the meanings of *PC*, $PC_{intrinsic}$, *PC′*, and $K_{SC/w}$ drawn from various sources. The matter has been clarified in the present analysis.

Anderson, Raykar, and coworkers[31,32] presented measurements of partition coefficients for lipids obtained by chloroform/methanol extraction from dry SC, and for delipidized SC. The former data represent direct measurements of $PC_{lip/w}$. The latter data are indicative of $PC_{cor/w}$ (correlated by Eq. 7), and also yield an alternate determination of $PC_{lip/w}$ by difference. These direct determinations of $PC_{lip/w}$ are presented (and converted to $K_{lip/w}$) in Table 3 and discussed below.

In drawing together data from all these sources, it is worth acknowledging the variability introduced by the variety of experimental conditions employed. If stated explicitly by the investigators, the temperatures at which partitioning experiments were conducted are listed in Table 2. Partition coefficients are generally temperature dependent, although Anderson et al.[30] found that temperature variations exceeding 20°C had little influence on their measured values of *PC′*. In the absence of comprehensive information regarding activation energies, we are forced to use the data reported without attempting any temperature corrections, as has been the inescapable policy for previous compilations.[29,37] Some of the investigators cited studied the effects of varying solute concentration in the donor solution.[30,35,36,38] The present analysis is tacitly restricted to the limit of dilute solutions, for which uptake is described by a (constant) partition coefficient representing the slope of the linear (low-concentration) regime of the equilibrium distribution isotherm.

Octanol/Water Partition Coefficients

Values of $K_{o/w}$ were obtained from the MEDCHEM database and (in the absence of a measured value therein) the CLOGP Program Vers. 2.0.0.[58] However, for the hydrocortisone esters[31,32] and methyl-substituted p-cresols[33] studied by Anderson, Raykar, and coworkers, their measured values of $K_{o/w}$ were used.

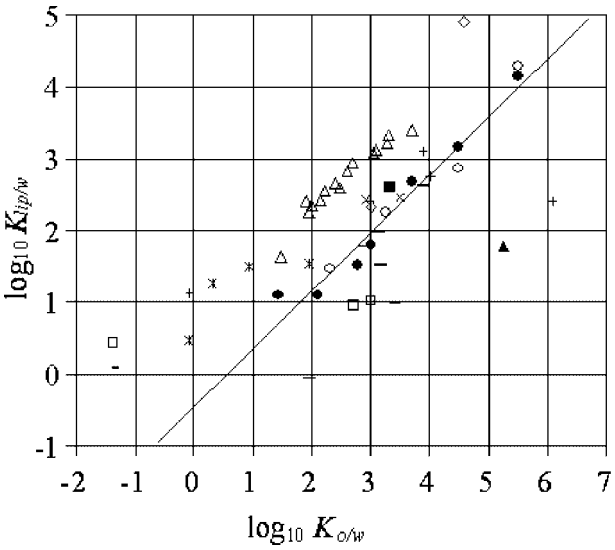

Figure 2. Dependence of $K_{lip/w}$ upon $K_{o/w}$. Filled circles (●) represent the direct determinations of $K_{lip/w}$ reported by Anderson et al.[32] The line represents Eq. 9 fitted to these data. All other symbols represent values of $K_{lip/w}$ estimated from data sets giving $K_{SC/w}$ using Eq. 8. Data sets are distinguished as follows: Scheuplein (1965)[27] (open squares (□)); Scheuplein (1969)[28] (long dashes (——)); R.A. Anderson et al. (1976)[30] (open triangles (△)); Raykar et al. (1988)[31] (open circles (○)); B.D. Anderson et al. (1989)[33] (asterisks (*)); Berner et al. (1989)[34] (short dash (-)); Surber et al. (1990a)[35] (crosses (+)); Surber et al. (1990b)[36] (ex's (×)); Wagner et al. (2002)[38] (filled triangle (▲)) Cross et al. (2003)[39] (open diamonds (◇)); Kasting et al. (2004)[41] (filled square (■)).

Preliminary Estimation of $K_{lip/w}$ from $K_{SC/w}$

Numerical values of $K_{cor/w}$ for each compound considered were estimated by substituting Anderson et al.'s[33] correlation for $PC_{pro/w}$ (Eq. 7) into Eq. B8. Derived data on $K_{lip/w}$ then followed by subtracting the implied corneocyte-phase holdup from $K_{SC/w}$, that is, by solving Eq. 3 for $K_{lip/w}$:

$$K_{lip/w} = (K_{SC/w} - \phi_{cor}\, K_{cor/w})/\phi_{lip}. \qquad (8)$$

Scatter in the correlation of $PC_{pro/w}$ (or $K_{cor/w}$) is magnified by division by the small number ϕ_{lip}. This procedure is particularly sensitive to the estimate of $K_{cor/w}$ for the less lipophilic solutes, for which corneocyte-phase holdup is significant or even dominant, given the large volume fraction of this phase and the significant degree of keratin binding. Thus, $K_{lip/w}$ represents the small difference between the nearly equal numbers $K_{SC/w}$ and $\phi_{cor}K_{cor/w}$.

For a number of solutes the estimated corneocyte-phase holdup $\phi_{cor}K_{cor/w}$ actually exceeds the reported value of $K_{SC/w}$. In such cases the (negative, untenable) value implied for $K_{lip/w}$ could not be calculated and reported in Table 2. This phenomenon, as well as the generally magnified scatter in derived values of $K_{lip/w}$ (see Fig. 2 below), is a natural consequence of the sensitizing process of having to estimate and subtract off corneocyte-phase solute holdup (Eq. 8). We gladly accept it in exchange for the opportunity of deducing $K_{lip/w}$ from the majority of data sets on partitioning into the SC,[27,28,30,34–36,38,39,41] for which the (lipid and corneocyte) phase-specific breakdown of $K_{SC/w}$ (cf. Anderson, Raykar, and coworkers[31–33]) has not been directly measured.

Regression of Data to Determine Final Recommended Coefficients in the Linear Free Energy Relationships for $K_{lip/w}$ and $PC_{pro/w}$

The proposed model giving $K_{SC/w}$ as a function of $K_{o/w}$ comprises Eqs. 3, 6, 7, and B8, together with

Table 3. Direct Determinations of SC Lipid/Water Partition Coefficients Reported by Anderson et al.[32]

Compound	$\log_{10} K_{o/w}$	$PC_{lip/w}$	$K_{lip/w}$	$\log_{10} K_{lip/w}$
1a	1.43	14	12.6	1.10
1d hy-hemisuccinate[a]	2.11	14	12.6	1.10
1g hy-6-OH-hexanoate	2.79	38	34.2	1.53
1h hy-propionate	3.00	69	62.1	1.79
1i	3.70	530	477	2.68
1j hy-hexanoate	4.48	1600	1440	3.16
1k hy-octanoate	5.49	16000	14400	4.16

$PC_{lip/w}$ is converted to $K_{lip/w}$ via Eq. B7. These data appear as filled circles (●) in Figure 2.
[a]This datum evidently refers to the case of low pH of the aqueous phase, at which the compound exists essentially completely in its unionized form, characterized by the large stated value of $\log_{10}K_{o/w}$.[31]

the values of w_{pro}, v, ϕ_{lip}, and ϕ_{cor} listed in Table 1 and the densities $\rho_{lip} = 0.9$ g/cm^3, $\rho_{pro} = 1.37$ g/cm^3, and $\rho_{water} = 1$ g/cm^3 from Appendix A. Best-fit values of coefficients appearing in Eqs. 6 and 7 were determined by systematic search to minimize the root-mean-square (rms) difference between calculated and measured values of $\log_{10}K_{SC/w}$. The data used were the values of $\log_{10}K_{SC/w}$ listed in Table 2 with equal weighting among all compounds.

RESULTS

Preliminary Assessment of $K_{lip/w}$

Figure 2 shows the derived values of $K_{lip/w}$ obtained from Eq. 8 (presented in Table 2), distinguished by investigator using different symbols for each data set. Also included are Anderson and Raykar's[32] direct determinations of $PC_{lip/w}$ (converted to $K_{lip/w}$ and listed for reference in Table 3).

As the only direct lipid-phase-specific determinations of $K_{lip/w}$, Anderson et al.'s data[32] (see also their previous study, Ref. 31) carry much weight. They are distinguished by filled circles (●) in Figure 2. The fact that the values of $K_{lip/w}$ derived from their overall $PC_{intrinsic}$ data via Eq. 8 (open circles (○)) match the direct determinations (●) is basically a restatement of their conclusion that SC/water partitioning is effectively explained by a protein and lipid phase-specific accounting of solute holdup in the SC. Linear regression of their direct measurements (filled circles) yields

$$\log_{10}K_{lip/w} = [-0.46] + \mathbf{0.81}\log_{10}K_{o/w} \quad \text{or}$$
$$K_{lip/w} = [0.35]\,(K_{o/w})^{0.81} \qquad (9)$$

as the best fit with correlation coefficient $r = 0.979$. The slope (or exponent) $\beta = 0.81$ is somewhat smaller than their suggested slope of 0.91.[31,32] Their value was obtained by regressing data reported in the earlier of their studies[31] for only compounds 1h–1j ($\log_{10}K_{o/w} \geq 3.00$). We consider the additional data points for compounds 1a, 1d, and 1g reported in their follow-up study,[32] in the interest of also representing less lipophilic compounds.

The slope $\beta = 0.81$ actually looks to be a reasonable descriptor of all the data sets. It is worth noting that derived values of $K_{lip/w}$ are probably unreliable for compounds for which $\log_{10}K_{o/w} \lesssim 2$. For these compounds the lipid phase accounts for under 16% of the total holdup in the SC, owing to the small volume fraction of the lipid phase and the significant degree of corneocyte-phase binding. Thus, only the more lipophilic compounds constitute a sound basis for deducing $K_{lip/w}$ from the difference between $K_{SC/w}$ and $\phi_{cor}K_{cor/w}$. The significant scatter is expected because this difference is subject to more uncertainty than either individual term. We regard the slope $\beta = 0.81$ as a more robust numerical result than the constant term -0.46 (or prefactor 0.35), which might have to be adjusted in order yield an optimal fit to all the data (as opposed to just the filled circles).

Data not shown indicate that there is no discernible correlation of $K_{lip/w}$ with molecular weight (MW). Thus, the experimental literature on SC/water partitioning presently available does not offer a basis for resolving molecular size/shape effects on partitioning into the lipid phase.

Final Recommended Coefficients in the Linear Free Energy Relationships for $K_{lip/w}$ and $PC_{pro/w}$

Our goal is to develop a useful, reasonably accurate, and physically sound analytical representation of all the partitioning data listed in Table 2. Toward this end, we accept the stated slopes (or exponents) in the linear free energy relationships for $PC_{pro/w}$ and $K_{lip/w}$ (**0.27** and **0.81** in Eqs. 7 and 9, respectively). As discussed above, these exponents are suggested convincingly by the available phase-specific partitioning data.[31–33] Moreover, the latter (lipid) value lies within the range of published estimates,[17,19,31,32] and is supported by Figure 2. The two prefactors (in square brackets) might require some adjustment, however, because absolute values of partition coefficients are generally more uncertain than the variation with $K_{o/w}$.

Absolute values are best quantified using as much data as possible. All values of $K_{SC/w}$ listed in Table 2 were included in the regression with a few (10) exclusions made for four reasons. (i) Water represents an exceptional case (discussed in Appendix C) for which $K_{SC/w}$ is essentially identical to the water volume fraction ϕ_{water} (≈ 0.78 for fully hydrated SC—see Table 1) irrespectively of the numerical coefficients in Eqs. 7 and 9. Thus, data for water do not furnish a basis for determining these coefficients. (ii) As addressed later in the Discussion, SC/water partition coefficients for highly lipophilic compounds are very sensitive to the lipid content and composition of SC samples (which vary significantly),[31,38] and to

experimental complications, and are therefore subject to high variability and uncertainty. We do not regard compounds for which $\log_{10} K_{o/w} > 5$ as a reliable basis for fitting parameters in our model. (iii) The datum for decanol[39] is well above the trend of the remaining compounds, and was excluded. (iv) The datum for Anderson, Raykar, and coworkers'[31,32] (acid) compound 1d was also excluded because the ionization states in the SC lipid and the pH 5.5 buffer used as the aqueous phase could have differed appreciably. With these exclusions (which left 72 data points), the recommended prefactors minimizing the rms error in $\log_{10} K_{SC/w}$ are given by the equations

$$\log_{10} K_{lip/w} = -0.37 + 0.81 \log_{10} K_{o/w} \quad \text{or}$$
$$K_{lip/w} = 0.43\,(K_{o/w})^{0.81}, \tag{10}$$

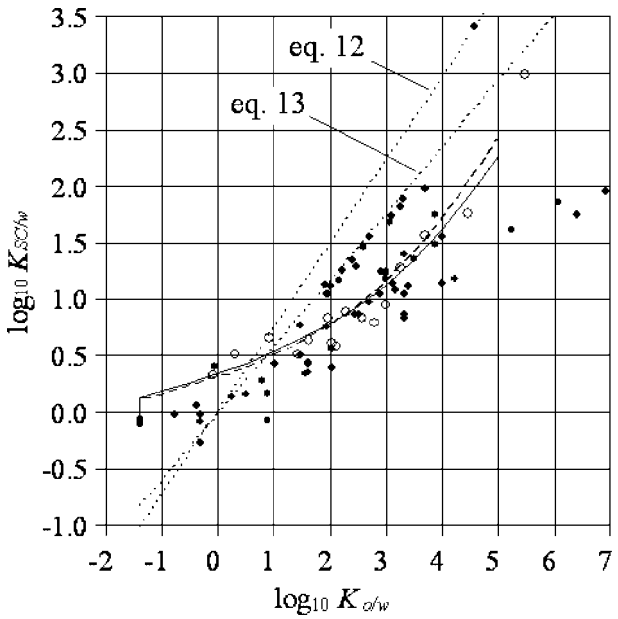

Figure 3. Log–log plot showing the dependence of $K_{SC/w}$ upon $K_{o/w}$. Open circles (○) represent data sets reported by Anderson, Raykar et al.[31,33] described by the model (dashed curve calculated from Eqs. 3, 10, 11, and B8) based on the SC composition reported by them[31] (compositional parameters from the fourth column of Tab. 1). Filled circles (●) represent all other data sets, described by the model (solid curve calculated from Eqs. 3, 10, 11, and B8) based on our average model of fully hydrated SC (compositional parameters from the third column of Table 1). All data points are shown, including those excluded from the final regression of $\log_{10} K_{SC/w}$ values. Dotted lines represent the predictions of Eqs. 12 and 13.

$$\log_{10} PC_{pro/w} = 0.73 + 0.27 \log_{10} K_{o/w} \quad \text{or}$$
$$PC_{pro/w} = 5.4\,(K_{o/w})^{0.27}. \tag{11}$$

The resulting best fit to the SC/w partitioning data is shown in Figure 3, the (minimum, best-fit) rms error being 0.302 among the points included in the fit.

DISCUSSION

It is worthwhile to recapitulate the representation of $K_{SC/w}$ in terms $K_{o/w}$ developed here, involving Eqs. 10 and 11 combined according to the formulas

$$K_{cor/w} = \frac{PC_{pro/w} w_{pro} + v}{(w_{pro}\,\rho_{water}/\rho_{pro}) + v}, \tag{B8}$$
$$K_{SC/w} = \phi_{lip} K_{lip/w} + \phi_{cor} K_{cor/w}. \tag{3}$$

Equation B8 (from Appendix B) expresses the fact that solute holdup in the corneocyte phase comprises both solute dissolved in the water of hydration, and solute adsorbed to keratin protein (and other corneocyte constituents). Equation 3 reflects the distribution of solute over the fractional volumes occupied by corneocyte and lipid phases. Requisite values of w_{pro}, v, ϕ_{lip}, and ϕ_{cor} are listed in Table 1; the appropriate density values are $\rho_{pro} = 1.37$ g/cm^3 and $\rho_{water} = 1$ g/cm^3 (Appendix A). Appendix C gives the modified form of Eq. B8 (see Eq. C1) needed to describe the special case of water as solute.

Efficacy of the Proposed Representation of $K_{SC/w}$

Figure 3 shows that the assumed functional form offers a reasonable description of the dependence of $\log_{10} K_{SC/w}$ upon $\log_{10} K_{o/w}$. As stated well by Raykar et al.,[31] nonlinearity of the curve "reflect[s] a change in mechanism from protein [corneocyte]-dominated uptake for hydrophilic solutes to lipid-dominated uptake for lipophilic solutes" (Ref. 31, p 149). The rms error in $\log_{10} K_{SC/w}$ is clearly not reducible below the minimum (0.302) owing to the scatter of the data and not to any weakness of the functional form.

Included in the figure are the predictions of two previously proposed correlations for $K_{SC/w}$, namely

$$\log_{10} K_{SC/w} = 0.74 \log_{10} K_{o/w} \quad \text{(Cleek and Bunge (1993),[23] Bunge and Cleek (1995)[24])}, \tag{12}$$

$$\log_{10} K_{SC/w} = -0.024 + 0.59 \log_{10} K_{o/w} \quad \text{(Roberts et al. (1996),[37] Pugh et al. (1996)[25])}, \tag{13}$$

which were formulated without any breakdown of the SC into separate lipid and corneocyte phases (having intrinsically different solubility properties). These previous correlations clearly overestimate $K_{SC/w}$ for lipophilic compounds and underestimate $K_{SC/w}$ for hydrophilic compounds. Part of the deficiency of Eq. 13 (the better of the two) may stem form a lack of distinction between the precise meanings of different types of partition coefficients (PC, $PC_{intrinsic}$, PC', $K_{SC/w}$) drawn from various sources in compiling the underlying database.[37] A single straight line (with empirically fitted coefficients significantly different from those in Eqs. 12 and 13) might also reasonably represent the data. However, it would suffer from the deficiency of not incorporating the minimum level of $K_{SC/w}$ guaranteed by solute dissolution (with a partition coefficient of unity) in the water of hydration, which is a significant point for hydrophilic compounds. Moreover, the use of such a purely empirical fit would offer no basis for quantifying the effects of varying degrees of hydration (see below) or supplying microscopic parameters for brick-and-mortar models of SC permeability.[13–21] These aspects constitute the real strengths of the present two-phase mechanistic approach, aside from the quantitative improvement over Eqs. 12 and 13 *per se*.

Comparison with Previous Estimates of $K_{lip/w}$

The final recommendation for $K_{lip/w}$ (Eq. 10) is similar to the simple correlation[17] $K_{lip/w} = (K_{o/w})^{0.76}$, as well as similar correlations in the literature.[17,19] It may be regarded as a recalibration of these linear free energy relationships based on judicious analysis of the latest, most extensive available database on SC/water partitioning presented here (Tab. 2), which suggests a somewhat smaller absolute value given by the prefactor 0.43 (as opposed to unity).

Highly Lipophilic Compounds

SC/water partition coefficients for highly lipophilic compounds are particularly sensitive to the lipid content and composition of SC samples (which vary significantly),[31,38] and are therefore subject to high variability and uncertainty. It is furthermore likely that, similar to shake flask measurements of octanol/water partition coefficients, experimental measurement of $K_{SC/w}$ for highly lipophilic permeants is complicated by imprecise measurements of the aqueous phase concentration. Not surprisingly, the data in Figure 3 show an increasing degree of (vertical) spreading with increasing $\log_{10} K_{o/w}$. The model developed here represents a reasonable compromise fit to all the data for $\log_{10} K_{o/w} \leq 5$. The estimation of $K_{SC/w}$ for very lipophilic compounds should be made with reference to a concomitant characterization of SC lipid content; this constitutes an important area for further study.

Effects of Delipidization

Anderson, Raykar, and coworkers[31–33] provided strong evidence for a scenario in which delipidization simply removes the strongly lipophilic fraction of the SC (and its associated solute uptake capacity), and does not appreciably alter the solute binding properties of the remaining keratin protein (plus any other nonextractable material associated with the corneocytes). Although this additive scenario was evidently true for their experiments, it is worth noting that Surber et al.[35,36] have reported a contradictory phenomenon, namely increased solute uptake upon delipidization, possibly attributable to the exposure of new regions not accessible to solute in the original undamaged membrane. The degrees of damage caused by delipidization processes, and the extent to which they affect the ability to identify corneocyte-phase properties with the properties of delipidized SC, clearly warrant further study.

Comparison of Fully and Partially Hydrated SC

Although no data exist on the subject, our analysis furnishes a reasonable basis for speculation about the relation between partition coefficients

for the cases of fully and partially hydrated skin. This comparison is of importance generally as part of the assessment of permeability of fully vis-à-vis partially hydrated SC. The comparison, shown in Figure 4, is made by substituting the values of w_{pro}, v, ϕ_{lip}, and ϕ_{cor} listed in the third and fifth columns of Table 1 into Eqs. 3 and B8. It is seen that $K_{SC/w}$ for partially hydrated SC (dashed curve) can be expected to be about three times the value for fully hydrated SC (solid curve). The underlying reason is that—with less water of hydration—the corneocyte phase comprises a greater fraction of protein, which is more favorable to the solute than water.

Water as solute represents an exceptional case: $K_{SC/w}$ for fully hydrated SC (filled circle) exceeds the value for partially hydrated SC (open circle). The reason is that the phenomenon of keratin binding simply establishes two states for the water in the corneocyte phase; it does not involve a favorable bound state increasing corneocyte-phase holdup as just discussed (see Appendix C). A smaller degree of hydration then simply decreases $K_{SC/w}$.

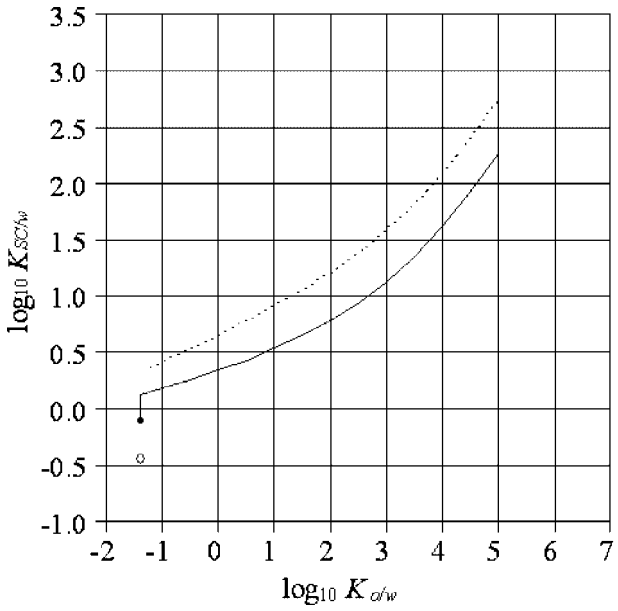

Figure 4. Comparison of $K_{SC/w}$ in fully and partially hydrated states predicted by the present model (Eqs. 3, 10, 11, and B8) using compositional parameters from the third and fifth columns of Table 1, respectively. Solid and dotted curves respectively represent fully and partially hydrated states for solutes other than water. Filled (●) and open (○) circles respectively represent fully and partially hydrated states for water as solute (see Appendix C).

Restriction to Dilute Solutions

It is worth emphasizing that—given the data upon which it is based—the model developed herein applies to dilute solutions, for which uptake by each phase of the SC is described by a constant partition coefficient, independent of solute concentration. Thus its application to uptake from a saturated drug solution is reliable only if the drug solubility is low. A case in point is application of the model in conjunction with water solubility to estimate solute solubility in partially hydrated skin (e.g., Eq. 41 of Ref. 59). This should only be attempted when equation of the partition coefficient to the ratio of SC and water solubilities is justified, that is, for low solubility compounds. Removal of this restriction is an important area for future investigation.

CONCLUSIONS

The preceding analysis has produced a significant recalibration of the linear free energy relationship quantifying $K_{lip/w}$ (Eq. 10) based on judicious analysis of the most comprehensive available database on $K_{SC/w}$ (Tab. 2). (The best-fit formula for $PC_{pro/w}$ (Eq. 11) differs negligibly from Anderson and Raykar's[33] original formula (Eq. 7).) Explicit recognition of the two-phase character of the SC yields a significant improvement over previously proposed analytical representations of $K_{SC/w}$ (Fig. 3), and furnishes a rational basis for treating the effects of varying hydration state (Fig. 4).

More broadly, our analysis underscores the importance of acknowledging the very substantial solute occupancy of the corneocyte phase, even for lipophilic compounds. There should be no reluctance to consider corneocyte-phase holdup, because corneocyte-phase holdup (most of which represents bound solute) is not synonymous with corneocyte-phase flux.

NOMENCLATURE

Definitions are given for the most important symbols used in the main text and appendices.

Roman symbols

$K_{i/w}$ partition coefficient for constituent or phase i (=lip, pro or cor) based on

molar concentrations, defined as (mol/vol. solute concentration in i)/(mol/vol. solute concentration in adjacent solution)

$K_{SC/w}$ SC partition coefficient based on molar concentrations, defined as (moles of solute absorbed in the SC per unit volume of the hydrated SC)/(mol/vol. solute concentration in adjacent solution)

m_i mass of constituent i (=lip or pro) in SC sample

m_{water} mass of water in (fully or partially hydrated) SC sample

$m_{SC,dry}$ $m_{lip}+m_{pro}$, mass of dry SC constituents

$PC_{i/w}$ partition coefficient for constituent i (=lip or pro) based on mass ratio concentrations, defined as (w/w solute concentration in i)/(w/w solute concentration in adjacent solution)

PC SC partition coefficient based on mass ratio concentrations and dry SC mass, defined as (mass of solute absorbed in the hydrated SC per unit mass of the original dry SC)/(w/w solute concentration in the adjacent solution)

$PC_{intrinsic}$ $PC-v$, modified version of PC that excludes solute contained in the water of hydration (see Eq. B5)

PC' SC partition coefficient based on mass ratio concentrations and hydrated SC mass, defined as (mass of solute absorbed in the SC per unit mass of the hydrated SC)/(w/w solute concentration in the adjacent solution)

v $m_{water}/m_{SC,dry}$, water (mass) content of SC per unit dry SC mass

w_i $m_i/m_{SC,dry}$, mass ratio of constituent i (=lip or pro) based on dry SC mass

Greek symbols

ρ density

ϕ volume fraction based on the total volume of the SC

φ volume fraction based on just the volume occupied by the corneocyte phase

Subscripts

cor referring to the corneocyte phase, considered as a composite medium comprising keratin protein and water

f referring to keratin microfibrils

lip referring to SC lipid

o referring to octanol

pro referring to keratin protein

water referring to water

/w (in partition coefficients) relative to an adjacent aqueous solution at a prescribed pH

ACKNOWLEDGMENTS

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APPENDIX A: COMPOSITIONAL PARAMETERS CHARACTERIZING SC MICROSTRUCTURE

The characterization of SC microstructure used here starts from the compositional parameters cited in the main text. For our default "average model" $w_{lip} \equiv m_{lip}/m_{SC,dry} = 0.1$ and $w_{pro} \equiv m_{pro}/m_{SC,dry} = 0.9$ (where $m_{SC,dry} = m_{lip} + m_{pro}$). The density of dry SC is $\rho_{SC,dry} = 1.3$ g/cm^3 (Refs. 60, 61) and a reasonable estimate for the density of SC lipids is $\rho_{lip} = 0.9$ g/cm^3. The relation

$$\rho_{SC,dry} = \frac{m_{lip}+m_{pro}}{(m_{lip}/\rho_{lip})+(m_{pro}/\rho_{pro})} = \frac{1}{(w_{lip}/\rho_{lip})+(w_{pro}/\rho_{pro})} \quad \text{(A1)}$$

expresses the fact that $\rho_{SC,dry}$ represents total mass per total volume of lipid and protein constituents, which individually contribute masses m_{lip} and m_{pro}, and volumes m_{lip}/ρ_{lip} and m_{pro}/ρ_{pro}, respectively. It leads to $\rho_{pro} = 1.37$ g/cm^3 as the value of ρ_{pro} that is consistent with the overall dry density $\rho_{SC,dry}$ and is used throughout our analysis.

For our default "average model" of the fully hydrated state, $v \equiv m_{water}/m_{SC,dry} = 2.75$.[40,52] Volume fractions of the three constituents i (=lipid, protein or water) follow from the relation

$$\phi_i = \frac{(m_i/\rho_i)}{(m_{lip}/\rho_{lip})+(m_{pro}/\rho_{pro})+(m_{water}/\rho_{water})}, \quad \text{(A2)}$$

and are respectively equal to 0.0316 (lipid), 0.1867 (protein), and 0.7817 (water). The hydrated

corneocyte phase can be regarded as a composite medium comprising the protein and water constituents. It occupies a fraction $\phi_{cor} = \phi_{pro} + \phi_{water} = 0.9684$ of the total SC volume. Keratin microfibrils occupy a fraction $\varphi_f = \phi_{pro}/\phi_{cor} = 0.1928$ of the corneocyte-phase volume. Two additional properties are as follows:

$$\begin{aligned}(\text{vol. of hydrated SC})/(\text{dry SC mass}) &= (w_{lip}/\rho_{lip}) + (w_{pro}/\rho_{pro}) + (v/\rho_{water}) \\ &= 3.518\ \text{cm}^3/\text{g},\end{aligned} \tag{A3}$$

$$\begin{aligned}\text{density of hydrated SC} &= \frac{1+v}{(w_{lip}/\rho_{lip}) + (w_{pro}/\rho_{pro}) + (v/\rho_{water})} \\ &= 1.066\ \text{g/cm}^3.\end{aligned} \tag{A4}$$

All these numbers are listed in Table 1 under the heading "Value for Average Model of Fully Hydrated SC."

Identical considerations based on different values of the ratios w_{lip}, w_{pro}, and v lead to corresponding numbers for the two further columns in Table 1.

APPENDIX B: RELATIONS AMONG MASS- AND VOLUME BASED PARTITION COEFFICIENTS REPORTED IN THE LITERATURE

Macroscopically observable partitioning of any given solute into the SC is quantified in several ways. Some investigators[35,36] report a quantity defined as the ratio

PC = (mass of solute absorbed in the hydrated SC per unit mass of the original dry SC)/(mass of solute per unit mass of water in the adjacent solution) (B1)

at equilibrium. This partition coefficient based on dry SC mass is given by the mass-average formula

$$PC = w_{lip}\,PC_{lip/w} + w_{pro}\,PC_{pro/w} + v, \qquad \text{(B2)},$$

in which the mass ratios w_{lip}, w_{pro}, and v have been defined previously, and

$PC_{lip/w}$ = (mass of solute absorbed in the lipid phase per unit mass of the lipid phase)/(mass of solute per unit mass of water in the adjacent solution), (B3)

$PC_{pro/w}$ = (mass of solute adsorbed on protein per unit mass of protein)/(mass of solute per unit mass of water in the adjacent solution). (B4)

In the last term, v has no multiplier because, as noted above, the aqueous part of the corneocyte phase has solvent properties effectively identical to those of the adjacent solution. It is for this reason that hydration of the corneocytes does not diminish the solute concentration in the adjacent solution in a solution depletion measurement starting with dry SC, as noted by Raykar et al.[31] Thus, the partition coefficients $PC_{intrinsic}$ reported by Anderson, Raykar, and coworkers[31,33] and other investigators[27,28,38] "do not include uptake due to water of hydration," and correspond to a modified version of Eq. B2 excluding the last term

$$PC_{intrinsic} = w_{lip}\,PC_{lip/w} + w_{pro}\,PC_{pro/w} = PC - v \tag{B5}$$

(see Ref. 31, p 145). Also reported[30,39] is a related measure of partitioning here denoted as PC', defined as the equilibrium ratio

PC' = (mass of solute absorbed in the hydrated SC per unit mass of the hydrated SC)/(mass of solute per unit mass of water in the adjacent solution). (B6)

The preceding partition coefficients contrast with the volume based coefficients introduced in the main text (Eqs. 2–5). By reconciling units, it is straightforward to develop relations between the mass- and volume based phase-specific partition coefficients, namely

$$K_{lip/w} = PC_{lip/w}\frac{\rho_{lip}}{\rho_{water}}, \tag{B7}$$

$$K_{cor/w} = \frac{PC_{pro/w}w_{pro} + v}{(w_{pro}\rho_{water}/\rho_{pro}) + v}, \tag{B8}$$

in which we take $\rho_{lip} = 0.9$ g/cm^3, $\rho_{pro} = 1.37$ g/cm^3, and $\rho_{water} = 1.0$ g/cm^3 (see Appendix A). In a hypothetical case where there is no solute adsorption to the protein ($PC_{pro/w} = 0$) Eq. B8 yields $K_{cor/w} = 1 - \varphi_f$, reflecting just volume exclusion from the fraction of the corneocyte phase occupied by keratin microfibrils. Numerical values of the various types of overall SC/w partition coefficient are related by the expressions

$$= PC/3.518 = (PC_{intrinsic} + 2.75)/3.518 \quad \text{(average model, fully hydrated)}, \tag{B9-a}$$

$$K_{SC/w} = PC/3.701 = (PC_{intrinsic} + 2.91)/3.701 \quad \text{(Raykar et al.}^{31}\text{ composition, fully hydrated)}, \tag{B9-b}$$

$$= PC/1.198 = (PC_{intrinsic} + 0.43)/1.198 \quad \text{(average model, partially hydrated)}, \tag{B9-c}$$

$$= PC' \cdot 1.066 \quad \text{(average model, fully hydrated)}, \tag{B10-a}$$

$$K_{SC/w} = PC' \cdot 1.056 \quad \text{(Raykar et al.}^{31}\text{ composition, fully hydrated)}, \tag{B10-b}$$

$$= PC' \cdot 1.194 \quad \text{(average model, partially hydrated)}. \tag{B10-c}$$

These formulas follow from the definitions of the partition coefficients (Eqs. 2, B1, B5, and B6) together with the entries in Table 1 giving the volume of hydrated SC per unit mass of the original dry SC, and the density of hydrated SC.

APPENDIX C: THE SPECIAL CASE OF WATER AS SOLUTE

Water represents an exceptional situation in which the solute under consideration is the same as the solvent that hydrates the SC. Keratin binding does not increase the holdup of water in the corneocyte phase. Rather, it simply partitions the total amount of water ($v = 2.75$ g water per g dry SC) into bound and free subsets, the bound subset represented as an annular volume surrounding each keratin microfibril in Figure 1d. (This two-state picture represents a simplified but nevertheless useful idealization of the true situation, which involves a continuous spectrum of states of water mobility.[40,52]) Thus, for water Eq. B8 should be replaced by

$$K_{cor/w} = \frac{v}{(w_{pro}\rho_{water}/\rho_{pro}) + v} \quad \text{(water as solute)}, \tag{C1}$$

which is tantamount to formally setting $PC_{pro/w} = 0$.

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