# Equilibrium Water Sorption in Human Stratum Corneum

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**ABSTRACT:** The water content of the stratum corneum (SC) is a key factor in skin barrier homeostasis; it is intimately related to both skin condition and skin permeability. Studies of water uptake in excised human SC show strong similarities and allow characterization of the equilibrium SC water sorption isotherm in terms of widely used theoretical models. At low water activities, SC water sorption resembles that in other keratinized tissues (i.e., wool and horn), whereas at high water activities, it resembles that in polymeric hydrogels. In this paper, theoretical water sorption models [Brunauer–Emmett–Teller (BET), D'Arcy–Watt, and Frenkel–Halsey–Hill] are fit to the combined human SC water sorption data from our laboratories and others. Each of these models provides a satisfactory description of the equilibrium water content of human SC over the water activity range 0.03–1.0. An accompanying paper discusses the implications of SC water sorption on water mobility in corneocytes and on SC permeability.  J Pharm Sci 92:1624–1631, 2003
**Keywords:** stratum corneum; water; sorption isotherm; hydration; clustering

## INTRODUCTION

The water handling properties of stratum corneum (SC) are key to both the barrier function and condition of the skin. Accounts of water sorption[1–8] and diffusion[5,8–12] in the SC have been published periodically, with various interpretations. Investigators agree on the general shape of the equilibrium water sorption isotherm for the SC; it can be described by a Type II or Type III isotherm, with a small amount of water persisting to very low relative humidity and a large increase at high relative humidity that is associated with pronounced swelling of the tissue.[1–8] Several of these datasets, plus additional values from our laboratory (see Methods), are shown in Figure 1. There is substantial agreement between SC water sorption values obtained from different laboratories using different techniques. We note that the data in Figure 1 reflect nonpalmar–plantar human SC maintained in the range 20–30°C. Palmar–plantar SC[1] and rodent SC[4] yield qualitatively similar results, but there are quantitative differences in the results in Figure 1. The temperature dependence of water sorption in the SC remains something of a puzzle, with conflicting results reported.[3,6,7] This discrepancy will be discussed later.

Spectroscopic[13,14] and calorimetric[15] characterization of partially hydrated SC has shown that the first 25–33% of the water entering dry SC (expressed as percent of total weight) becomes tightly associated with the tissue, as judged by narrowing of infrared (IR) and nuclear magnetic resonance (NMR) line widths and the lack of a melting endotherm at 0°C. These values are alternatively expressed as 0.33–0.5 g water/g dry tissue. A comparison of these values with the data in Figure 1 indicates that water sorbed up to a relative humidity of 90% may be considered "bound" water on this basis. Additional water entering the tissue at higher relative humidity does appear to melt like bulk water,[15] but NMR spin–spin relaxation times suggest a gradual, rather than abrupt, transition to the bulk state.[14] This concept is discussed in some detail below, and

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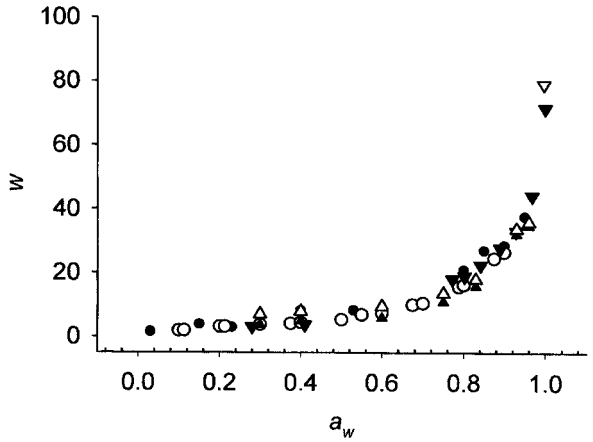


**Figure 1.** Equilibrium water sorption data for human SC, expressed as weight % water in the tissue versus water activity. Key: (●) Anderson et al.,[3] 30°C; (○) El Shimi et al.,[5] 23.3°C; (▲) Spencer et al.,[6] 20°C; (△) Spencer et al.,[6] 30°C; (▼) this study (Table 1), 30°C; (▽) Talreja,[39] 30°C.

the implications for water mobility are described in the accompanying paper.

The following sections present the equilibrium water sorption data for human SC and three models that satisfactorily describe this behavior. The objective is to provide a quantitative framework on which the effect of hydration on the permeability of the skin to water and other solutes can be discussed.

## THEORY

Water sorption in the fibrous, keratinized tissues wool and horn was initially described by the Brunauer–Emmett–Teller (BET) isotherm and modifications thereof.[16–19] The derivation for a biopolymer system begins by considering a model in which $A$ moles of water are distributed on $B$ moles of localized sites at an equilibrium water activity (or relative pressure) $x = p/p_0$. If one considers that $X$ moles of water are strongly bound to the polymer matrix and the remaining $A - X$ moles are adsorbed on top of these sites, a statistical thermodynamic analysis leads to the following relationships:[16,18,20]

$$x = (A - X)/A \tag{1}$$

and

$$(A - X)/(B - X) = \gamma X^2. \tag{2}$$

The factor $\gamma$ involves the binding energy, $W$, of the localized molecules and the ratio, $r_j$, of the internal partition functions of the free and localized water molecules:[16–18]

$$\gamma = r_j e^{-WRT} \tag{3}$$

For the water–wool system at room temperature, Cassie[16] estimated $W = 3500$ cal/mol, $r_j = 33$, and $\gamma = 0.1$. Combination of eqs. 1 and 2 leads to the BET isotherm:[16,19]

$$A/B = \frac{cx}{(1-x)(1-x+cx)} \tag{4}$$

in which $c = 1/\gamma$. Equation 4 is mathematically equivalent to the more common form of the BET equation arising from gas adsorption theory,

$$\frac{v}{v_m} = \frac{cx}{(1-x)(1-x+cx)} \tag{5}$$

in which $v$ is the adsorption volume and $v_m$ is the volume of an adsorbed monolayer. Equations 4 and 5 have been widely used to describe Type II sorption isotherms. The appropriateness of the model is often assessed by examining the following linear transformation:

$$\frac{x}{v(1-x)} = \frac{1}{(v_m c)} + \frac{(c-1)}{(v_m c)}x \tag{6}$$

The BET model considers only short-range interactions between adsorbate (i.e., water) and adsorbent. Adsorbed molecules beyond the first layer are assumed to interact as in the bulk liquid. Modifications of the BET model with longer range interactions have been proposed; for example, Windle's analysis of water sorption in wool which distinguished three forms of sorbed water.[18] However, more success in the wool–water system has been achieved with multilayer models deriving from Enderby[21] and King,[22] as discussed by several authors,[19,23,24] or solution models based on modifications of Flory–Huggins theory.[25–27] The two competing approaches of D'Arcy–Watt[23] and Pierlot[27] may be summarized in eqs. 7 and 8, respectively:

$$A = \frac{B\tilde{b}x}{1+\tilde{b}x} + Ex + \frac{C\tilde{c}x}{1-\tilde{c}x} \tag{7}$$

$$\ln x = \ln \varphi_1 + \varphi_2 + \chi\varphi_2^2 + F \tag{8}$$

In eq. 7, $A$ and $x$ have the same meanings as in eq. 4 and $\tilde{b}, B, \tilde{c}, C$, and $E$ are disposable constants. In eq. 8, $\varphi_1$ and $\varphi_2$ are the volume fractions of water and polymer, respectively, $\chi$ is a water–polymer interaction factor, and $F$ is a factor that corrects the Flory–Huggins isotherm at low water activity for volume changes and glass transition effects.

The latter has different and somewhat complicated forms in various theories.[26,27]

Because of its relative simplicity compared with eq. 7 or 8, we consider also a general approach deriving from potential theory that allows for continuous variation of the energy of adsorption with distance from the adsorption site.[28] Assuming that the potential energy of a molecule varies as the inverse $n^{\text{th}}$ power of its distance from the surface, Halsey[29] deduced an isotherm of the form

$$(v/v_m)^n = -\tilde{A}/\ln x \tag{9}$$

now known as the Frenkel–Halsey–Hill (FHH) equation.[28,30] In this equation, $x$, $v$, and $v_m$ have their previous interpretations and $\tilde{A}$ is a constant. In many systems, $n$ is found to have a value between 2 and 3,[29,30] corresponding to potentials that fall with distance at a rate intermediate between an inverse square and an inverse cubic power. However, values of $n$ as low as 1 have been reported for some adsorption systems.[28] Equation 7 may also be written in the linear form

$$\ln v = k - \frac{1}{n}\ln\ln(1/x) \tag{10}$$

where $k = \ln(\tilde{A}^{1/n} v_m)$, which allows a determination of $n$ from a plot of $\ln v$ versus $\ln\ln(1/x)$. [Note on units: When fitting sorption models such as eqs. 5 and 9 to experimental data, considerable flexibility exists in the choice of units for $v$ and $v_m$. For the keratin–water system, the common choice has been to express both $v$ and $v_m$ as (grams of water)/(gram of dry tissue).[16–18,24] We have adopted this convention.]

Other isotherm relationships express water content differently. The experimental results in this report (Figure 1, Table 1) are given as weight percent water ($w$). Equation 8 employs the volume fraction water, $\varphi_1$. The various expressions for water content are related by

$$v = \frac{w}{100 - w} = \frac{\rho_1 \varphi_1}{\rho_{mem}(1 - \varphi_1)} \tag{11}$$

in which $\rho_1$ is the density of water (1.0 g cm$^{-3}$) and $\rho_{mem}$ is the membrane dry density. For the present work, the value $\rho_{mem} = 1.3$ g cm$^{-3}$ has been employed throughout.[8]

The cluster theory of Zimm[31] and Zimm and Lundberg[32] may be applied to these isotherms to gain insight into the structure of water in the SC. According to this theory, the cluster function

$$\varphi_1 \frac{G_{11}}{V_1} = (1 - \varphi_1)\left[\frac{\partial \ln \varphi_1}{\partial \ln a_1}\right]_{P,T} - 1 \tag{12}$$

expresses the number of type 1 molecules in excess of the mean concentration of type 1 molecules in the neighborhood of a given type 1.[19] Letting type 1 be water, as in eq. 8, $\varphi_1$ is volume fraction water, $G_{11}$ is a cluster integral, $V_1$ is the partial molal volume of water, and $a_1$ is water activity. With the aid of eq. 11 and the identification $a_1 = x$, the right-hand side of eq. 12 may be evaluated for any of the isotherm models (i.e., eqs. 4–10). The result for the FHH model (eq. 9 or 10) is

$$\varphi_1 \frac{G_{11}}{V_1} = -\frac{(1-\varphi_1)^2}{n \ln x} - 1 \tag{13}$$

To see this, note that $(\partial \ln \varphi_1/\partial \ln a_1) = (1/\varphi_1)(\partial \varphi_1/\partial \ln x) = (1/\varphi_1)(\partial v/\partial \ln x)(\partial \varphi_1/\partial v)$. Evaluation of the partial derivatives in the rightmost expression, followed by substitution into eq. 12, leads to eq. 13. This expression will be used in conjunction with the isotherm data in Figure 1 compare the present results with earlier work in rat[4] and human[7] SC.

**Table 1.** Uptake of Water by Cryopreserved Human Stratum Corneum at 30°C

| $a_w$ | Wt % $H_2O$ ($w$)[a] |
|---|---|
| 0.28 | 3.1 ± 3.6 |
| 0.41 | 3.7 ± 1.2 |
| 0.77 | 18.1 ± 8.2 |
| 0.80 | 19.0 ± 9.0 |
| 0.84 | 22.5 ± 8.6 |
| 0.89 | 27.8 ± 9.8 |
| 0.93 | 33.1 ± 8.3 |
| 0.97 | 44.2 ± 9.0 |
| 1.00 | 71.6 ± 16.4 |

[a] Mean ± SD, $n = 5$–6.

## EXPERIMENTAL

### Water Sorption

Cryopreserved, split-thickness human skin (back, abdomen, or leg) was obtained from Ohio Valley Tissue and Skin, Inc. (Cincinnati, OH). Frozen samples were thawed in lukewarm water, and the epidermis was heat-separated from the remaining dermis (2 min at 60°C).[33] Isolated SC was prepared from the epidermal samples by trypsin digestion[33] and stored in a dessicator over Drierite® for several days prior to use. The dried samples (4–5 mg) from each skin donor were carefully weighed (± 0.01 mg) and placed in glass

Petri dishes, which were subsequently transferred to dessicators maintained at 30°C and relative humidities (RHs) ranging from 30 to 100%. RH was controlled with NaCl and LiCl solutions.[34] Samples were removed from the dessicators daily, quickly weighed, and replaced. Sorption equilibrium, as judged by constancy of weight, was reached in 1–2 days.

### Data Analysis

Weight percent water sorption $w$ was converted to adsorption volume $v$ or volume fraction $\varphi_1$ using eq. 11. Sorption models were fit to the combined SC water sorption data [i.e., log $v$ versus water activity ($a_w$)], shown in Figure 2, using nonlinear regression (SigmaPlot, Jandel Scientific). For the BET and FHH models (eqs. 5 and 9, respectively), the value of $x$ was replaced by $x = a_w/a_0$, where the value of $a_0$ is an adjustable parameter. Thus, the equations to which the parameters were fit were, respectively, slightly modified BET and FHH isotherms as follows:

$$\log v = \log v_m + \log\left\{\frac{c(a_w/a_0)}{(1-a_w/a_0)[1+(c-1)a_w/a_0]}\right\} \quad (14)$$

and

$$\log v = \log\left(\tilde{A}^{1/n} v_m\right) - \frac{1}{n}\log \ln(a_0/a_w) \quad (15)$$

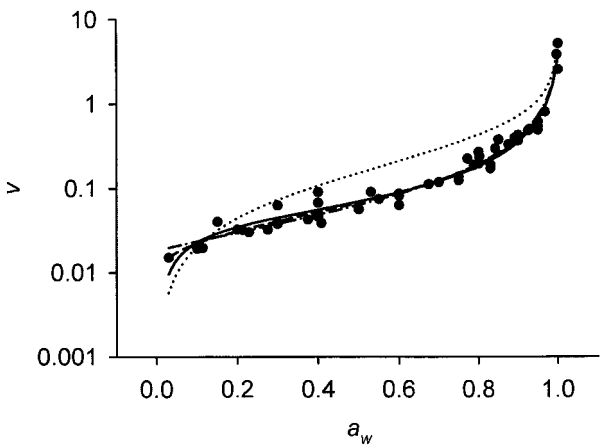


**Figure 2.** Equilibrium water sorption data for human SC, expressed as g $H_2O$/g dry tissue. The smooth curves are fits of sorption isotherm models to these data; regression parameters are given in Table 2. Key: (solid curve) BET (eq. 14); (dashed curves) FHH (eq. 15) and modified D'Arcy–Watt (eq. 16); (dotted curve) Pierlot (eq. 8) with $F = 0$, $\chi = 0.4$.

This procedure was chosen in lieu of Cassie's "reduced isotherm" approach[16] to provide a reasonable limit to sorption at unit water activity. The values found for $a_0$ ($a_0 \approx 1.01$) were only 1% larger than the value $a_0 = 1$, expected for an unconstrained system; thus, this modification has minimal impact on most of the isotherm. The alternative to this approach involves constructing a model for the restoring forces in the swollen keratin matrix,[16] which was problematical for the water–wool system[19] and may also be for SC. This procedure was not required for the D'Arcy–Watt model (eq. 7) in which $x = a_w$. Here the limit to sorption at high water activity is provided automatically because the value of $\tilde{c}$ can be slightly <1. However, the five-parameter model represented by eq. 7 was found to have no advantage in degree of fit versus the simpler, three-parameter model (in logarithmic form)

$$\log v = \log\left(v_m + \frac{C\tilde{c}x}{1-\tilde{c}x}\right) \quad (16)$$

in which the Langmuir term in the right-hand side of eq. 7 has been replaced by $v_m$ and the linear term $Ex$ has been omitted. (Note that the substitution $x = a_w/a_0$ used in eqs. 14 and 15 has no impact on the cluster function in eq. 13; this phenomenon may be seen by applying the chain rule to eq. 12 and noting that $\partial \ln x/\partial \ln a_w = 1$.)

## RESULTS

The results of the human SC water sorption studies conducted in our laboratories are shown in Table 1. The values are in good agreement with those from previous studies, as can be seen from Figure 1. The data in Figure 1 were combined and replotted in Figure 2 as adsorption volume $v$ (g $H_2O$/g dry tissue) versus $a_w$. Sorption was nearly log linear over most of the water activity range, as has been noted previously for rat SC.[35,36]

The BET, D'Arcy-Watt, and FHH models were fit to the data in Figure 2 as discussed in the Experimental section. The resulting equations are plotted in Figure 2. Linearized plots of these data, constructed according to eqs. 6 and 10 with $x = a_w/a_0$, are shown in Figure 3 and the regression parameters are given in Table 2. All three models provided highly satisfactory overall fits to the dataset, with $r^2 > 0.97$. Thus, any model can be used to calculate equilibrium water content in

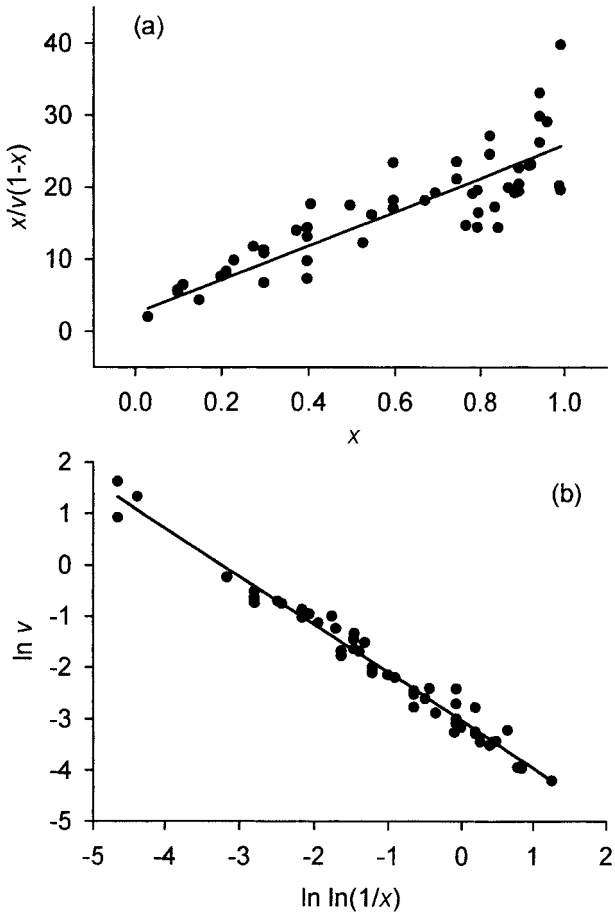


**Figure 3.** Plots of SC water sorption data according to the linearized forms of the BET and FHH equations: (a) BET model, eq. 5; (b) FHH model, eq. 9. The regression parameters are shown in Table 2.

SC over the full range of water activities. The linearized BET analysis is quite sensitive to variation in the dataset, as can be seen from Figure 3a. The FHH analysis provided slightly better predictions than either the BET or simplified D'Arcy–Watt isotherms (eq. 16), according to the PRESS and Durbin–Watson statistics. These features, in combination with the excellent linearity of the FHH plot in Figure 3b, highlight the advantages of this analysis. More importantly, the FHH approach avoids the dilemma of trying to reconcile the BET or D'Arcy-Watt monolayer volumes, $v_m = 0.039$ and $0.0183$ g $H_2O$/g dry tissue, respectively, with bound water measurements in SC, as discussed later.

The modified Flory–Huggins model (eq. 8) could not successfully describe SC water sorption over the full range of water activity unless a very large correction factor $F$ was assumed. An example of an uncorrected ($F = 0$) Flory–Huggins isotherm is shown in Figure 2. Because the corrections suggested for wool keratin by Rosenbaum[26] and Pierlot[27] apply primarily at low $a_w$, it seems difficult to justify this approach for the case of human SC.

Using the parameters in Column 4 of Table 2 and the substitution $x = a_w/a_0$, the cluster function, eq. 13, was calculated for water in human SC according to the FHH model. The results are shown in Figure 4. The value of $\varphi_1 G_{11}/V_1$ rose gradually from approximately $-1$ at $a_w = 0$, became positive at $a_w \approx 0.5$, and then rose steeply to a maximum value of 3.5 at $a_w = 0.97$. The other

**Table 2.** Regression Parameters from Least Squares Fits of Water Sorption Models to SC Water Sorption Data in Figure 2[a]

| Parameter | BET, Eq. 14 | D'Arcy–Watt, Eq. 16 | FHH, Eq. 15 |
|---|---|---|---|
| Regression coefficients | | | |
| $a_0$ | $1.010 \pm 0.002$ | [1] | $1.010 \pm 0.002$ |
| $v_m$[b] | $0.0386 \pm 0.0018$ | $0.0183 \pm 0.0020$ | [0.0386][c] |
| $c$ or $\tilde{c}$ | $10.4 \pm 2.3$ | $0.9871 \pm 0.0021$ | — |
| $C$[b] | — | $0.0464 \pm 0.0027$ | — |
| $\tilde{A}$ | — | — | $1.278 \pm 0.024$ |
| $n$ | — | — | $1.071 \pm 0.038$ |
| Regression statistics | | | |
| $r^2$ | 0.9703 | 0.9712 | 0.9742 |
| $s$ | 0.103 | 0.102 | 0.096 |
| $F$ | 785 | 809 | 906 |
| Durbin–Watson | 1.80 | 1.76 | 1.95 |
| PRESS | 0.685 | 0.616 | 0.565 |

[a]The error estimates are standard parameter errors calculated from the regression analysis.
[b]The parameters $v_m$ and $C$ have units of g $H_2O$/g dry tissue. The other parameters are dimensionless.
[c]The value of $v_m$ was fixed at the BET value in order to determine $\tilde{A}$.

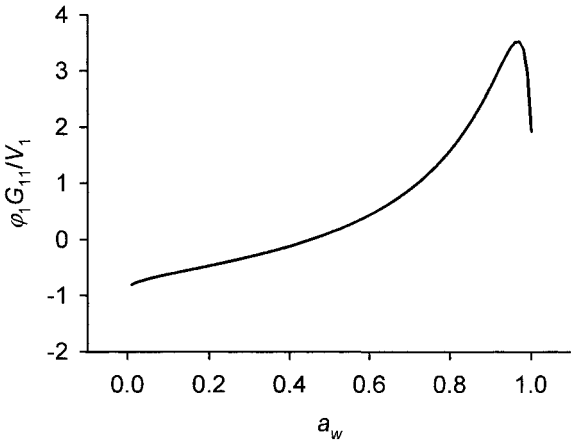


**Figure 4.** Cluster function for water in human SC, calculated according to eq. 13, which is derived from eq. 12 and the FHH isotherm, eq. 9. The parameters are given in Table 2.

isotherm models, eqs. 5 and 16, led to similar profiles (data not shown).

## DISCUSSION

The general form of the water sorption profile for human SC (Figures 1 and 2) has been known for some time. The models presented herein summarize these data in a manner useful for further computations. In addition, they offer some insights into the nature and number of water binding sites in SC and the range of the intermolecular forces.

The number of carbonyl groups in dry keratin (as wool) has been estimated to be 1.11 mol/100 g.[16] Alternatively, Windle[18] cites the values 0.84–0.87 mol/100 g of peptide groups plus 0.42 mol/100 g of polar side chain groups, giving a total of ~1.3 mol/100 g of polar functionalities in keratin. Taking these values to be representative of the corneocyte phase of SC (~85% of SC dry weight), and assuming 1–3 mol of water binding sites per mole of SC lipid (15% of SC dry weight) projects to a density of $H_2O$ binding sites in SC of ~1.2 mol/100 g dry tissue. BET analysis of the SC water sorption data (Table 2) yielded $v_m = 0.039$ g $H_2O$/g dry tissue, corresponding to $B = 0.21$ mol $H_2O$/100 g dry tissue in eq. 4. This value represents only 18% of the potential $H_2O$ binding sites in the tissue. Furthermore, the amount of "bound" water in SC estimated by other techniques (see Introduction) is 1.8–2.8 mol $H_2O$/100 g dry tissue, which exceeds the projected number of binding sites by a substantial margin. Thus, the BET adsorption model, which assumes a tightly bound monolayer of water overlaid by successive layers of "bulk" water, does not explain SC water sorption from a mechanistic standpoint. The same may be said for the modified D'Arcy-Watt isotherm (eq. 16) because the monolayer value $v_m$ estimated using this equation was even less than that from the BET model.

The FHH model, eq. 9, offers a somewhat more satisfactory explanation, albeit still semiempirical. The FHH model is rigorous only for extremely flat surfaces, in which $v_m$ is a true monolayer volume and $n = 3$, corresponding to the $1/r^3$ rate of decline of a van der Waals potential from a plane.[28,30] Smaller values of $n$ found in real systems[28] imply long-range potentials and correspondingly long-range intermolecular forces. The value $n = 1.07$ estimated for human SC (Table 2) suggests a $1/r$ potential such as found for electrostatic interactions; this agreement is likely coincidental because water molecules are uncharged. Nevertheless, it is consistent with the fact that bound water in SC appears to extend well beyond monolayer adsorption to polar binding sites. The value of the monolayer volume $v_m$ selected for the FHH isotherm is arbitrary because it cannot be deconvolved from the constant $\tilde{A}$. The value of the monolayer volume is commonly taken as the BET value, as we have done in Table 2; however, it is not necessary to assign it physical significance. Thus, the FHH interpretation of SC water sorption also removes the need to quantitatively explain this factor.

The cluster function plot (Figure 4) is similar to that observed in rat SC[4] and in a number of hydrophilic synthetic polymers.[19] For $a_w \leq 0.7$, the value of $\varphi_1 G_{11}/V_1$ is in close agreement with the values calculated by El Shimi and Princen[7] for a sample of human SC (to show this, one must convert the values to the related function $G_{11}/V_1$, the quantity plotted by these workers). At higher values of $a_w$, our values are lower than those of El Shimi and are in qualitative agreement with the suggestion of Haly and Snaith[37] for wool (see also ref. 24) that $\varphi_1 G_{11}/V_1$ rises to ~3 for high values of $a_w$. For SC, according to Watt,[24] "The large negative values of the cluster function at low humidities indicate segregation of the water molecules... The steadily increasing values of the cluster function in the intermediate humidity range support the concept of multilayer sorption proceeding concurrently with a localized adsorption. Substantial clustering occurs only at high relative humidities..." The present data support this interpretation.

The overall picture that emerges from this and previous[14,15] analyses is that of a tissue that absorbs only a small amount of tightly bound water at low water activities. Hansen and Yellin call this "primary water of hydration",[14] which is likely to be associated with strongly polar functional groups in both protein and lipid phases. As $a_w$ increases and the tissue hydrates and swells, more water binding sites in the keratin matrix become exposed. Water sorbed in this region (up to $a_w \approx 0.90$, corresponding to $v \approx 0.4$ g $H_2O$/g dry tissue or $w \approx 30\%$) consists of additional primary water of hydration plus a less tightly bound species, which may be hydrogen bonded to the primary water or to dipolar sites on the protein.[14] This water still does not freeze, even at temperatures as low as $-50^\circ$C.[14,15] Water sorbed at even higher values of $a_w$ ($\geq$0.90) does freeze, but has lowered mobility versus bulk water based on spin–spin relaxation times.[14] The FHH isotherm, which incorporates a continuous variation of adsorption energy with distance,[28] satisfactorily correlates experimental water sorption data in SC and provides a plausible framework for describing the various forms of water observed spectroscopically.

We considered and discarded the idea of correcting the water sorption data in Figure 1 for temperature effects. Spencer and co-workers reported that SC water sorption at low relative humidity increased with temperature,[6] suggesting an endothermic reaction of water with dry keratin. However, Anderson's group reported the opposite effect, including unpublished calorimetric results suggesting the exothermic nature of the reaction.[3] El Shimi and Princen[7] also calculated an exothermic $\Delta H$ based on water sorption data at 32 and 36°C. Equilibrium sorption and calorimetric data on the wool–water system[16,19,38] clearly establish the exothermic nature of water sorption in this system. The bulk of the data support an exothermic reaction with a $\Delta H$ value that approaches the latent heat of condensation as the SC water content approaches 20%.[7] Further investigation of this subject may be warranted.

## CONCLUSIONS

Equilibrium water sorption in human SC can be described by physical chemical models common to polymer–water systems. At low humidities, the results can be interpreted in terms of segregated water molecules tightly bound to polar groups in keratin and intercellular lipids. At higher humidities, more keratin sites are exposed, leading to more "bound" water, and the molecules tend to cluster. Spectroscopic evidence that even the large amount of water incorporated at very high humidities has decreased mobility relative to bulk water can be rationalized on the basis of the FHH sorption isotherm, which allows for long-range interactions between the solute and the tissue.

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