In vivo measurement of the surface energy of human fingernail plates

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SYNOPSIS

The surface energy of the human nail plate is expected to influence the adhesion of microorganisms (and subsequent colonisation and infections) as well as that of medicines (and subsequent drug permeation) and of cosmetics. The aim of the study was therefore to measure the surface energy of nail plates in vivo. The surface energy of healthy human fingernails (untreated, hydrated and abraded) and of hoof membranes (often used as a model for the nailplate) was estimated from contact angle measurements of liquids (water, formamide, diiodomethane and glycerol) on the nail plate and subsequent computation using the Lifshitz-van der Waals/acid-base (LW-AB) approach. The surface energy of untreated fingernail plates was found to be 34 mJ/m². Most of this total energy was from the apolar Lifshitz-van der Waals component. When the polar component of the surface energy was analysed, the electron-donor component was considerably larger than the electron-acceptor one. Hydrating the nail plate had no significant influence on the surface energy. In contrast, abrasion caused a small, but statistically significant increase in the apolar surface energy component. The surface energy of bovine hoof membrane was similar to that of the fingernail plate. We conclude that the human fingernail plate is a low-energy surface, and that bovine hoof membranes may be used as a substitute for the nail plate in certain experiments.

Keywords: nail, surface energy, hydration, abrasion, hoof
INTRODUCTION

The human nail unit has diverse functions, such as protection of the delicate tips of fingers and toes against trauma. It allows us to manipulate objects, enhances the sensation of fine touch, and is used for scratching and decoration [1-5]. The nail is also subject to diseases, such as fungal infections, and a range of topical medicines, such as solutions, paints, lacquers and patches, have been formulated [6-8]. Despite the advantages of local drug delivery such as, drug targeting to the site of action, and avoidance of systemic side effects and drug interactions, the success of local drug therapy is limited [9]. In their review on the topical delivery of drugs to the nail, Sun et al, in 1999, concluded that the topical treatment of onychomycosis was a drug delivery problem [10]. To date, optimisation of topical drug formulations remains a priority.

While considerable literature exists on the bulk properties of the nail plate (summarised briefly in [11]), its surface properties have not been extensively studied. A greater understanding of the nail plate surface properties would be invaluable to the drug/cosmetic formulator as interfacial energies between topical formulations and the nail plate are expected to govern their contact. For drug delivery, this would influence ungual (pertaining to the nail; used interchangeably with onycheal) drug permeation, while for cosmetics, this would influence residence and consumer acceptability. Interfacial forces between the nail plate and micro-organisms are also expected to influence the adhesion of microorganisms onto the nail plate and subsequent colonisation and infections. Despite the important role of the nail plate’s surface
properties on local infections and their treatment, the nail plate’s surface energy has not been extensively investigated. Our aim was therefore to measure the surface energy of nail plates in vivo.

Surface energy was determined from contact angle measurements of appropriate liquids against the nail plate surface as this widely used method is believed to be the simplest [12]. The contact angle that a liquid droplet makes on a solid surface (Fig. 1) can be treated as a result of the mechanical equilibrium under the action of three surface tensions – the solid-vapour interfacial tension (\(\gamma_{SV}\)), the liquid-vapour interfacial tension (\(\gamma_{LV}\)), and the solid-liquid interfacial tension (\(\gamma_{SL}\)) [13], as follows:

\[
\gamma_{LV} \cos(\theta) = \gamma_{SV} - \gamma_{SL}
\]

Thus, \(\gamma_{SV}\) can be calculated from the above equation by measuring the contact angle \(\theta\) that a liquid makes on the solid surface, and from knowing the surface free energies \(\gamma_{LV}\) and \(\gamma_{SL}\) from reference sources [14]. A number of approaches to compute a solid’s surface energy following contact angle measurements have been developed, as reviewed in [12]. In this paper, the Lifshitz-van der Waals/acid-base (LW-AB) approach has been used as it is currently one of the most commonly used approaches, it has been successfully applied in many instances, including in the evaluation of the surface energy of biological surfaces such as the skin [15, 16]. The LW-AB approach was developed by van Oss et al. who suggested that the surface energy of a solid or liquid \((\gamma)\) can be considered as the sum of the apolar Lifshitz-van der Waals (\(\gamma^{LW}\)) and the polar acid-base (\(\gamma^{AB}\)) components [17-20] as follows:

\[
\gamma = \gamma^{LW} + \gamma^{AB}
\]
Where $\gamma_{\text{LW}}$ is the sum of the London dispersion forces, Debye induction and Keesom orientation interactions, and

$$\gamma_{\text{AB}} = 2(\gamma^+ \gamma^-)^{1/2}$$  \hspace{1cm} (3)

Where $\gamma^+$ and $\gamma^-$ are the electron accepting and electron-donating surface energy parameters respectively, determining the material's ability to exert acid-base interactions on a comparative scale with respect to the reference water, for which the ratio $\gamma^+/\gamma^-$ is arbitrarily set to 1.

Equation (1) can be solved if the solid-liquid interfacial free energy is expressed in terms of Lifshitz-van der Waals and acid-base components, and according to van Oss et al. [17-19] $\gamma_{\text{SL}}$ can be re-written as:

$$\gamma_{\text{SL}} = \gamma_{\text{LW}}^{\text{S}} + \gamma_{\text{LW}}^{\text{L}} - 2(\gamma_{\text{LW}}^{\text{S},\text{L}})^{1/2} + 2[(\gamma_{\text{S}}^{+} \gamma_{\text{L}}^{-})^{1/2} + (\gamma_{\text{S}}^{-} \gamma_{\text{L}}^{+})^{1/2} - (\gamma_{\text{S}}^{+} \gamma_{\text{L}}^{-})^{1/2} - (\gamma_{\text{S}}^{-} \gamma_{\text{L}}^{+})^{1/2}]$$  \hspace{1cm} (4)

where subscripts "S" and "L" denote for a solid and a liquid respectively.

Rewriting equation 1 using equations 2-4 above gives:

$$\gamma_{\text{L}}(1 + \cos \theta) = 2(\gamma_{\text{LW}}^{\text{S},\text{L}})_{\text{S}}^{1/2} + 2(\gamma_{\text{LW}}^{-})_{\text{S}}^{1/2} + 2(\gamma_{\text{LW}}^{+})_{\text{S}}^{1/2}$$  \hspace{1cm} (5)

The solid surface parameters $\gamma_{\text{LW}}^{\text{S}}, \gamma_{\text{S}}^{+}$ and $\gamma_{\text{S}}^{-}$ can be computed following measurements of contact angles of droplets of three different liquids (two polar and one apolar) whose surface free energy components are known.

In our experiments, we used the polar glycerol, formamide and distilled water and the apolar diiodomethane; the literature values of these liquids' surface tension and components are shown in Table I. In addition to untreated nails, we also measured
the surface energy of hydrated and abraded nail plates. The latter were evaluated as in practice, patients abrade their nail plates prior to applying medicines in an attempt to increase ungual drug permeation. Hydrated nails were evaluated as topical nail medicines may increase nail plate hydration by occluding the nail plate surface and reducing trans-onychial water loss [21-23]. Any changes in the surface properties of nails due to abrasion or hydration could affect the interfacial interactions between the nail plate and the topical preparation, and hence contact. In addition, the surface energy of bovine hoof membranes was measured as these are often used as models for nail plates in drug delivery studies, e.g. in [24-28].

**MATERIALS AND METHODS**

Glycerol was obtained from Ransom (Herts, UK); formamide and diiodomethane were from Sigma. Bovine hooves, obtained from an abattoir, were cleaned and cut into thin sections. A contact angle goniometer (FTA 1000, First Ten Angstroms, USA) was used to measure the contact angle made by a liquid on a bovine hoof membrane/ fingernail plate in volunteers.

**Measurement**

Following approval by the London School of Pharmacy’s ethics committee, volunteers (8 females and 9 males, aged 23-51 years old) with healthy fingernails were recruited. The measurements were conducted in a laboratory where the room temperature ranged from 20 to 26 °C and the relative humidity from 19 to 29%. Volunteers washed
their hands with tap water and a liquid hand cleanser, dried them with tissue paper and sat in the measurement laboratory for 20 minutes prior to measurement. To measure contact angle, the volunteer’s finger was placed on a support underneath the syringe holder and a liquid droplet (volume 5-10µL) was dispensed from a micrometer syringe (Gilmont Instruments) fitted to a 20 gauge blunt needle and allowed to fall gently onto the fingernail. The distance between the needle tip and the nail was kept to a minimum such that the drop of liquid could fall without interference from the needle, but did not spread on the nail upon contact due to kinetic energy. The liquid droplet was allowed to stabilize on the nail surface, all the while video recording the droplet. For each video, a series of droplet images were analysed, and the triple point at the intersection of the liquid, solid and vapour phases on both sides of each image was manually identified. The contact angles were calculated and averaged for the two sides on each video image. Subsequently, contact angle versus time was plotted to enable visualization of the stable contact angles, which were then averaged to obtain a mean contact angle. For each liquid, the experiment was repeated at least three times on each nail, with handwashing, drying and equilibrating prior to each repetition.

To measure the contact angle of liquid droplets on hydrated nails, the fingernail (washed as above) was immersed in distilled water at room temperature for ten minutes, then blotted dry with paper towels, before immediate measurement as described above. Ten minutes was considered sufficient to achieve hydration saturation in vivo as shown by trans-onychial water loss (TOWL) measurements [23].

To measure the contact angle of liquid droplets on abraded nails, the dorsal surface of
the nail plate was rubbed using an abrasive file provided in a pack of Curanail®
(amorolfiné nail lacquer). Rubbing was performed at least three times, ensuring that
the entire nail surface on which a liquid droplet would sit was abraded. The nail plate
was then wiped gently with tissue paper before immediate measurement as described
above.

The contact angles of two polar liquids and one apolar liquid obtained on each nail
plate was then used to calculate its surface energy by the LW-AB method using the
goniometer software. Use of water, glycerol, formamide and diiodomethane liquids
enabled the computation of the nail plate’s surface energy using the following two
liquid combinations : water-formamide-diiodomethane (WFD) and water-glycerol-
diiodomethane (WGD). It was intended that for each nail, the contact angle of all
four liquids would be determined, and that in each volunteer the surface energy of all
the ten fingernails would be measured. This was not always possible, however, as a
stable droplet of a particular liquid could not always be obtained, e.g. due to high
curvature of some fingernails. In addition to curvature, the surface smoothness of the
nail plate was taken into account. For each volunteer, the flattest and smoothest nail
plates were used.

The surface energy of bovine hoof membranes was also measured as described above,
except for the use of bovine hoof membranes instead of nails. Measurements were
conducted on four hoof membranes. On each membrane, the contact angles made by
water, glycerol and formamide (ten drops for each liquid) were measured.
Although temperature is known to affect contact angles of liquids [29], the errors were expected to be small [16]. Surface roughness - also known to influence contact angles - is discussed later.

**Statistical analysis**

Contact angles: To analyse the influence of nail plate hydration and abrasion on the contact angle formed by the four liquids, repeated measures ANOVA was conducted on the contact angle data for nails which had been measured at all three conditions – untreated, hydrated and abraded.

Surface energy: General linear model was performed to investigate the influence of volunteer, hand side, digit and liquid combination used, on the nail plate surface energy. One way ANOVA was conducted to investigate the influence of hydration and abrasion on the nail plate surface energy. SPSS 18 was used for all statistical calculations.
RESULTS AND DISCUSSION

Contact angles of liquid drops on human fingernail plate in vivo

The contact angles formed by distilled water, glycerol, diiodomethane and formamide on untreated, hydrated and abraded fingernail plates are shown in Fig. 2. Diiodomethane (the liquid with the lowest surface tension of 50.8 mN/m) spread on the nail plate surface to the greatest extent and gave the lowest contact angle. Diiodomethane was followed by formamide (surface tension of 58.2 mN/m), which was itself followed jointly by glycerol (surface tension of 64.0 mN/m) and water (surface tension of 72.8 mN/m). The inverse relationship between a liquid’s surface tension and its extent of spreading on the nail plate is expected: high liquid surface tension would oppose liquid spreading (to minimise the liquid-air interfacial area) and thereby minimise the total surface energy. Our results reflect the inverse relationship between \( \cos \theta \) (where \( \theta \) is the contact angle) and the surface tension of liquids reported by Zisman [30]. Water’s greater surface tension compared to that of glycerol could have led to the expectation of a greater contact angle by water. However, the relationship between \( \cos \theta \) and liquid surface tension is known to be not linear for high surface tension liquids [30].

Standard deviations of between 6° and 8° were obtained (Fig. 2). Such variability is due to a number of factors including the inherently inexact [31] manual location of the triple point (where the solid, liquid and vapour phases meet) on the goniometer images and intra- and inter-individual variability, compounded by the nail plate’s
curvature [32] and surface roughness. Interestingly, similar contact angles (and standard deviations) for these liquids on the skin have been reported [16, 33].

**Influence of nail plate hydration and abrasion on contact angles**

As can be seen from Fig. 2, the contact angles of the different liquids on the nail plate surface did not differ greatly when the nail plate was hydrated or abraded. For a number of nails, the contact angle formed by the four liquids had been measured at all three conditions – untreated, hydrated and abraded (Table II). Repeated measures ANOVA on this data showed that, unlike abrasion, hydration did not significantly alter the contact angle of any of the four liquids (p>0.01). Immersion of the nail in water is known to increase its water content, such that nails become soft, and the surface water content, measured by opto-thermal radiometry (OTTER) was found to increase from 19 to 24% after a 15-minute immersion in water [34]. It seems that such a small increase in water content does not affect the nature of the nail plate surface in a sufficient manner to influence the contact angles of water, glycerol, formamide and diiodomethane.

On the other hand, abrading the nail plate surface (which greatly increased surface roughness as shown in Figure 3) reduced the contact angles of diiodomethane and of formamide (p<0.01), but had no influence on those of water and glycerol (p>0.01). Surface roughness is known to influence the contact angle of a liquid on a solid surface in complex ways [35-39]. Our results show an influence of surface roughness on the contact angles of the two liquids which formed the lowest contact angles.
Surface energy of fingernail plates

Surface energy of fingernail plates (untreated, hydrated and abraded) was calculated for the fingernails of digits 1-4, on the right and left hands in 15 volunteers, using two combinations of liquids (diiodomethane-formamide-water or diiodomethane-glycerol-water). General linear model showed no influence of volunteer, hand side or digit on the nail plate surface energy (p>0.01), but a significant influence of the nail state and of the liquid combination used (p ≤ 0.01). The mean surface energy for the different nail states (untreated, hydrated and abraded) and the two liquid combinations was thus calculated by grouping the digits of the right and left hands in all the volunteers (Table III). A number of negative values for $\gamma^+$ and $\gamma^{AB}$ can be seen in Table III.

Negative values for surface energy components are known to occur when the LW-AB method is used [40], although the proponents of the LW-AB method have demonstrated how small changes in the contact angles, e.g. by as little as $0.5^\circ$ (due to slight error in measurement) could have led to a positive value for $\gamma^+$ (and hence for $\gamma^{AB}$) instead of a negative one [41]. Thus, the small negative energies could arise from ‘noise’ in the contact angle data, as well as from imperfect knowledge of $\gamma^{AB}$ components [42], and are taken by some authors to be zero [43]. It can also be seen that the two liquid combinations (WFD and WGD) gave similar, but not identical surface energy values. Different surface energy values obtained by the use of different liquid combinations are also not unusual [12, 15, 16, 40], and is another criticism of the LW-AB approach, although in our experiments, the values of surface energy computed
by water-formamide-diiodomethane and water-glycerol-diiodomethane combinations were fairly close (Table III).

The mean surface energy of the untreated fingernail was calculated to be 35.5 ± 4.7 mJ/m² and 32.6 ± 6.2 mJ/m² using WFD and WGD combinations respectively. Averaging the surface energy values obtained with these two combinations gives a mean surface energy of 34.1 ± 5.5 mJ/m². These are similar to, although slightly lower than, those reported for untreated skin using the same liquids and LW-AB method [16]. The nail surface energy values show that the nail plate, like other organic substances, and like the skin, is a low-energy surface. The apolar Lifshitz-van der Waals (\( \gamma^\text{LW} \)) part is the major component of the nail plate’s total surface energy (Table III). When the polar acid-base (\( \gamma^\text{AB} \)) is analysed, the nail plate’s electron acceptor constituent (\( \gamma^+ \)) is negligible, in contrast to the considerable electron donor constituent (\( \gamma^- \)). The nail plate surface seems to be a largely basic surface. It must be noted that most solid surfaces, including the skin, have been found to be overwhelmingly basic when the LW-AB approach has been used [12, 17, 40, 41].

**Influence of hydration and abrasion on nail plate surface energy**

Hydration caused very small changes in the nail plate’s surface energy and its components, all of which were statistically insignificant (ANOVA; \( p<0.01 \)). This is not surprising given that hydration did not significantly alter the contact angle of any of the four liquids as discussed above. A small increase in the water content of the nail plate,
following immersion in water for ten minutes, does not seem to affect its surface energy.

In contrast, the changes in the nail plate’s surface energy due to abrasion were somewhat more complicated due to the fact that abrading the nail plate surface changed the contact angle of formamide but not that of glycerol (as discussed earlier), which led to differences in the computation of the nail plate surface energy by the two liquid combinations water-formamide-diiodomethane and water-glycerol-diiodomethane. The water-formamide-diiodomethane combination showed that abrasion increased the nail plate’s surface energy (p<0.01) while the water-glycerol-diiodomethane showed no significant change (p>0.01). However, for both liquid combinations, when the surface energy components were considered, abrasion resulted in a statistically increased apolar Lifshitz-van der Waals ($\gamma_{\text{LW}}$) component (p<0.01), but caused no significant change in the polar acid-base, electron acceptor and electron donor components (p>0.01).

**Surface energy of hoof membranes**

The surface energy of bovine hoof membranes is shown in Table IV. The total surface energy and its components are similar to those of the nail plate shown in Table III. As for the nail plate, the apolar component is the greatest constituent of the total surface energy of the hoof membrane, the electron acceptor constituent ($\gamma^+$) is negligible, while the electron donor constituent ($\gamma^-$) is considerable. The surface energy
similarities of the nail plate and of the hoof membrane are likely due to the structural similarities between the two keratinic tissues [44, 45].

CONCLUSIONS

The surface energy of healthy nail plates were determined in vivo from contact angle measurements, using the Lifshitz-van der Waals/acid-base (LW-AB) approach. Fingernail plate surface energy was found to be 34 mJ/m² and to have a significant apolar Lifshitz-van der Waals component (the major component), and like most solid surfaces, considerable electron-donicity, and negligible electron-accepticity.

Hydrating the nail plate had no significant influence on the contact angles of any of the liquids, and consequently, on the nail plate’s surface energy. In contrast, abrasion decreased the contact angles of diiodomethane and of formamide, but caused no change in the contact angles of water and glycerol. The latter differences resulted in different computations of the surface energy of abraded nails by the two liquid combinations. Thus, the water-formamide-diiodomethane combination showed that abrasion increased (albeit to a small extent) the nail plate’s surface energy, while the water-glycerol-diiodomethane showed no significant change. When the surface energy components were considered, for both liquid combinations, abrasion increased the apolar Lifshitz-van der Waals component, but caused no change in the polar acid-base components.
The surface energy of bovine hoof membranes was found to be similar to that of the nail plate. This shows that the hoof membrane could be used as a model for the nail plate in experiments related to the latter’s surface energy properties, for example, when testing adhesion and residence of nail preparations to the nail plate.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

Table I: Surface tension (and its components) of liquids used [21]

<table>
<thead>
<tr>
<th>Liquid</th>
<th>$\gamma_{\text{total}}$</th>
<th>$\gamma^{\text{LW}}$</th>
<th>$\gamma^{\text{AB}}$</th>
<th>$\gamma^+$</th>
<th>$\gamma^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>72.8</td>
<td>21.8</td>
<td>51.0</td>
<td>25.5</td>
<td>25.5</td>
</tr>
<tr>
<td>Formamide</td>
<td>58.0</td>
<td>39.0</td>
<td>19.0</td>
<td>2.28</td>
<td>39.6</td>
</tr>
<tr>
<td>Glycerol</td>
<td>64.0</td>
<td>34.0</td>
<td>30.0</td>
<td>3.92</td>
<td>57.4</td>
</tr>
<tr>
<td>Diiodomethane</td>
<td>50.8</td>
<td>50.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table II: Comparison of contact angles of four liquids on nails in the untreated, hydrated and abraded states. * signifies statistical difference between the contact angle of the abraded nail plate compared to the control (untreated).

| Liquid         | n = | Contact angle (degrees) on:
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>untreated</td>
</tr>
<tr>
<td>Diiodomethane</td>
<td>61</td>
<td>50.8 ± 7.1</td>
</tr>
<tr>
<td>Formamide</td>
<td>53</td>
<td>59.3 ± 6.9</td>
</tr>
<tr>
<td>Glycerol</td>
<td>63</td>
<td>75.3 ± 9.6</td>
</tr>
<tr>
<td>Water</td>
<td>62</td>
<td>77.4 ± 7.3</td>
</tr>
</tbody>
</table>
Table III: Surface energy (and its components) of untreated, hydrated and abraded nail plates computed using the two combinations water-formamide-diiodomethane (WFD) and water-glycerol-diiodomethane (WGD). Means ± sd are shown. Number of nails = 50-78 in 9-15 volunteers.

<table>
<thead>
<tr>
<th>Liquid combination used</th>
<th>state of the nail</th>
<th>Total surface energy (mJ/m^2)</th>
<th>Surface energy components (mJ/m^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total surface energy (mJ/m^2)</td>
<td>Lifshitz-van der Waals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ</td>
<td>γ_LW</td>
</tr>
<tr>
<td>WFD</td>
<td>untreated</td>
<td>35.5 ± 4.7</td>
<td>34.0 ±3.9</td>
</tr>
<tr>
<td></td>
<td>hydrated</td>
<td>34.2 ±3.6</td>
<td>33.6 ±3.8</td>
</tr>
<tr>
<td></td>
<td>abraded</td>
<td>39.2 ±3.9</td>
<td>37.0 ±4.2</td>
</tr>
<tr>
<td>WGD</td>
<td>untreated</td>
<td>32.6 ±6.2</td>
<td>34.1 ±3.9</td>
</tr>
<tr>
<td></td>
<td>hydrated</td>
<td>31.5 ±6.0</td>
<td>33.4 ±3.8</td>
</tr>
<tr>
<td></td>
<td>abraded</td>
<td>32.9 ±4.8</td>
<td>37.0 ±4.0</td>
</tr>
</tbody>
</table>
Table IV: Surface energy of bovine hoof membranes, measured using water, glycerol and diiodomethane. Means ±sd are shown. N=4 hoof membranes.

<table>
<thead>
<tr>
<th>Liquid combination used</th>
<th>Total surface energy of hoof membrane (mJ/m²)</th>
<th>surface energy components (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\gamma$</td>
<td>Lifshitz-van der Waals $\gamma^{\text{LW}}$</td>
</tr>
<tr>
<td>WGD</td>
<td>34.8 ± 2.6</td>
<td>37.3 ± 2.8</td>
</tr>
</tbody>
</table>
Figure 1a: The contact angle (θ) that a liquid droplet makes on a solid surface can be treated as a result of the mechanical equilibrium under the action of three surface tensions – the solid-vapour interfacial tension (γ_{SV}), the liquid-vapour interfacial tension (γ_{LV}), and the solid-liquid interfacial tension (γ_{SL}). 1b: A photograph of a water droplet sitting on a nail plate in vivo during contact angle measurement.
Figure 2: Contact angles formed by a drop of diiodomethane, formamide, glycerol and water, on untreated, hydrated and abraded fingernail plates (digits 1-4). Means and standard deviations are shown. The means were obtained from 57-86 fingernails in 11-17 volunteers.
Figure 3: Scanning electron microscopy shows the increase in surface roughness of the nail plate upon abrasion.