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# **Effects of iontophoresis, hydration and permeation enhancers on human nail plate: infrared and impedance spectroscopy assessment**

**Ian Benzeval<sup>1</sup>, Christopher R. Bowen<sup>2</sup>, Richard H. Guy<sup>1</sup>, M.Begoña Delgado-Charro<sup>1,3</sup>**

<sup>1</sup> Department of Pharmacy & Pharmacology, <sup>2</sup> Department of Mechanical Engineering,  
University of Bath, Bath, UK

## **<sup>3</sup>Corresponding author:**

M. Begoña Delgado-Charro, PhD.

Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath,  
BA2 7AY, UK.

Fax: 44 (0)1225 383969

Phone: 44 (0)1225 386114

e-mail: B.Delgado-Charro@bath.ac.uk

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## **Abstract**

Infrared and impedance spectroscopies were used to investigate the effects of hydration, iontophoresis and chemical enhancer (N-acetyl-L-cysteine) treatment on the healthy human nail. Although significant shifts to higher wavenumbers were observed for the symmetric and asymmetric  $-\text{CH}_2$  stretching vibrations, the fact that these changes were essentially the same for the three treatments suggested that they were principally due to hydration alone. Spectral changes associated with amide bonds from nail protein were particularly evident post-treatment with N-acetyl-L-cysteine. The alternating current conductivity and permittivity of the nail, particularly at low frequencies, increased with hydration. Iontophoresis increased the low frequency ac conductivity of the nail but had less effect on the nail capacitance/permittivity. Further, the effects seemed to return gradually to baseline after termination of current passage. Treatment with N-acetyl-L-cysteine produced a greater perturbation, leading to increased low-frequency conductivity and a shift of the frequency-dependent conductivity region to a higher frequency. Overall, the effects of iontophoresis on both the IR and impedance spectroscopic profiles of the nail were attributable simply to increased hydration and similar to those observed after skin iontophoresis. In contrast, both spectroscopy techniques indicated that N-acetyl-L-cysteine disrupted nail structure in line with the enhancer's known effect on keratin.

**Keywords:** N-acetyl-L-cysteine, nail hydration, iontophoresis, infrared spectroscopy, impedance spectroscopy.

## **Abbreviations:**

IR: Infrared spectroscopy

IS: Impedance spectroscopy

## Introduction

Management of nail diseases such as onychomycosis and psoriasis represents an important unmet clinical need. Oral and topical drug therapies are currently available, as well as painful intralesional injections in nail psoriasis, with nail avulsion as a last choice strategy (1-3). However, systemic drug treatment results in significant side effects and potential drug-drug interactions and, in the case of nail psoriasis, is not recommended unless there is substantial skin involvement too (1). While topical treatments minimize systemic adverse effects, and avoid the pain associated with intralesional injections, they have limited efficacy (1-4) due primarily to the nature and structure of the nail plate that severely limit drug penetration to the target site of action (5,6). Much research has examined strategies to improve drug delivery to and across the nail (6-7) including the use of penetration enhancers, such as N-acetyl-L-cysteine which breaks disulphide bonds in keratin, the main constituent of the nail plate (6-9). Another approach is iontophoresis (10) which involves drug administration to the nail with the application of a small electrical current to enhance molecular transport. Indeed, iontophoresis of terbinafine has shown promising results in the treatment of onychomycosis (11).

Both attenuated total reflectance, Fourier transform infrared spectroscopy (ATR-FTIR) and impedance spectroscopy (IS) have been used successfully to elucidate how chemical enhancers and iontophoresis perturb skin barrier function and the mechanisms by which they promote molecular transport across this membrane (12-16). However, application of these two techniques to the nail has been infrequent. Photoacoustic and ATR-FTIR spectroscopies have identified absorption bands from the lipid and protein present in different layers of the nail (17). More recently, ATR-FTIR has been used (via analysis of the amide I region of the spectrum) to assess changes in the secondary structure of nail proteins in patients with chronic fatigue syndrome (18). Elsewhere, some differences in the amide I region of the spectra of nails from diabetic and non-diabetic patients have been noted (19), whereas another FTIR study revealed no changes in the spectral features associated with disulphide bonds in nails post-hydration (9).

The electrical properties of skin and the effects of current passage, hydration and enhancers on this biological membrane have been characterized using impedance spectroscopy (14-16). While both current passage and hydration reduced skin impedance, iontophoresis induced a rapid decrease in the low-frequency resistive impedance component but had a much smaller effect on lipid/protein domains associated with skin capacitance. The effects of iontophoresis on skin impedance were increased following pre-treatment with penetration enhancers such as Azone, sodium lauryl sulphate and propylene glycol, suggesting that these compounds had disrupted stratum corneum structure. With respect to the nail, immersion in water increased its conductance, mostly at frequencies above 10Hz (20), and the latter effect was later shown to be a function of frequency and of absolute water content (21). However, little is known about the effects of iontophoresis and penetration enhancers on the electrical properties of the human nail. Importantly, the appendageal route, which provides a key pathway for current passage across the skin (22), is not present in the nail plate making the extrapolation of skin impedance spectroscopy data to the nail problematic.

This work aimed to use ATR-FTIR and IS to investigate the effects of hydration, iontophoresis, and N-acetyl-L-cysteine (a recognised penetration enhancer) on the healthy nail.

## Materials and Methods

**Materials:** Ag wire (1 mm, 99.99%), AgCl (99.999%), Pt (0.5 mm wire, 99.99%), NaCl (99.5%) and N-acetyl-L-cysteine (99.0%) were purchased from Sigma Aldrich (Gillingham, UK).

**Nails:** Nail clippings, at least 8 mm long, were donated by healthy volunteers after informed consent (Bath REC 06/Q2001/80).

**Iontophoresis:** Side-by-side glass diffusion cells equipped with a Teflon nail adaptor (Side-Bi-Side, PermeGear Inc., Bethlehem, PA) were used. The area of the nail exposed to current passage during either iontophoresis or impedance spectroscopy experiments was 0.2 cm<sup>2</sup>. Ag/AgCl electrodes were made as previously described (23) and positioned approximately 2 cm from the nail surface. Iontophoresis was performed using a power supply (Kepco APH 1000M, Flushing, NY). The current was set to 0.1 mA (0.5 mA.cm<sup>-2</sup>) in most experiments (see below) and the voltage limited to a maximum of 60 V. The electrolyte in both the anodal and cathodal chambers was 154 mM NaCl in deionised water.

**ATR-FTIR:** A Perkin Elmer Spectrum 100 with a diamond universal ATR attachment and an MCT detector was used to scan the nail samples. The reflectance crystal was 2 mm in diameter. Each spectrum was the average of eight scans from 4000-650 cm<sup>-1</sup> with an interval of 1 cm<sup>-1</sup>. Two to five sites on the same nail were scanned and averaged unless otherwise indicated. Each scan took 30 seconds and the time required for completing five scans was approximately 4-5 minutes. Preliminary work indicated that, while spectral changes occurred within the time required for data acquisition (probably due to nail dehydration), these were less than those due to inter-nail variability. In the iontophoresis experiments, “exposed” and “edge” scans were taken from the area (0.2 cm<sup>2</sup>) directly exposed to the passage of current and from the adjacent areas, respectively. A force (40±3 N) was applied to the nails to facilitate good contact with the crystal. Five peak positions, the asymmetric and symmetric -CH<sub>2</sub> vibrations (~2920 and 2850 cm<sup>-1</sup>), and the amide I, II and III bands (~1640, 1540 and 1240 cm<sup>-1</sup>) from protein, were determined from the first derivatives of the scans.

**Impedance Spectroscopy (IS):** IS was performed using a Solartron 1296 Impedance / Gain-Phase Analyzer and a 1260 Dielectric Interface. A small alternating voltage of varying frequency was

applied across the nail and the conductive and capacitive components of the nail altered the resulting current amplitude and phase angle. Sweeps were taken from 1Hz to 1MHz, 10 points per decade, with amplitude of  $0.25 V_{rms}$  and 0 V offset.

The alternating current (ac) conductivity ( $\sigma$ , admittance) was calculated using Eqn.1:

$$\sigma = \frac{Z'}{Z'^2 + Z''^2} \cdot \frac{t}{A} \quad (\text{Eqn.1})$$

where  $Z'$  and  $Z''$  are the real and imaginary parts of the impedance,  $A$  is the sample area and  $t$  is the sample thickness. The real part of the relative permittivity ( $\epsilon_r$ ) was calculated using Eqn. 2:

$$\epsilon_r = -\frac{Z''}{Z'^2 + Z''^2} \cdot \frac{t}{\omega \cdot A \cdot \epsilon_0} \quad (\text{Eqn.2})$$

where  $\omega$  is the angular frequency and  $\epsilon_0$  is the permittivity of free space ( $8.8541 \times 10^{-12} \text{ Fm}^{-1}$ ).

**Experiments:** Two types of experiments were performed and used 22 nails in total. A first group of experiments used FT-IR to compare the effects caused on the nail by iontophoresis, hydration and by the penetration enhancer, N-acetyl-L-cysteine. These experiments used a total of 18 nails collected from four volunteers. The second group of experiments used impedance spectroscopy to examine the effects of iontophoresis, N-acetyl-L-cysteine and hydration on the frequency dependent conductivity and permittivity. Four nails collected from two volunteers were used for these experiments.

**ATR-FTIR experiments** compared the effects of hydration, iontophoresis and treatment with the penetration enhancer, N-acetyl-L-cysteine. These experiments comprised two series of studies. In the first, the nails were initially hydrated for 10 minutes in deionised water and an ATR-FTIR spectrum of each was taken as detailed above. In most cases, five spectra were taken from various positions on the same nail. The nails were then divided randomly into three groups of 6, which were examined as detailed in Table I. In the second series, range-finding experiments were conducted similarly to those just described with modification of either the intensity or the duration of current application, as outlined in Table I.

**IS experiments** were likewise performed in two discrete series. In the first, three nails underwent sequential steps of hydration, iontophoresis and N-acetyl-L-cysteine treatments. After each step, the

nails were dried at 45°C and then rehydrated during which impedance sweeps were recorded. The protocol followed was: (a) The nails were hydrated for 10 minutes in deionised water and maintained at room temperature for 1 hour. They were then sandwiched between the two halves of the iontophoresis cells and rehydrated (154 mM NaCl) from dry with impedance sweeps from 1 Hz - 1 MHz being taken every 5 minutes for the next 15 hours. (b) Immediately after and while still hydrated, the nails were submitted to 45 minutes of iontophoresis at 0.1 mA (154 mM NaCl). Then, and again keeping the nails hydrated, impedance sweeps from 1 Hz - 1 MHz were performed every 5 minutes for 1 hour. The nails were next removed from the cells and dried at 45°C for 20 hours. The nails were subsequently replaced in the cells and fully hydrated once more with impedance sweeps from 1 Hz - 1 MHz again recorded every 5 minutes for 15 hours. Finally, the nails were removed from the cells. (c) Still hydrated, the nails were soaked in 10% w/v N-acetyl-L-cysteine for 5 hours and then placed in deionised water for 2 hours (with the water refreshed at 20 and 60 minutes) to remove the penetration enhancer. Afterwards, the nails were dried at 45°C for 5 hours, then replaced in the cells and hydrated with impedance sweeps from 1 Hz - 1 MHz performed every 5 minutes for 15 hours.

The second series of experiments addressed the fact that the previous protocol had applied iontophoresis to nails which have been extensively hydrated, whereas in practical applications much shorter periods (than the 15 h used before) are likely. Two nails were therefore subjected to iontophoresis using a modified procedure as follows: (i) The nails were sandwiched between the two halves of the iontophoresis cells and hydrated for 10 minutes in water with an impedance sweep (from 1 Hz to 1 MHz) taken every minute. (ii) Iontophoresis was then applied immediately for 45 minutes at 0.1 mA. Impedance spectroscopy was undertaken as soon as iontophoresis stopped with sweeps every five minutes (1Hz-1MHz) for 15 hours. (iii) The nails were removed from the cells, dried at 45°C for 20 hours, replaced in the cells and then rehydrated with further impedance sweeps from 1 Hz - 1 MHz performed every 5 minutes for the next 15 hours.

**Statistics:** The data are presented as mean  $\pm$  standard deviation of the sample (SD). Five two-way ANOVAs on the factors "treatment" and "nail" compared the frequencies assigned to the  $-\text{CH}_2$  symmetric,  $-\text{CH}_2$  asymmetric vibrations and to the amide I, II and III peaks before (control, 10

minutes hydration) and after treatment (iontophoresis edge, iontophoresis middle, penetration enhancer, hydration). The level of statistical significance was fixed at  $p \leq 0.05$ . The shifts in the peak maxima for the  $-\text{CH}_2$  symmetric,  $-\text{CH}_2$  asymmetric vibrations and for the amide I, II and III bands were calculated as:

$$\text{Frequency shift (cm}^{-1}\text{)} = \text{Peak maxima}_{\text{post treatment}} - \text{Peak maxima}_{\text{control, 10 minutes hydration}}$$

using each nail as its own control. Subsequently, the frequency shifts were compared by five one-way ANOVAs followed by the corresponding Bonferroni's multiple comparison tests. The level of statistical significance was also fixed at  $p \leq 0.05$

## Results

**ATR-FTIR experiments:** Representative ATR-FTIR spectra of a dry nail, a control nail (after 10 minutes hydration), of treated nails and of the enhancer N-acetyl-L-cysteine are shown in Figure 1. Nails had to be hydrated for a short while before application of iontophoresis (10) so that they became sufficiently flexible to fit into the diffusion cells and, in this work, to facilitate contact with the ATR-FTIR crystal. While it was possible to scan dry nails, the ATR-FTIR spectra obtained in this way have insufficient quality, probably due to poor contact. However, nails became considerably hydrated within 10 minutes, and it has been reported that this uptake can be as much as 0.3-0.5 g  $\text{H}_2\text{O}$  / g dry tissue (21,24,25). Wessel et al. (24) used NIR-FT-Raman to investigate the kinetics of water penetration into nails and reported a saturation of water uptake after 10 minutes. Martinsen et al. (21) also reported a rapid change in the electrical properties of the nail when the relative humidity was increased from 54% to 68%, with the effects reaching a plateau after ~1 hour. Consequently, to discriminate the effects of iontophoresis from those due to the 10 minute hydration, and thereby enable a better comparison between the three treatments, all nails were submitted to the same 10 minutes hydration time. Similarly, all nails were submitted to a 10 minute hydration post-treatment before recording the ATR-FTIR spectra to bring all the samples to comparable hydration conditions and close to those in which the control measurements had been made. Therefore, there is a

possibility that any reversible effects of current and N-acetyl-L-cysteine are no longer detectable after this 10 minute hydration.

Figure 2 shows the peak maxima ( $-\text{CH}_2$  symmetric,  $-\text{CH}_2$  asymmetric and amide I) determined for individual nails, before and after treatment, and illustrates the level of inter- and intra- nail variability observed in these experiments. Table II shows, for the first series of IR experiments, the averaged wavenumbers of the  $-\text{CH}_2$  symmetric,  $-\text{CH}_2$  asymmetric vibrational peak frequencies, as well as those of the amide I, II and III signals before and after treatment. The series of two-way ANOVAs indicated that the factor “nail” (i.e., the inter-nail variability) was always significant ( $p < 0.05$ ) with the only exception being the “amide II” for the edge- and exposed- iontophoresis groups. In fact, the inter-nail variability explained 61-81% of the variance associated with the lipid peaks, 40-91% for the amide I and III peaks, and 23-62% for the amide II peaks. The effects of N-acetyl-L-cysteine, hydration and the passage of current (through exposed areas) were significant ( $p < 0.05$ ) in some cases. As expected, iontophoresis caused no changes to the “edge” area of the nails not directly exposed to current passage. Both the  $-\text{CH}_2$  symmetric and asymmetric absorbances shifted significantly ( $p < 0.05$ ) to higher wavenumbers with hydration and when treated with the penetration enhancer; on the other hand, iontophoresis only increased significantly ( $p < 0.05$ ) the frequency of the asymmetric  $-\text{CH}_2$  vibration. The three treatments significantly ( $p < 0.05$ ) shifted the amide I peak to a lower wavenumber whereas hydration and N-acetyl-L-cysteine significantly ( $p < 0.05$ ) increased the frequency of the amide II and III peaks. Table III and Figure 3 show the results of the second series of IR experiments; the only significant change ( $p < 0.05$ ) was a shift to higher wavenumbers for the asymmetric  $-\text{CH}_2$ .

Figure 4 shows the frequency shifts caused by current passage, N-acetyl-L-cysteine and hydration in the five regions of interest. The five one-way ANOVAs found no significant differences among the treatments with the exception of a greater shift ( $p < 0.05$ ) caused by the enhancer on the amide II band.

**IS experiments:** The changes in ac conductivity and relative permittivity as a function of frequency for a representative nail sample (Nail 1) during its complete hydration in the first set of IS experiments are shown in Figures 5A and 5B, respectively. Both parameters increased with time,

particularly at low frequency, and most change occurred within the first hour of hydration, followed by a more gradual evolution thereafter. The ac conductivity (Figure 5A) is almost frequency independent at low frequencies ( $<10^2$ Hz); however, at higher frequencies ( $>10^3$  Hz), it increases essentially linearly, following ‘universal’ power law behaviour (26) such that,

$$\sigma(\omega) = \sigma(0) + A\omega^n$$

where  $\omega$  is angular frequency,  $n$  is a constant ( $0 < n < 1$ ),  $A$  is a constant and  $\sigma(0)$  is the low frequency (dc) conductivity. A similar power law dispersion is observed for the relative permittivity (Figure 5B), which can be expressed as:

$$\varepsilon(\omega) = D\omega^{n-1} + \varepsilon_\infty$$

where  $D$  is a constant and  $\varepsilon_\infty$  is the limit of permittivity at high frequency. For the nail plate there is a rapid decrease of the relative permittivity with frequency and  $\varepsilon(\omega)$  approaches a constant value at high frequency ( $\sim 10^6$  Hz in Fig 5B).

Figure 6 shows single impedance sweeps for three nails, including Nail 1, at different steps in the first series of IS experiments. The ac conductivity increased following iontophoresis, but slowly returned towards the value measured after full hydration (Figure 6A); the permeation enhancer caused a larger increase in the conductivity at low frequency.

The change in relative permittivity with iontophoresis (Figure 6B) was less significant than the change in conductivity. However, the change after treatment with the permeation enhancer was greater. The magnitude of the change was larger at low frequencies and then converged at higher frequencies ( $\sim 10^6$  Hz). Under most of the conditions studied, there were two inflections of the permittivity-frequency curves, seen at approximately 1 kHz and 30 kHz, while the post-permeation enhancement curves show inflections at approximately 20 kHz and 200 kHz.

Figure 7 shows the change in nail impedance at a fixed frequency (10 kHz) as a function of time of hydration for two different nails. The two curves correspond to the 10 minute period of hydration, to the IS sweep after iontophoresis, and to the second hydration in the second series of IS experiments. The gap (solid line) in the data coincides with the period of iontophoresis when it

was impossible to collect impedance data. It can be seen that the rate of hydration was broadly similar whether iontophoresis had occurred before or not.

## Discussion

The peak frequencies of the symmetric and asymmetric  $-\text{CH}_2$  vibrational bands were similar to those reported previously for human nail clippings (17) and for human stratum corneum (27,28); those of the amide peaks also agreed well with earlier observations (17-19). In one published case, however, the amide II absorption was not seen in the IR spectra of some healthy nails (but did appear in those taken from diabetic patients) (19), perhaps due to the fact that the nails were ground and combined with KBr into a pellet before recording the spectra. Alternatively, this may simply be an extreme reflection of the inter- and intra-nail variability observed in the experiments of the current work: all IR peaks investigated showed significant variation between nails from different volunteers and between positions on the same nail from a single individual (Figure 2).

Tables II and III, and Figure 4, show the changes in the IR spectra of nail clippings caused by current passage, N-acetyl-L-cysteine and hydration. In the case of iontophoresis, significant ( $p < 0.05$ ) frequency shifts were only detected in that part of the nail directly exposed to current passage; the “edge” data therefore provide additional control measurements. The results of the simple hydration experiment permitted the effects due to current passage and to the penetration enhancer to be discriminated since both of these treatments also involved exposure of the nail to aqueous solutions for prolonged times.

In general, hydration, iontophoresis and penetration enhancer treatment each increased (modestly, but with statistical significance) the  $-\text{CH}_2$  stretching frequencies; only the shifts recorded in the nail “edge” control experiments were negligible. In stratum corneum (SC), such ‘blue’ shifts have been associated (following carefully designed IR experiments coupled with sensitive differential scanning calorimetry) with increased intercellular lipid acyl chain disorder (29). The effects of iontophoresis on the  $-\text{CH}_2$  vibrational bands from the nail have not been reported before, but the small increases in peak frequencies observed are in agreement with those measured in

human SC *in vitro* and *in vivo* after application of current densities up to 0.5 mA/cm<sup>2</sup> (27,28,30). Nevertheless, it is noteworthy that the impact of iontophoresis on the –CH<sub>2</sub> vibrational bands was no different from that of either hydration alone or treatment with N-acetyl-L-cysteine (Table II, Figure 4), suggesting that all the changes observed were simply the result of hydration. However, even this interpretation has been questioned when similar results from the SC were obtained. Hydration significantly increases the broad O-H vibrational band centred around 3300 cm<sup>-1</sup> and this has the effect of displacing the –CH<sub>2</sub> signals, which sit on the shoulder of the O-H absorbance to higher wavenumbers (an effect demonstrated when the SC was hydrated with D<sub>2</sub>O rather than H<sub>2</sub>O) (31). In short, therefore, the perturbation of the relatively low lipid content of the nail by hydration, iontophoresis and penetration enhancer treatment is at most rather small and unlikely, it appears, to play much of a role in terms of altered permeability.

The amide I band, primarily due to the stretching vibration of the carbonyl functional group, typically appears in the range 1600-1690 cm<sup>-1</sup> depending on the protein backbone conformation and the hydrogen bonding pattern (32). This band is significantly modified by the presence of water (30,32,33). The amide II band (1480-1575 cm<sup>-1</sup>) primarily reflects in-plane N-H bending (40-60%) and C-N stretching vibrations (18-40%) (32), and is less sensitive to water than the amide I band (30,32,33). Finally, the origin of the amide III band (1229-1301 cm<sup>-1</sup>) is more complex and depends, for example, on the nature of amino acid side-chains and hydrogen bonding (32). Hydration and especially penetration enhancer treatment significantly decreased the absorbance maximum of the amide I band and significantly increased those of the amide II and amide III bands; iontophoresis also decreased amide I but had no significant effect on the other two (Table II, Figure 4), a finding which is consistent with previous IR measurements on SC protein post-current passage (27,30), and with earlier work on human nails using near-IR FT-Raman spectroscopy that revealed only minor changes in protein-associated bands post-hydration (24). The effects of N-acetyl-L-cysteine on the amide signals are the most notable of the treatments considered in the current work, in accord with this enhancer's ability to break disulphide bonds and to provoke conformational changes and increased water access to additional sites in the protein (6,7,8). Corroborating this

deduction is recent evidence from scanning electron microscopy and mercury intrusion porosimetry (9) that N-acetyl-L-cysteine increases the apparent surface porosity of human nails to a significantly greater extent than simple hydration.

The type of universal frequency response observed in the impedance spectroscopy experiments summarised in Figure 5 is typical of a resistor-capacitor (R-C) network (34). The nail can be considered as a complex network of conductive (R) and capacitive (C) sites. For biological materials such as skin and nail, the conductive sites can be considered as current pathways (35) and the capacitive sites are non-conductive regions; in skin, the lipid milieu has been suggested as a source of reactive (capacitive) contributions (15). If the frequency response of the individual components in the network is considered, the conductivity of the resistors ( $R^{-1}$ ) are frequency independent while the conductivity (admittance) of the capacitors ( $\omega C$ ) increase linearly with frequency. At low frequencies,  $R^{-1} \gg \omega C$  and currents flow preferentially through the conductive sites and the ac conductivity is frequency independent, as observed at frequencies below  $10^2$  Hz in Figure 5A. As the frequency is increased, the capacitor admittance becomes increasingly more dominant and, when  $\omega C \gg R^{-1}$ , a frequency dispersion is observed (see frequencies above  $10^4$  Hz in Figure 5A). The end result is that the ac conductivity rises with fractional powers of frequency, as in Figure 5A, and the network capacitance falls (Figure 5B). When the nail is hydrated, and the water content increases, the fraction of conductive sites increases and the frequency-independent conductivity increases in magnitude (Figure 5A). The greater degree of hydration also leads to the frequency dispersion moving to higher frequencies, which must be achieved before the capacitive sites can contribute to conductivity (i.e., the condition  $\omega C \gg R^{-1}$ ).

The nail also exhibits a higher relative permittivity with increasing hydration, as has been noted before (21,36), particularly at low frequencies ( $< 10^2$  Hz) where very high values of  $\epsilon_r$  (in excess of  $10^4$ ) are achieved. These high relative permittivity values at low frequency are likely to be a result of significant conductivity in the material and have been observed in other conductor-insulator systems (34,37), including a number of biological tissues (38). At high frequencies, where currents flow preferentially through the capacitive sites, the relative permittivity eventually converges

to a low value ( $\sim 30$ ), which is independent of the level of hydration. This suggests that, while hydration increases the overall conductivity of the conductive pathways in the nail, and thus both the dc conductivity and low frequency permittivity, it influences the higher frequency permittivity to a lesser extent. It has been proposed (21) that the capacitance is also dependent upon the mobility of the keratin chains.

The permeation enhancer produced a large increase in the conductivity at low frequency (Figure 6A) and a shift to higher frequency of the frequency-dependent region. The effect of iontophoresis is to increase the low frequency conductivity compared to its initial fully hydrated state, but the increase is relatively small in comparison to that of the permeation enhancer and the change from dry to fully hydrated. Comparing Figures 6A and 6B, it can be seen that iontophoresis increases the low frequency conductivity of nail but that it has fewer effects on lipid/protein domains associated with capacitance, in agreement with work on skin (14-16). Nail keratins are a complex mixture of hard and soft keratins, keratin filament-associated proteins and other proteins and the structure is stabilized via inter- and intra- molecular disulfide bonds, as well as hydrogen, peptide and other bonds (39). It has been suggested (24) that hydration loosens the nail structure, by modifying the van der Waal's forces, hydrogen bonding and ionic interactions between matrix and fibre proteins. In fact, water has been considered as a nail permeation enhancer (7). The treatment with N-acetyl-L-cysteine further disrupts the structure by breaking disulphide bonds and causes increased swelling (40). On the other hand, both hydration and N-acetyl-L-cysteine alter nail microstructure, increasing its porosity (9). This is consistent with ion transport being facilitated (i.e., increased conductivity) through the swollen, more porous structure as implied by the IS data since the fraction of conductive sites is increasing. The larger conductivity changes observed for the N-acetyl-L-cysteine treated nails indicates that the structure has been more perturbed which is in good agreement with previous findings (9) and with the IR data in Figure 4 and Table II. In the case of iontophoresis, ion transport is further facilitated and the nail conductivity at low frequency is slightly increased due to the increased concentration of ions in the membrane.

Both IS and IR data point out that iontophoresis effects are mostly caused through enhanced hydration of the nail. Further, the relatively quick return to baseline values observed 45 minutes post-iontophoresis (Figure 5) is in agreement with the recovery of transonychia water loss post nail iontophoresis observed *in vivo* (41). Similarly, both techniques suggest that N-acetyl-L-cysteine provokes a more intense disruption of nail structure. Finally, it should be stressed that the findings reported here concern nail clippings from healthy human volunteers; it follows that further research will be necessary to characterize the spectroscopic properties of diseased nails and the extent to which they are modified by the drug transport enhancement techniques employed.

## **Conclusions**

IR and impedance spectroscopies have been used to characterize the effects of hydration, iontophoresis, and N-acetyl-L-cysteine on human nail plate. The results from the two approaches were complementary and consistently demonstrated that (a) the impact of iontophoresis did not significantly differ from that of hydration alone, and (b) the penetration enhancer disrupted nail structure to a measurable extent. It follows, therefore, that IR and IS tools may prove useful in the development and optimisation of topical drug delivery systems to treat nail disease.

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**Table I:** ATR-FTIR experimental design

ATR-FTIR experiments	Procedures (number of replicate measurements)
<b>Series 1<sup>a</sup></b>	(a) Immerse in 154 mM NaCl for 1 hour (n = 6 nails x 5 sites/nail)
	(b) Iontophoresis in 154 mM NaCl at 0.1 mA <sup>b</sup> for 1 hour (n = 6 nails x 2-5 sites/nail)
	(c) Soak in 10% w/v PE <sup>c</sup> for 5 hours, wash for 2 hours <sup>d</sup> (n = 6 nails x 5 sites/nail)
<b>Series 2<sup>a</sup></b>	(a) Iontophoresis in 154 mM NaCl at 0.1 mA <sup>b</sup> for 0.5 hour (n = 1 nail x 2 sites)
	(b) Iontophoresis in 154 mM NaCl at 0.1 mA <sup>b</sup> for 2 hours (n = <sup>e</sup> 2 nails x 1 site)
	(c) Iontophoresis in 154 mM NaCl at 1.5 mA <sup>f,g</sup> for 1 hour (n = 1 nail x 2 sites )

<sup>a</sup>After each of these treatments, the nails were hydrated in deionised water for 10 minutes and FTIR spectra were recorded to determine the shifts in the position of the lipid-associated asymmetric and symmetric –CH<sub>2</sub> absorbances, and of the amide I, II and III peaks from protein.

<sup>b</sup>Equivalent to 0.5 mA cm<sup>-2</sup>.

<sup>c</sup>PE = N-acetyl-L-cysteine.

<sup>d</sup>Nails were washed twice in deionised water, which was refreshed twice at 20 and 60 minutes into the washing procedure.

<sup>e</sup>Two sections of a large thumbnail were used in two replicate experiments

<sup>f</sup>The maximum voltage output of the DC power supply was increased for the 1.5 mA experiment to allow precise delivery of a constant current.

<sup>g</sup>Equivalent to 7.5 mA cm<sup>-2</sup>.

**Table II:** Maxima (mean  $\pm$  SD; n=6 nails, 2-5 measurements per nail) of five IR spectral absorbances recorded in the first series of ATR-FTIR experiments (see Table 1). Upper values in italics are pre-treatment control measurements taken after a 10-minute period of hydration. Lower values are the results following each treatment.

Absorbance	<i>Control (edge)<sup>a</sup></i> 1h – 0.1 mA (edge) <sup>a</sup>	<i>Control (exposed)<sup>b</sup></i> 1h – 0.1 mA (exposed) <sup>b</sup>	<i>Control</i> 1h - Hydration	<i>Control</i> 5h – 10% w/v N-acetyl-L-cysteine
Asymmetric -CH <sub>2</sub>	<i>2918.8 <math>\pm</math> 1.0</i> 2918.8 $\pm$ 0.7	<i>2919.1 <math>\pm</math> 1.0</i> 2919.7 $\pm$ 1.2 <sup>c</sup>	<i>2920.0 <math>\pm</math> 1.4</i> 2920.4 $\pm$ 1.5 <sup>c</sup>	<i>2919.9 <math>\pm</math> 0.9</i> 2920.7 $\pm$ 1.2 <sup>c</sup>
Symmetric -CH <sub>2</sub>	<i>2850.8 <math>\pm</math> 0.7</i> 2850.6 $\pm$ 0.6	<i>2851.1 <math>\pm</math> 0.7</i> 2851.4 $\pm$ 0.8	<i>2851.4 <math>\pm</math> 0.8</i> 2851.7 $\pm$ 0.8 <sup>c</sup>	<i>2851.5 <math>\pm</math> 0.6</i> 2851.8 $\pm$ 0.7 <sup>c</sup>
Amide I	<i>1641.3 <math>\pm</math> 2.7</i> 1641.4 $\pm$ 0.6	<i>1641.4 <math>\pm</math> 3.4</i> 1639.9 $\pm$ 3.1 <sup>c</sup>	<i>1636.5 <math>\pm</math> 4.6</i> 1635.5 $\pm$ 4.5 <sup>c</sup>	<i>1636.1 <math>\pm</math> 2.5</i> 1633.4 $\pm$ 2.0 <sup>c</sup>
Amide II	<i>1536.8 <math>\pm</math> 0.3</i> 1536.6 $\pm$ 0.6	<i>1537.0 <math>\pm</math> 0.4</i> 1537.5 $\pm$ 0.3	<i>1536.4 <math>\pm</math> 0.9</i> 1536.9 $\pm$ 0.8 <sup>c</sup>	<i>1535.9 <math>\pm</math> 0.8</i> 1538.0 $\pm$ 1.3 <sup>c</sup>
Amide III	<i>1239.6 <math>\pm</math> 1.7</i> 1239.4 $\pm$ 1.5	<i>1240.8 <math>\pm</math> 2.0</i> 1241.7 $\pm$ 2.3	<i>1239.38 <math>\pm</math> 0.8</i> 1241.2 $\pm$ 1.5 <sup>c</sup>	<i>1240.1 <math>\pm</math> 1.1</i> 1242.8 $\pm$ 2.1 <sup>c</sup>

<sup>a</sup>“Edge” scans were recorded on areas of the nail sandwiched between the two halves of the diffusion cell and were not exposed to current passage.

<sup>b</sup>“Exposed” scans were recorded from the area of nail across which current passage occurred.

\*Significantly different (P < 0.05) than the corresponding control.

**Table III:** Mean IR spectral absorbance maxima recorded in the range finding, second series of ATR-FTIR experiments (see Table 1). Top values in italics correspond to control FT-IR scans taken 10 minutes hydration. Bottom values correspond to FT-IR scans taken after the corresponding iontophoretic treatment. The scans were taken in the area of the nail exposed to current passage.

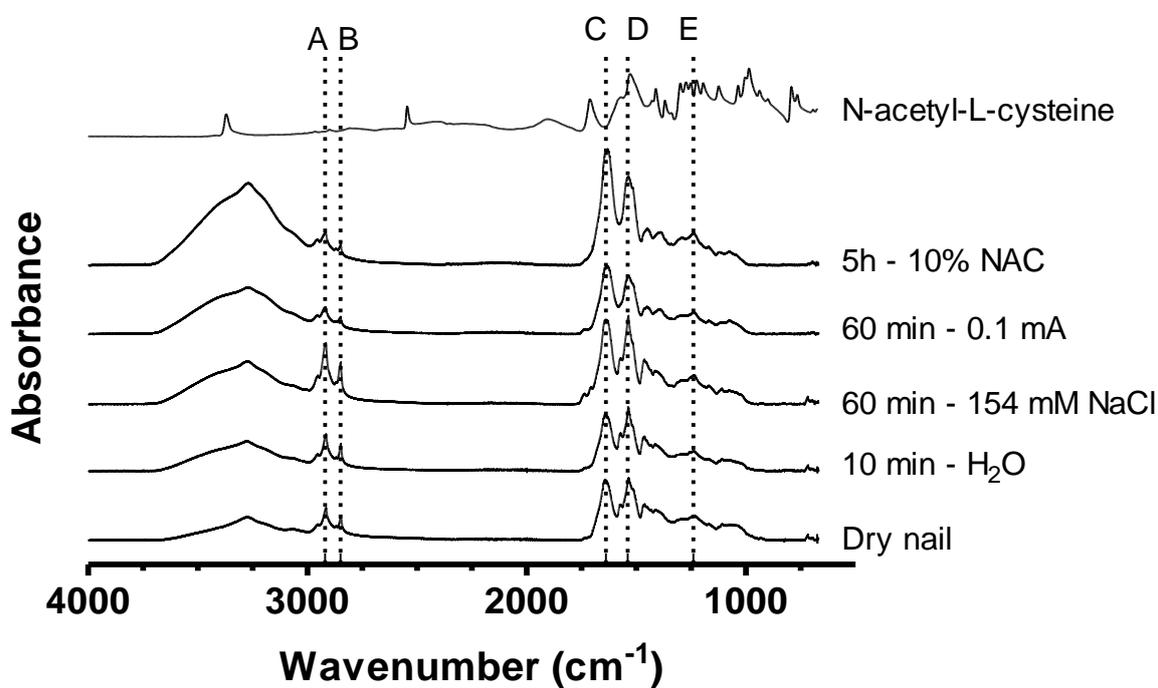
Absorbance	<sup>a</sup> <i>Control</i>	<sup>a</sup> <i>Control</i>	<sup>a</sup> <i>Control</i>
	<sup>b</sup> 2h - 0.1 mA	<sup>a</sup> 0.5h - 0.1 mA	<sup>a</sup> 1h - 1.5 mA
Asymmetric	<i>2919.4</i>	<sup>c</sup> <i>2918.8</i>	<i>2919.6</i>
-CH <sub>2</sub>	2920.4	<sup>c</sup> 2918.8	2921.3
Symmetric	<i>2851.3</i>	<sup>c</sup> <i>2850.8</i>	<i>2851.6</i>
-CH <sub>2</sub>	2851.8	2852.6	<sup>c</sup> 2852.3
Amide I	<i>1639.6</i>	<i>1639.9</i>	<i>1638.3</i>
	1640.2	1640.6	1633.0
Amide II	<i>1537.1</i>	<i>1537.1</i>	<i>1537.4</i>
	1537.1	1536.1	1537.5
Amide III	<i>1239.8</i>	<i>1242.6</i>	<i>1240.5</i>
	1238.8	1240.4	1242.3

<sup>a</sup>n = 1 nail x 2 sites.

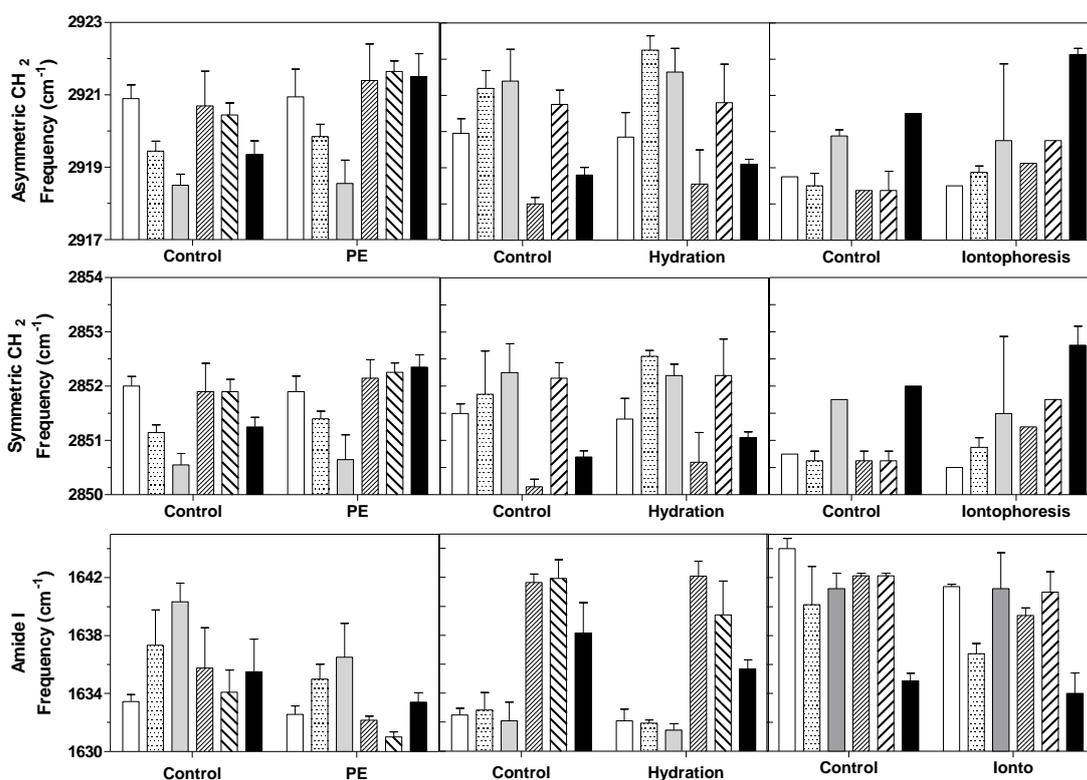
<sup>b</sup>n = 2 nails x 1 site.

<sup>c</sup>n = 1 nail x 1 site.

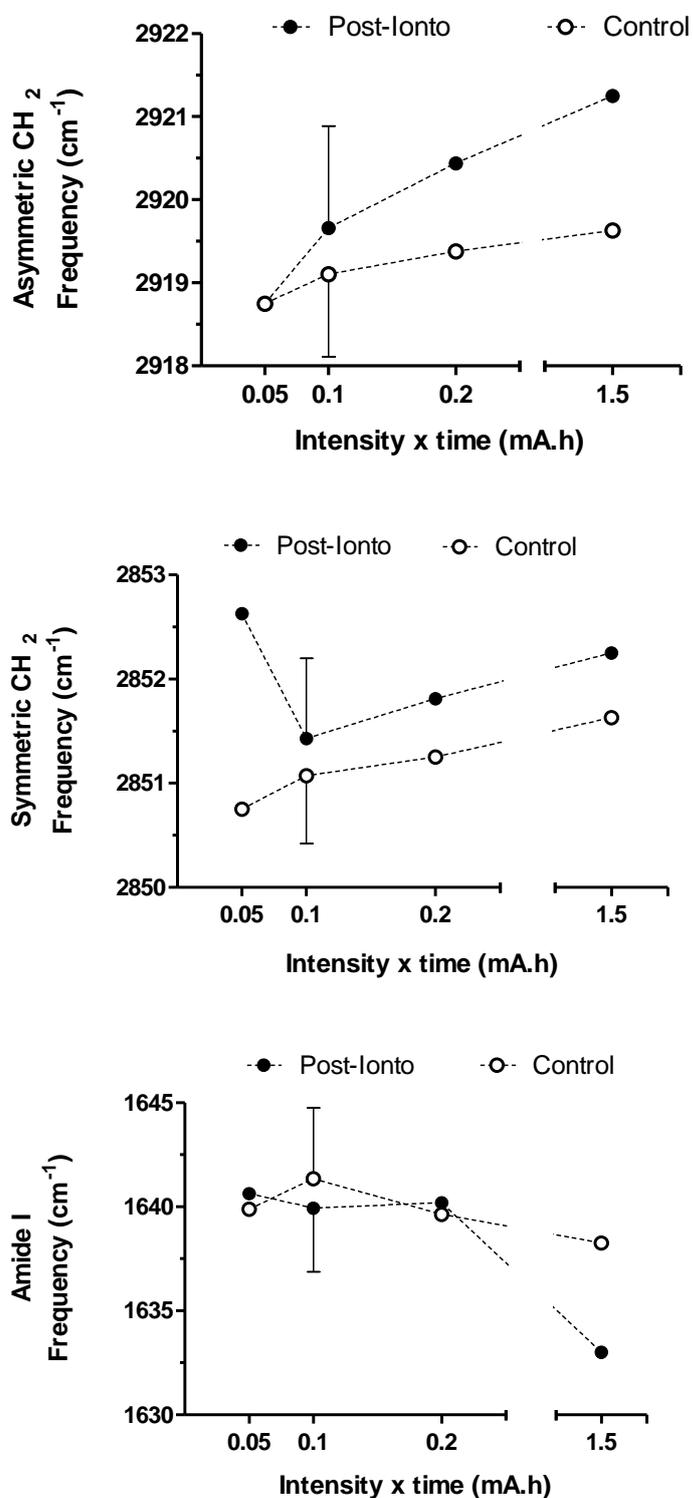
**Figure 1.** Representative ATR-FTIR spectra of [a] human fingernail clippings (dry, after 10 minutes hydration; 1 hour in 154 mM NaCl; 1h – 0.1 mA iontophoresis and 5 h in 10% N-acetyl-L-cysteine) and [b] pure N-acetyl-L-cysteine. Dotted lines indicate peak positions targeted: A: asymmetric  $-\text{CH}_2$  stretching; B: symmetric  $-\text{CH}_2$  stretching; C: amide I; D: amide II; E: amide III.



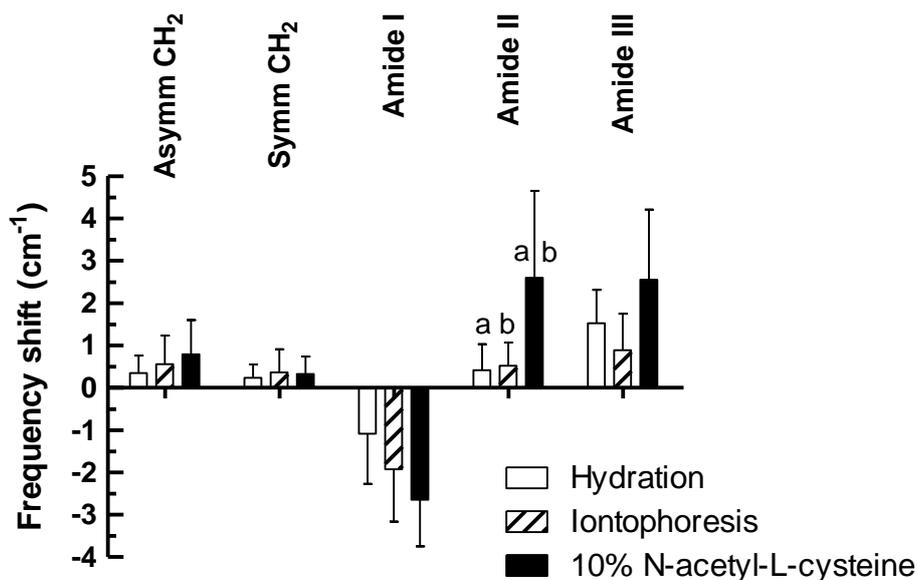
**Figure 2.** Inter- and intra- variability of the IR peak maxima assigned to the asymmetric  $-\text{CH}_2$  stretching; symmetric  $-\text{CH}_2$  stretching and amide I band for control (10 minutes hydrated) and treated nails: PE (exposure for 5 hours to a 10% w/v aqueous solution of N-acetyl-L-cysteine), hydration (1 hour in 154 mM NaCl) and iontophoresis (1 hour at 0.1 mA). Each bar corresponds to one nail; bars with the same pattern in each panel correspond to the same nail.



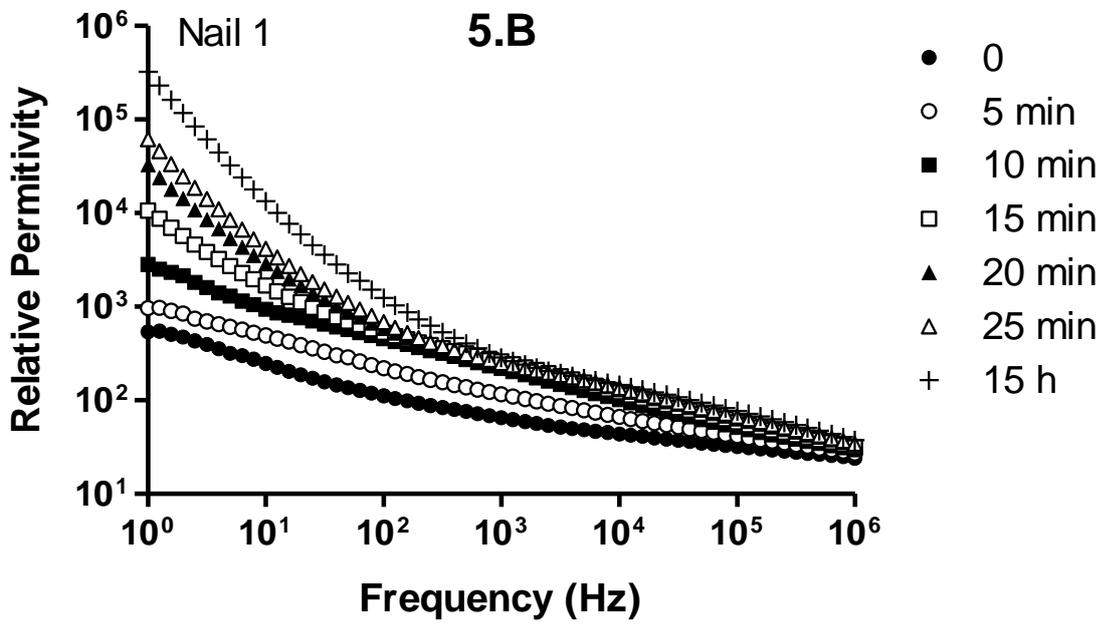
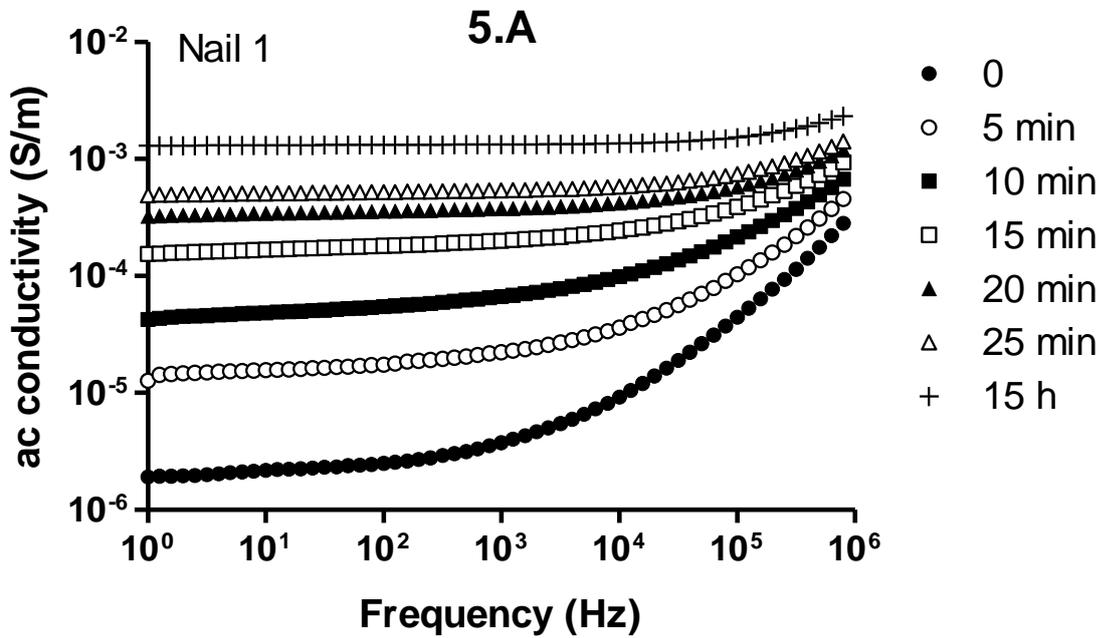
**Figure 3.** Maxima of  $-\text{CH}_2$  symmetric,  $-\text{CH}_2$  asymmetric and amide I peaks recorded from nails exposed to different iontophoresis 'doses' (expressed as intensity x time (mA·h)). The shifts were measured in the area of nail across which current passage occurred.



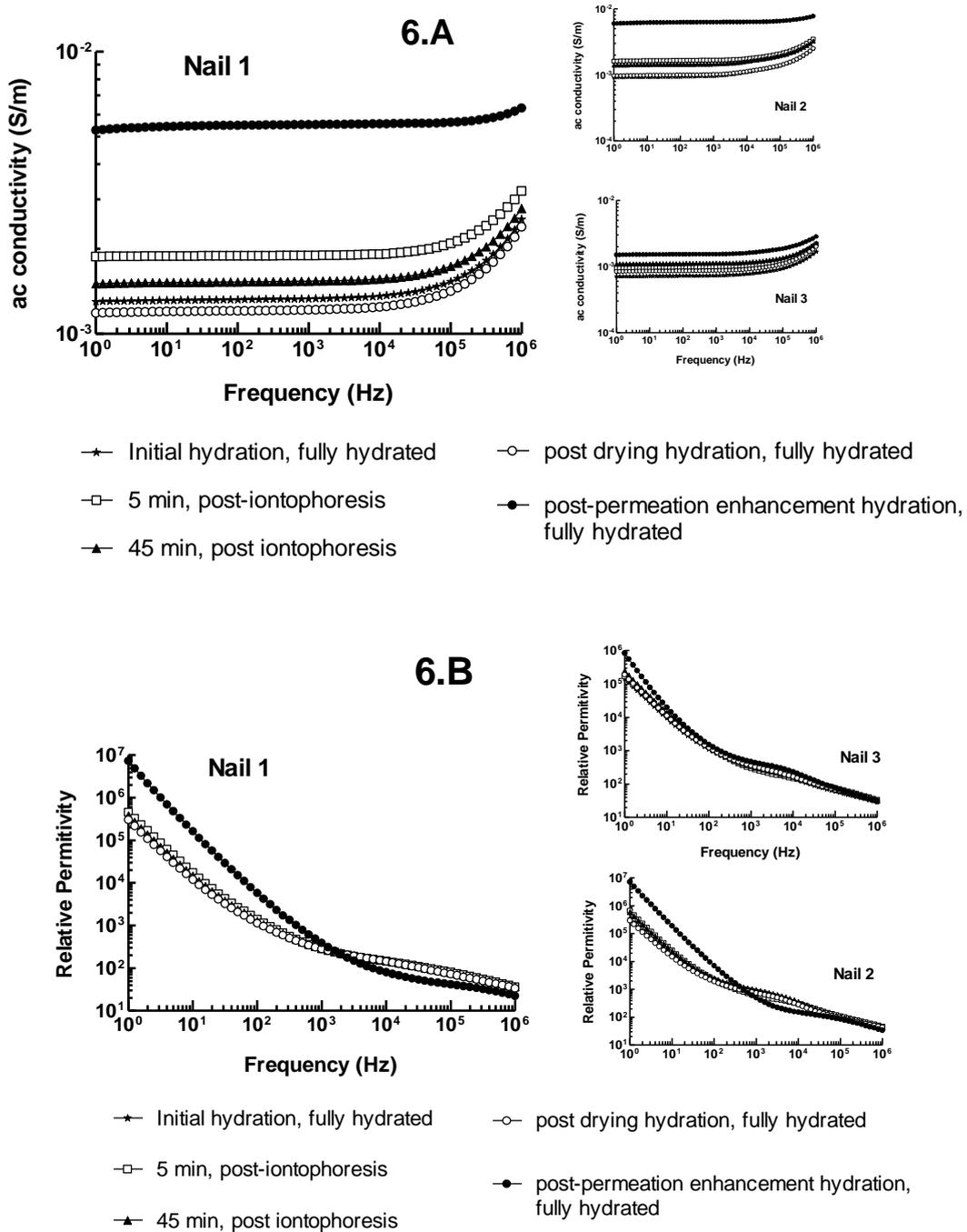
**Figure 4.** Shifts in IR peak maxima (mean  $\pm$  SD; n=6 nails) following (a) hydration for 1 hour in 154 mM NaCl, (b) iontophoresis for 1 hour at 0.1 mA, and (c) exposure for 5 hours to a 10% w/v aqueous solution of N-acetyl-L-cysteine. The control, pre-treatment values were determined on the same nails after 10 minutes hydration in water. (a) and (b) identify pairs of treatments causing a significantly ( $p < 0.05$ ) different shift.



**Figure 5.** Conductivity (A) and relative permittivity (B) of a nail (Nail 1) during hydration over a 15-hour period.



**Figure 6.** Conductivity (A) and relative permittivity (B) of three nails measured during the first series of impedance spectroscopy experiments.



**Figure 7.** Nail impedance at 10 kHz recorded for two nails during various stages of hydration during protocol the second series of impedance spectroscopy experiments.

