Lipid loses and barrier function modifications of the Brown to White hair transition

Journal:	<i>Skin Research and Technology</i>
Manuscript ID	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Oliver, Marc; Instituto de Quimica Avanzada de Cataluna, COSMETIC AND TEXTILE INNOVATIONS Martí, Meritxell; IQAC (CSIC), Chemical and Surfactants Technology Coderch, Luisa; IQAC, Carrer, Victor; Instituto de Quimica Avanzada de Cataluna Kreuzer, Martin ; ALBA Synchrotron Barba, Clara; IQAC (CSIC),
Keywords:	white hair, lipid distribution, hair treatment, barrier function, grey hair, spectroscopy

Lipid loses and barrier function modifications of the Brown to White hair transition

Modifications of the Brown-White hair transition

Marc Adrià Oliver¹, Meritxell Martí¹, Luisa Coderch¹, Victor Carrer¹, Martin Kreuzer² and Clara Barba¹

1. Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Jordi Girona 18-26, Barcelona, 08034, Spain

2. ALBA Synchrotron, Carrer de la Llum 2-26, 08290 Cerdanyola, Spain

Acknowledgements

The authors wish to thank the 2009 SGR 2012 (AGAUR) for providing support. FTIR microspectroscopy was performed on the MIRAS beamline at the ALBA Synchrotron in collaboration with ALBA staff. Additionally, the authors acknowledge the technical support provided by Isabel Yuste.

Abstract

BACKGROUND: The main objective of this study is to determine the lipid profile of brown and white Caucasian hair fibres and the effects of lipids on the properties of fibres. **MATERIALS AND METHODS:** To determine the structures of white and brown hair lipid bilayers, cross-sections of fibres of both hair types were examined using synchrotron-based μ -FTIR mapping. Dynamic vapour sorption analyses were also performed to determine the differences in the barrier function of both fibres. **RESULTS:** Spatial identification of lipids showed that a great amount of lipids was present in the medulla of fibres of both hair types, but important differences were also observed between cuticles of the different fibres. The cuticle of a white hair fibre showed a significant decrease in its lipid content, but did not show differences in the lateral packing order with respect to the cuticle of a brown hair fibre. The cortex and medulla of the white hair fibre also exhibited a significant decrease in its lipid content but with a higher lateral packing order than brown hair. Using dynamic vapour sorption analysis, it was found that the water dynamics of white hair fibres differed from those of brown hair fibres, showing a decrease in their total capacity to absorb water and an increase in the velocity of the exchange of water with the environment. **CONCLUSION:** The results of both techniques demonstrated a high correlation between the characteristics of the lipids located in the cuticle and the water dynamics of the fibres.

Keywords: white hair; lipid distribution; hair treatment; barrier function; grey hair; spectroscopy

1. Introduction

Hair has several protective and sensory functions, among others, in mammals, but in humans, hair plays an additional, important role in social interaction. Human hair fibres consist of three major regions: the cuticle, the cortex and the medulla. The outermost part of the fibre contains a thick protective covering called the cuticle. The cuticle consists of flat cells that overlap the core of the fibre and plays an important role in both chemical protection against external substances and in the functioning of the fibre barrier ($\pm 5 \mu\text{m}$ thick) (1). The main core of hair is the cortex, which contains most of the mass of the fibre (95% of the dry fibre weight). The cortex is mainly formed by keratin proteins, keratin-associated proteins and the matrix. The cortex plays an important role in providing strength and flexibility to the fibre and contains melanin in pigmented hairs (2). The medulla, which is not always present, is located in the inner part of the hair and is formed by loosely packed cells with a variable thickness of 5-10 μm . Some studies have detected the presence of lipids in the medulla, but the function of that region or the importance of the lipids has not been determined as of yet (3; 4).

Although the study of hair properties has largely addressed the fraction of protein within the fibres, only slight differences in the amino acid composition of hairs from humans of different ethnicities have been found, which cannot explain the physical differences that have been previously demonstrated (5). Recently, the importance of lipids within the hair fibre has become apparent (6), even though lipids comprise less than 9% of the fibre dry weight (7). Some studies (8; 9) have been conducted to identify the effects of lipids on the properties of fibres, which have found that lipids play a key role in maintaining adequate water permeability and the hydrophobic character of the fibre surface. These effects were primarily related to the lipid amounts and the order within the cuticle region. Changes in the lipid quantity or composition could modulate the dynamics of water in the fibre and thereby generate regions with distinct resistance (10).

Loss of colour or greying of hair is a phenomenon related to ageing and is one of its most obvious signs (11). The loss of melanin not only produces a loss of colour but also has relevance to hair's hygroscopic character and its affinity for metals (12). During age-related loss of colour, the lipid composition of hair may also be altered at the same time that its melanin content decreases, which may lead to a modification of the fibre properties. To date, studies of hair lipids have been performed mainly by comparing hairs derived from different ethnic groups or by exposing hair to external factors, but very few studies have focused on the processes underlying the loss of pigmentation (13). Despite the importance of lipids to the properties of hair, no studies have been performed that have profiled the lipids present in both pigmented and un-pigmented fibres. Differences in lipid content or in the fluidity of the lipids involved in the functioning of the barrier in hair fibres could explain the appreciable physical differences between pigmented and un-pigmented fibres (14).

The main objective of this study is to determine the lipid profile of brown and white Caucasian hair fibres and the effects of lipids on the properties of fibres. To this end, the properties of the lipids in

both types of fibres were evaluated using synchrotron-based Fourier transform infrared microspectroscopy (μ -FTIR). The combination of a conventional infrared spectrometer with the synchrotron infrared source and a microscope allows the spatial determination of the chemical composition of the samples, and several studies have been performed using μ -FTIR (15; 3; 16). Differences in the lipid order or quantity in both types of hair were examined. In previous studies, attenuated total reflection Fourier transform infrared (ATR-FTIR) was used to study the differences in the amounts of lipids and the lipid order in hair fibres (8). The ATR-FTIR has the inconvenience of the non-spatial resolution and the low penetrance in the sample, for these reasons the current study used a combination of transmittance microscopy FTIR with the ALBA synchrotron infrared light source. The μ -FTIR allows a higher spatial resolution and thereby permitted the analysis of the lipid fraction across the structure, enabling discern the amount and the organization of the lipids on the different layers of the hair fibre (17).

In addition, dynamic vapour sorption (DVS) was performed to determine the permeation characteristics of both types of hair fibres. DVS was used to measure differences in the humidity content, the binding energies of water to the different components of the fibre (GAB model) and the water diffusion/velocity during the exchange of water with the environment (D_A); all of these factors contribute to the functioning of the barrier of the fibre (18). The GAB model also provides the value of the water monolayer (W_m), which is the sorbed monolayer moisture content (g) per 100 g of dry sorbent and the energy (c_g), which is a constant that represents the difference between the free enthalpy of the water molecules in a pure liquid state and in the monolayer. This is proportional to the ratio of the attachment and the escape rate constants of the primary sites. K is the ratio between the standard vapour pressure of the liquid and the vapour pressure of the sorbate in the secondary (upper) layers, which is proportional to the ratio of the attachment and the escape rate constants for all higher layers. DVS was used frequently to analyse the water dynamics properties of hair fibres (9; 19; 8), but non previous comparison between white and brown hair was performed. The choice of both techniques was performed for their complementary approaches. DVS and μ -FTIR combination allow describe the interactions of the fibres with the ambient and describe the lipids in the region that modulate that exchange.

2. MATERIALS AND METHODS

2.1 Hair samples

The hairs used in this study were natural virgin brown Caucasian hair and white Caucasian hair, both without any pretreatment, that were supplied by De Meo Brothers (Passaic, New Jersey, USA). The hair samples were washed with 3% diluted Pantene Pro-V commercial shampoo (Procter & Gamble, USA) at a 1/30 (hair/solution) ratio, rinsed 3 times with water and dried at room temperature.

2.2 Synchrotron-Based Fourier Transform Infrared Microspectroscopy (μ FTIR) and Data Acquisition.

Hair tufts of 1 cm in diameter were embedded in Optimal Cutting Temperature (Oct) compound (Bright Instruments, Bedfordshire, UK) and immediately frozen in liquid N₂. Sample blocks were cut using a Cryostat CM3050 S (Leica Biosystems Nussloch, Germany) to 5 µm thick cross sections. The sections were placed on circular CaF₂ windows that were 1 mm thick and 13 mm in diameter (Crystran, Dorset, UK).

µFTIR was performed at the MIRAS beamline at the ALBA synchrotron (Cerdanyola del Vallès, Spain) (17) using a Hyperion 3000 microscope (Bruker, USA) equipped with a 36× magnification objective and a condenser coupled to a Vertex 70 spectrometer (Bruker, USA) and a 50 µm HgCdTe (MCT) detector that was continuously purged with N₂ gas. The measuring range was 4000–900 cm⁻¹, and the spectra collection was carried out in transmission mode at 4 cm⁻¹ resolution with an aperture with dimensions of 10 µm × 10 µm (minimum to avoid high level saturation of spectra), a step size of 10 µm and 128 coadded scans. Background spectra were collected from a clean area of the CaF₂ window after every 20 measured points. At least one map of each sample was made in parallel to isolated points from the cuticle cortex and medulla of more than 10 cross-sections of different fibres.

The OPUS software (Version 7.5, Bruker, USA) was used to obtain individual spectra from each region. Classification by precedence (cuticle, cortex or medulla) was performed manually by overlapping the optical microscope image with the measurement points. Due to the small width of the cuticle layer, all spectra from areas inside the hair and close to the border have been considered to be derived from cuticle regions. Cortex and medulla spectra were easily distinguished by their position within the cross-section. Spectra with high saturation in the lipid zone (3000–2800 cm⁻¹) were discarded, as well as spectra that contained no signal. Due to the greater coloration of Brown cross-sections, a greater number of spectra were discarded by saturation. Chemical maps were performed using the same software by calculating the second derivatives of the spectra using the Savitsky-Golay algorithm with a second polynomial order and 21 smoothing points to avoid saturated peaks of the map. The integrated areas of the lipid CH₂ peak symmetric stretching are shown (CH₂ Sym., 2860–2840 cm⁻¹) (20; 21).

Unscrambler X software (Version 10.5, CAMO Software, Norway) was used to perform the statistical analysis. Spectra were corrected using the baseline offset and linear baseline correction algorithms provided by the software (22), and the principal component analysis (PCA) was performed based on the spectral region of the CH₂ sym peak. All PCA were performed with cross validation test to check the statistical reproducibility of the analysis. Average spectra were calculated with the reduce algorithms provided by the software using the baseline corrected spectra. (22) Score and loading values, as well as the mean values, were used for data interpretation (21). Average spectra of both hair types were analysed using UnscramblerX software. Peak position and amplitude of each spectrum (685 in total) were determinate using Gaussian/Lorentzian fitting using a self-written python code.

2.3 Dynamic vapour sorption

Dynamic vapour sorption (DVS) was performed using a thermogravimetric balance Q5000SA Sorption Analyser (TA Instruments, New Castle, USA) with a controlled humidity chamber to measure vapour absorption and desorption. Experiments were conducted using 10 ± 1 mg of sample with a total gas flow of 200 ml/min at 25°C according to a previously described procedure (9). In brief, initial drying was performed at 60°C and 0% relative humidity (RH) overnight. Pre-stabilization was then performed at 25°C and 0% RH, followed by the measurement of the initial absorption kinetics at 5% RH and the determination of an *absorption curve* by subjecting the sample to a series of absorption tests that increased in steps of 10% until reaching 95% RH. Each test step lasted until the mass of sample reached equilibrium, which was arbitrarily defined as a change in mass of less than 0.02% per minute for 10 minutes. For the *desorption curve*, the sample that was previously stabilized at 95% RH after the conclusion of the absorption tests was subjected to a series of desorption tests that decreased in steps of 10% until reaching 5% RH. The sample was then stabilized at 5% RH. As in the absorption tests, the sample remained in each stage until its mass was fully equilibrated.

Mathematical models based on empirical and/or theoretical criteria that describe sorption isotherms have been previously published, and the GAB model is one of the most frequently used. Because of its theoretical basis, its parameters better describe the physical aspects of the sorption process compared to other empirical models. The monolayer moisture concept is the basis of the GAB model and provides a value for the monolayer moisture content of the material. In the present study, the sorption isotherm data were modelled according to the GAB model, which is consistent with previous studies. The coefficient of determination (R^2) was also evaluated to determine the goodness of fit of the model (18).

When moisture absorption/desorption tests are performed with hygroscopic samples, equilibration takes a very long time; however, the greatest interchange of water occurs during the initial steps. To shorten the testing time, some conditions were optimized that allowed the final regain (R_f) of each step to be used to calculate the apparent diffusion coefficients despite the fact that equilibrium was not reached.

The DVS measures differences in the humidity content, the binding energies of water in relation to different components of the fibre (GAB model) and the water diffusion/velocity when exchanging water with the environment (D_A); all of these factors contribute to the functioning of the barrier of the fibre. The regain measures the capacity of the fibre to absorb water, and the humidity content at 95% relative humidity ($R_{g95\%}$) represents the maximal amount of water that can be absorbed by the fibre.

The method applied by Vickerstaff (23) to study the diffusion of dyes within fibres was used to determine the diffusion coefficient and is represented by an expression derived from Fick's equation that is applied to moisture diffusion. Satisfactory results for the early stages of moisture absorption were obtained using this expression, similarly to results obtained for dye diffusion. The fraction of absorbed water plotted against the square root of the absorption time should produce

a straight line with a slope that represents the square root of the apparent diffusion coefficient, DA. The apparent diffusion coefficient is represented as min^{-1} divided by the sample's mass:

$$R(t)/R_f = \sqrt{D_A} \sqrt{t}$$

3. Results & Discussion

3.1 Synchrotron-Based Fourier-Transform Infrared Microspectroscopy (μ FTIR)

Detection of the vibrations of the alkyl chain within lipids and the resulting determination of the lipid conformational order and alkyl chain packing in both brown and white Caucasian hair was the main objective of this investigation. To this end, cross-sections of both hair types were evaluated using μ FTIR analysis. The two main peaks in the IR spectra that reflect the phase of the lipids are the CH_2 asymmetric stretching (CH_2 asym) and the CH_2 symmetric stretching (CH_2 sym) peaks, which had maxima at approximately 2920 and 2850 cm^{-1} , respectively; both of these peaks allow for the measurement of the lipid amounts and the conformational order and packing of the chains. In both peaks, a shift to higher wavenumbers indicated an increase in lipid disorder. The 2850 cm^{-1} peak is more sensitive, with values between 2847 – 2855 cm^{-1} ; a maximum at less than 2850 cm^{-1} indicates the presence of an orthorhombic (OR) lipid chain conformation, while a hexagonal (HEX) chain conformation produces a maximum between 2850 - 2852 cm^{-1} and a liquid crystalline (LIQ) chain conformation produces a maximum at a wavenumber greater than 2852 cm^{-1} . (24; 19).

The optical microscopic image of the brown hair cross-sections allowed for the observation of hair pigmentation, and the three hair layers, cuticle (the outermost dark layer), cortex and medulla (dark spot on the centre), were easily distinguished (Fig. 1a). Synchrotron-based FTIR measurements were used to obtain FTIR chemical maps of the hair regions. After conversion of the spectra within the map based on the second derivative, the integrated values of the CH_2 sym bands were used to qualitatively map the distribution of the lipids in the different regions (Fig. 1b). As observed in previous reports (3; 25; 26; 27), the highest concentration of lipids was found in the cuticle and medulla layers in brown Caucasian hair, while lower concentrations of lipids were found in the cortex (Fig. 1b-c).

Cross sections ($n=2$) of different brown hairs were studied simultaneously at identical single points within each region from different cross sections ($n=10$ per region). Measurements were taken with a $10 \times 10 \text{ }\mu\text{m}$ aperture to avoid supersaturation, having in mind that large aperture could mean additional noise on the narrow regions (cuticle and medulla). All of the spectra that were obtained were grouped based on the different hair regions from which they were obtained. The average spectra for each hair region were calculated in order to analyse the CH_2 sym peak (Fig. 1c). The average spectra from the medulla of brown hair exhibited the most intense CH_2 sym peak, followed by that from the cuticle and then the cortex, of brown hair, which had the lowest peak intensity, as it is consistent with reports from previous studies (25). A slight wavelength shift between the regions was also observed. Brown hair cuticles exhibited lower CH_2 sym peaks than

those obtained for the other regions, which indicated that lipids in the cuticle region had a higher conformational order. The analysis of the average spectra allowed for a greater understanding the overall features of each of the regions; however, due the complexity of the hair structure, a more specific analysis was required.

Spectra were classified according to their origin and analysed using principal component analysis (PCA). PCA is a powerful multivariate analytical approach that can be used to identify correlations between spectroscopic peaks by calculating the principal components (PCs) (28). The results of PCA analysis are represented in the form of score plot and the corresponding loadings (Fig. 1d,e). Each point (score) in the score plot corresponds to one spectrum.

PCA analysis of the CH₂ sym peaks derived from the spectra obtained from brown hair shows differences between the regions. The distribution of the points along the PC-1 axis confirms that there is a high difference (95%) in the amount of lipids in each of the three hair regions (Fig. 1d). The loadings plot for PC-1 shows that samples with positive scores for PC-1 have a correspondingly greater CH₂ sym peak intensity and, consequently, a higher lipid amount (15) (Fig. 1e). The highest amounts of lipids are present in the hair medulla, with the cortex of the hair layer containing fewer lipids. Despite the fact that the PC-2 axis explains only 4% of the differences between the spectra, the brown hair cuticle exhibits higher values than the other regions (Fig. 1d). Spectra with corresponding high values for PC-2 have peaks that are shifted to a lower wavenumber, as shown in the PC-2 loading plot (Fig. 1e), where negative values correspond to a wavenumber of 2857 cm⁻¹ and positive values correspond to a wavenumber of 2848 cm⁻¹. PCA demonstrates that the brown hair cuticle layer contains lipids with a higher conformational order, which was as also observed for the average spectra.

When observing the optical microscope images obtained from the white hair, a clear lack of pigmentation is found (non-presence of brown granules), and the three hair layers can be easily distinguished (Fig. 2a). The white hair chemical map (obtained from the integrated areas of the CH₂ sym. peak) shows a specific lipid distribution. The highest lipid concentration was found in the medulla, followed by the cuticle, which had an apparently heterogeneous distribution. The lowest concentration was found in the cortex (Fig. 2b).

Similarly, to brown hair, the spectra derived from the map (n=3) and from single points (n=20) from white hair samples were grouped by region (cuticle, cortex and medulla), and the average spectrum from each region was calculated in order to obtain the mean CH₂ sym peak (Fig. 2c). In accordance with the chemical map (Fig. 2b) the average spectrum for the white hair medulla exhibits a much higher CH₂ sym peak intensity compared with that from the other regions. However, the cuticle and cortex-derived spectra show similar CH₂ sym peak intensities (Fig. 2c). The difference between the chemical map and the average spectra could be due to the high number of spectra included in the average spectrum. Shifts in the peak positions were not observed.

When PCA was performed on spectra derived from white hair, it was found that in contrast to the analysis of the brown hair, the main differences between the regions were due to the lipid order. The samples of the three regions are separately grouped with respect to PC-1 (68%). The loadings plot shows that the differences were due to shifts of the CH₂ sym peak and therefore resulted from differences in the order of the lipids within the different regions (Fig. 2d, e). The results derived from the white hair samples showed differences in the conformational order of the lipids compared to those derived from brown hair samples. The lipids in the medulla had the highest conformational order, while the lipids in the cuticle had the lowest conformational order. The PC-2 component, which explains 22% of the variance between the spectra, indicates that the medulla is the layer with highest amount of lipids (Fig. 1e). Small differences in the lipid order were observed between the cortex and cuticle of the white hair, as indicated by small shifts in the positions of the peaks in the average spectra (Fig. 1c).

To further investigate differences in the conformational order of lipids between brown and white Caucasian hair, comparative analyses of the different hair regions were performed. In this report, only the results from the cuticle region are shown since the greatest differences were found in this region between both of the studied hairs. PCA of both cuticle regions (Fig. 3a, b) demonstrated the differences between them, as observed in the map analysis (Fig. 1a and 2a). The C-1 distribution (83% of sample variance), which described the differences in the lipid content, confirmed that cuticles from **brown** hair had greater lipid amounts; however, no differences were found in the order of the lipids between the **brown** and **white** cuticles (PC-2: 8% of sample variance).

As explained previously, the lipid order plays as important a role as does the lipid content. The fluidity of the lipids can modulate the rates of penetration and egress of substances. For that reason and with the aim of confirming the PCA results, the exact positions and amplitude of the CH₂ sym peaks for each spectra (685 in total) were analysed (Table I). The peak positions and amplitudes were obtained from Gaussian-Lorentzian fit of the CH₂ sym. peak spectra. A lower wavelength value indicated the presence of more highly ordered lipids, and higher amplitude values indicated a higher lipid amount (24).

The analysis of the **brown** hair showed that the cuticle had more highly ordered lipids (2851.32 cm⁻¹) than the interior regions (cortex 2852.01 cm⁻¹ and medulla 2851.99 cm⁻¹), despite non-significant differences were found. Small differences were found between the peak positions of the cortex and medulla. CH₂ sym amplitudes obtained for **brown** hair showed significant differences between the three regions, the highest amount of lipids was present in the medulla (0.28 a.u.) followed by the cuticle (0.21 a.u.) and finally the cortex (0.18 a.u.). These results confirmed the interpretation of the PCA analysis of the **brown** hair layers (Fig. 1d), demonstrating that the region within **brown** hair that has the most highly ordered lipids is the cuticle, while the greatest amount of lipids are found in the medulla and, to a lesser degree, the cuticle.

In addition, lipids in **white** hair cuticles exhibited a slightly lower order, based on the CH₂ sym values (2851.07 cm⁻¹) than in the cortex and medulla (2849.89 and 2850.56 cm⁻¹, respectively),

which was also apparent based on the PCA. The **white** hair medulla also has the highest amount of lipids, based on CH₂ sym amplitude (0.17 a.u.), in contrast to the cuticle and cortex (0.06 and 0.08 a.u., respectively). The values obtained for the lipid amounts and chain conformational order based on the average spectra derived from **white** hair are comparable with those obtained from the PCA on **white hair** (Fig. 2d).

This investigation found important differences between the two hair types. White hair demonstrated a clear reduction in the lipid content within all of the layers, this being more evident in the cuticle. On the other hand, the position of the CH₂ sym peaks revealed an increase in the conformational order of lipids within the inner layers of white hair compared brown hair; however, no differences were found in the outermost layers.

3.2 Water dynamics

To clarify the effects of the lipid differences that we found during the FTIR analysis between both hair types, the barrier functions of both types of hair were determined using water dynamics. The main differences observed between **brown** and **white** hairs in their cuticle regions could affect the barrier function (9; 19). Changes in the amounts and order of the lipids in the cuticle region may modify the water dynamics of the fibre, producing alterations in its behaviour in response to environmental changes and the penetration of exogenous substances (29; 30).

The DVS analyses of the hairs allowed for greater understanding of the dynamics of water absorption/desorption within the fibres. Differences in these parameters may be produced by variations in both physical (i.e., texture, damage) and chemical (i.e., composition, order) properties. Results of the GAB analyses for the two types of fibres are presented in Table II. **Brown** hair fibres had a lower degree of W_m, or water directly bound to the structure, when compared with **white** fibres (0.0772% and 0.0789, respectively). On the other hand, the energy constants (c_g and K) reflected the fact that the strength of water bonding was higher in the **brown** hair (Table II). The lower W_m value and higher energy constants found for **brown** hair may indicate a higher degree of attraction of water, which may lead to an increase in the number of water layers. One possible explanation could be the presence of melanin in **brown** hair, which may increase the attraction of water to the entire fibre due to its hygroscopic properties (31).

Based on the GAB results, the **brown** fibres also demonstrated a greater R_g95% level compared with **white** fibres (26.25 and 24.85, respectively), indicating that the pigmented fibre absorbs more water at higher levels of humidity. When variation of the regain value and humidity was compared between the types of fibres (Fig. 4a), the main differences were observed in the last steps of the absorption and desorption processes. These results indicate a higher maximum absorption and slower release of water for **brown** fibres. One factor that could lead to these differences is the presence of melanin; its hygroscopic properties may increase the attraction of water and produce a more highly hydrated fibre.

The level of water absorption may change the properties of fibres, not only in terms of swelling but also by changing their mechanical properties (32). Changes in the physicochemical properties of fibres could explain the differences in the regain values. The differences in the lipid content and distribution within the entire fibre as well as within the medulla that was observed from the μ FTIR analysis may also explain the differences in the regain values.

Another important parameter that may explain the water dynamics is the diffusion coefficient (D_a). The plot of the D_a values obtained experimentally showed a slight difference in the absorption process between the two types of fibres, with the maximal difference found in the Rg55% values (Fig. 4b), as has been observed in previous studies (9). The variation in the Rg55% between the hair types were appreciable based on the D_a values obtained during the entire process. The diffusion coefficients were always higher for **white** hair compared with **brown** hair. Diffusion can be related to the barrier properties of the fibre, particularly with those of the cuticle, which play key roles in the diffusion process. Changes in diffusion could be produced by changes in the amounts and order of the lipids in the cuticle. Another possible explanation was made by Bianchini *et al.* (13), who postulated that the presence of a larger medulla could create shorter paths for diffusion and thereby increase the D_a value.

The differences observed in the DVS analysis reflect differences in the behaviour of the two types of fibres. It should be noted that higher level of diffusion and a lower regain value at 95% RH was observed in **white** hair. These changes could be due to chemical or physical differences between the types of fibres. The μ FT-IR analysis revealed that **white** fibres had lower lipid amounts within all three regions, but mainly in the cuticle. Similar observations were made in previous studies (8), where cuticle lipids were extracted from brown Caucasian hair, which produced delipidised fibres with higher levels of diffusion and lower regain. The similarities between the behaviour of the white hair and delipidised fibres would confirm that the changes observed in the DVS analysis were due to differences in the lipid amounts within the cuticle.

4. Conclusion

The μ FT-IR technique has been previously demonstrated to be useful in the determination of differences in lipids between different hair types, which allowed for the comparison of the amounts and conformational order of lipids in different hair layers. The μ FT-IR analysis has demonstrated that **brown** hair possesses a greater amount of lipids in the medulla and the cuticle. This investigation has also determined that the conformational orders of the lipids in the inner layers of **brown** hair are decreased. When **white** hair was analysed, important differences between it and **brown** hair were found. The un-pigmented fibres were shown to have decreased amounts of lipids in all of the layers; this decrease is more critical within the cuticle. On the other hand, an increase in lipid chain packing in the cortex and medulla was observed. However, the cuticle of un-pigmented hair showed no differences in the conformational order of lipids compared with the pigmented cuticle.

Cuticles from white hair, containing lower amounts of lipids but having lipids with a similar order, were observed to have changes in the rate of the penetration of substances to the inner part of the fibre and to produce faster modifications to their properties upon changes in the relative humidity. DVS analysis indicated that the water dynamics of white fibres differed from those of brown fibres, resulting in a decrease in the total capacity to absorb water and an increase in the velocity of water exchange with the environment. The results of both techniques demonstrated a high correlation between the characteristics of the lipids located in the cuticle and the water dynamics of the fibres.

5. References

1. *Fine details on the surface of human hair.* **Swift, J. A.** 143-159, s.l. : International Journal of Cosmetic Science, 1991, Vol. 13.

2. *Transfer of melanin granules from melanocytes to the cortical cells of human hair.* **JA., SWIFT.** 203:976-7, s.l. : Nature, 1964, Vol. 29.

3. *Profiling lipids across Caucasian and Afro-American hair transverse cuts, using synchrotron infrared microspectrometry.* **L. Kreplak, F. Briki, Y. Duvault, J. Doucet, C. Merigoux, F. Leroy, J. L. Lvque, L. Miller, G. L. Carr and G. P. Williams and P. Dumas.** 2001, International Journal of Cosmetic Science, pp. 23, 369-374.

4. *Organic reagent interaction with hair spatially characterized by infrared microspectroscopy using synchrotron radiation.* **J - L . BANTIGNIES, G . FUCHS, G.L. CARR, G . P . WILLIAMS, D. LUTZ and S . MARULL.** 381-394, s.l. : International Journal of Cosmetic Science, 1998, Vol. 20.

5. *Human hair: A unique physicochemical composite.* **Leszek J., Wolfram.** 106-114, s.l. : Journal of the American Academy of Dermatology, 2003, Vol. 48.

6. *The influence of hair lipids in ethnic hair properties.* **Mart M, Barba C, Manich AM, Rubio L, Alonso C, Coderch L.** 2016 , Int J Cosmet Sci., pp. 38(1):77-84.

7. *Characterization of the lipid composition at the proximal root regions of human hair.* **Yoshinori Masukawa, Hirofumi Narita, and Genji Imokawa.** 1-16, s.l. : Journal of cosmetic science, 2005, Vol. 56.

8. *Exogenous and endogenous lipids of human hair.* **L. Coderch, M. A. Oliver, V. Martnez, A. M. Manich, L. Rubio, M. Mart.** 2017, Skin Res Technol, pp. 23(4):479-485.

9. *Moisture sorption/desorption of protein fibres.* **C. Barba, M. Mart, J. Carilla, A.M. Manich, L. Coderch.** 2013, Thermochimica Acta, pp. 70– 76.

10. *The ethnic differences of the damage of hair and integral hair lipid after ultra violet radiation.* **Ji JH, Park TS, Lee HJ, Kim YD, Pi LQ, Jin XH, Lee WS.** 54-60, s.l. : Ann Dermatol., 2013, Vol. 25.

11. *Aging of hair.* **RM, Trueb.** 60-72, s.l. : J Cosmet Dermatol., 2005, Vol. 4.

12. *Current Understanding of the Binding Sites, Capacity, Affinity, and Biological Significance of Metals in Melanin.* **Lian Hong, and John D. Simon.** 7938-7947, s.l. : J. Phys. Chem. B, 2007, Vol. 111.

13. *Grey hair: clinical investigation into changes in hair fibres with loss of pigmentation in a photoprotected population.* **Bianchini, P. D. Kaplan T. Polefka G. Grove S. Daly L. Jumbelic D. Harper M. Nori T. Evans R. Ramaprasad R.** 171-182, s.l. : International Journal of Cosmetic Science, 2011, Vol. 33.

14. *Chemical and physical properties of pigmented and non-pigmented hair ('grey hair').* **B. Hollfelder, G. Blankenburg, LJ Wolfram, H. Hcker.** 2, s.l. : International Journal of Cosmetic Science, 1995, Vol. 17.

15. *Synchrotron-based v-XRF mapping and -FTIR microscopy enable to look into the fate and effects of tattoo pigments in human skin.* **Ines Schreiber, Bernhard Hesse, Christian Seim, Hiram Castillo-Michel, Julie**

- Villanova, Peter Laux, Nadine Dreiaek, Randolph Penning, Remi Tucoulou, Marine Cotte & Andreas Luch.** s.l. : Scientific Reports, 2017, Vol. 7:11395.
16. *Elucidation of penetration enhancement mechanism of Emu oil using FTIR microspectroscopy at EMIRA laboratory of SESAME synchrotron.* **Mansour RSH, Sallam AA, Hamdan II, Khalil EA, Yousef I.** 1-10, s.l. : Spectrochim Acta A Mol Biomol Spectrosc, 2017, Vol. 185.
17. *MIRAS: The Infrared Synchrotron Radiation Beamline at ALBA.* **I. Yousef, L. Ribó, A. Crisol, I. Šics, G. Ellis, T. Ducic, M. Kreuzer, N. Benseny- Cases, M. Quispe, P. Dumas, S. Lefrançois, T. Moreno, G. García, S. Ferrer, J. Nicolas & M.A.G. Aranda.** s.l. : Synchrotron Radiation News, 2017, Vols. Vol. 30, No. 4.
18. *Modifications of the Brunauer, Emmett and Teller Equation.* **Anderson RB, Hall WK.** 1727-1734, s.l. : J Am Chem Soc., 1948, Vol. 70.
19. *Interrelationship between the hair lipids and the hair moisture.* **Nishimura, K., Nishino, M., Inaoka, Y., Kitada, Y. and Fukushima, M.** 134-139, s.l. : J. Cosmet. Sci. Soc. Jpn, 1989, Vol. 13.
20. *Vibrational Spectroscopic Investigation of the Phase Diagram of a Biomimetic Lipid Monolayer.* **Sylvie Roke, Juleon Schins, Michiel Müller, and Mischa Bonn.** s.l. : PHYSICAL REVIEW LETTERS, 2003, Vols. 90, 128101 .
21. *Distinguishing cell types or populations based on the computational analysis of their infrared spectra.* **Francis L Martin, Jemma G Kelly, Valon Llabjani, Pierre L Martin-Hirsch , Imran I Patel, Júlio Trevisan, Nigel J Fullwood & Michael J Walsh.** 1748-1760, s.l. : Nature Protocols, 2010, Vol. 5.
22. **AS, CAMO SOFTWARE.** [Online] [Cited: November 20, 2018.] <https://www.camo.com/downloads/user-manuals.html>.
23. *The Physical Chemistry of Dyeing.* **T, Vickerstaff.** s.l. : London: Oliver and, 1954.
24. *Determination of molecular conformation and permeation in skin via IR spectroscopy, microscopy, and imaging.* **Richard Mendelsohn, Carol R. Flach , David J. Moore.** 2006, Biochimica et Biophysica Acta, pp. 923–933.
25. *Fourier Transform Infrared Imaging of Human Hair with a High Spatial Resolution Without the Use of a Synchrotron.* **K.L.A. Chan, S.G. Kazarian, A. Mavraki, and D.R. Williams.** 149-155, s.l. : Applied Spectroscopy, 2005, Vols. Vol. 59, Issue 2.
26. *The use of synchrotron infrared microspectroscopy in biological and biomedical investigations.* **Paul Dumas, Lisa Millerb.** 3-21, s.l. : Vibrational Spectroscopy, 2003, Vols. Volume 32, Issue 1.
27. *Imaging capabilities of synchrotron infrared microspectroscopy.* **P. Dumas, N. Jamin, J. L. Teillaud, L. M. Millerd and B. Beccard.** s.l. : Faraday Discussions, 2004, Vol. 126.
28. *Understanding the molecular information contained in principal component analysis of vibrational spectra of biological systems.* **Bonnier, F. & Byrne, H. J.** 322–32, s.l. : Analyst, 2012, Vol. 137.
29. *Keratins and lipids in ethnic hair.* **Cavaco-Paulo, C. F. Cruz M. M. Fernandes A. C. Gomes L. Coderch M. Martí S. Méndez L. Gales N. G. Azoia U. Shimanovich A.** 244-249, s.l. : International Journal of Cosmetic Science, 2013, Vols. Volume35, Issue3.
30. *Determination of physicochemical properties of delipidized hair.* **Roger L. McMullen, Donna Laura, Susan Chen, Donald Koelmel, Guojin Zhang, and Timothy Gillece.** 355-370, s.l. : Journal of cosmetic science, 2013, Vol. 64.
31. *The physical and chemical properties of eumelanin.* **Meredith P, Sarna T.** 2006, Pigment Cell Res., pp. 19(6):572-94.
32. *Caucasian Hair, Negro Hair, and Wool: Similarities and Differences.* **J. MENKART, Ph.D., L..1. WOLFRAM, Ph.D., and IRENE MAO, M.D.** 1966, J. Soc. Cosmetic Chemist, pp. 17, 769-787.

Table I Whavelengt values and amplitude with SD of the CH₂ symmetric stretching peak (CH₂ Sym.) from Brown and White Caucasian hair fibers in the Cuticle, Cortex and Medulla. Abbreviations: λ=Wavelength, a.u.= Amplitude in arbitrary units

CH ₂ Sym.				
	Brown		White	
	λ	a.u.	λ	a.u.
Cuticle	2851.32±0.85	0.21±0.09*†	2851.07±2.23†	0.06±0.03*†
Cortex	2852.01±0.61*	0.18±0.08*†	2849.89±1.25*†	0.08±0.03*†
Medulla	2851.99±0.28*	0.28±0.07*†	2850.56±0.90*	0.17±0.07*†

* Student's t test p<0.05 between same zone of Brown and White hair; † Student's t test p<0.05 between different zones of the same sample

Table II. DVS gravimetric analysis of Brown and White fibers. Maximum moisture regain at 95% RH (RG95%) , GAB monolayer capacity (Wm), GAB energy monolayer constant (Cg), GAB deeper layers constant (K), GAB determination coefficient (R^2), total time to reach equilibrium (t), apparent diffusion coefficient (Da) and apparent diffusion coefficient at 55% of RH (Da 55RH) for Caucasian Brown and White hair fibers.

DVS	Brown	White
RG95%	26.25	24.85
Wm (%)	0.0772	0.0789
Cg	5.915	5.813
K	0.7367	0.72095
R^2	0.9980	0.9986
t(min)	3317.6	3236.0
Da (min ⁻¹ x10 ⁻³)	20.46	21.04
Da 55RH	24.1	25.1

Fig. 1 Distribution and order analysis of Brown Caucasian hair cross-sections using μ -FTIR. (a) Cross-section observed by optical microscopy of Brown Caucasian hair selected to analyze by μ -FTIR, regions were manually determined. (b) Chemical map of second derivative obtained at 2850cm^{-1} (CH_2 symmetric stretching). (c) Average spectra at 2850cm^{-1} for each region (d) PCA score plot of PC-1 vs. PC-2 with boxplot for each PC in the opposite site. (e) Loading plots of PC-1 and PC-2 of PCA. Boxplot represents 25/75% quintile, whisker 5/95% quantile and central bar represents median. Abbreviations: Cu=cuticle; Co=cortex; M=medulla

156x84mm (300 x 300 DPI)

Fig. 2 Distribution and order analysis of White Caucasian hair cross-sections using μ -FTIR. (a) Cross-section observed by optical microscopy of White Caucasian hair selected to analyze by μ -FTIR, regions were manually determined. (b) Chemical map of second derivative obtained at 2850 cm^{-1} (CH_2 symmetric stretching). (c) Average spectra at 2850 cm^{-1} for each region (d) PCA score plot of PC-1 vs. PC-2 with boxplot for each PC in the opposite site. (e) Loading plots of PC-1 and PC-2 of PCA. Boxplot represents 25/75% quintile, whisker 5/95% quantile and central bar represents median. Abbreviations: Cu=cuticle; Co=cortex; M=medulla

156x84mm (300 x 300 DPI)

Fig. 3 Multi variant analysis of Brown vs. White Caucasian cuticles. (a) PCA score plot of PC-1 vs. PC-2 with boxplot for each PC in the opposite site. (b) Loading plots of PC-1 and PC-2 of PCA. Boxplot represents 25/75% quintile, whisker 5/95% quantile and central bar represents median.

156x42mm (300 x 300 DPI)

Fig. 4 (a) Water sorption isotherms and (b) change in apparent diffusion coefficient in DVS analysis of Brown and White Caucasian hairs.

156x42mm (300 x 300 DPI)