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Water sorption of nails treated with wool keratin proteins and peptides

C. Barba · M. Martí · A. Roddick-Lanzilotta · A. Manich · J. Carilla · J. L. Parra · L. Coderch

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Abstract Water has a considerable effect on human keratin fibres, such as nails, and is therefore crucial for their cosmetic performance. Wool proteins are mild, natural, biodegradable and are sustainably produced with multiple functionalities. They have a potential for use in the cosmetic and detergent markets. The effectiveness of two wool keratin ingredients in restoring the water sorption characteristics of nails was determined. Acetone treatment modified nail water sorption, resulting in an increase in water sorption capacity and in nail permeability. The application of keratin peptides and proteins to healthy and damaged nails improved water sorption properties, reducing permeability, especially in the case of wool keratin protein treatment.

Keywords Thermogravimetric balance · Water absorption · Desorption · Diffusion · Nails · Wool proteins

Introduction

The nail unit consists of three parts. The nail matrix or root, the nail bed, which underlies and nourishes the nail plate, and the nail plate itself. The skin, which surrounds the nail plate, is termed the nail fold. The nail plate is a hard keratin structure which is regarded as an indicator of overall health. Keratin is the tough, fibrous, insoluble protein that forms the structure of fingernails. The complex keratin structure is held together by characteristically disulphide linkages of cystine, hydrogen bonds and polar linkages [1]. Like hair, the nail plate consists of hard keratin and lipids [2]. The degree of hydration is considered to be the most important factor that influences the physical properties of the nail [3]. Frequent washing of nails can increase their brittleness [4]. It has been reported that repeated hydration and dehydration of nail plates causes delamination, dryness and brittleness, which is a condition known as lamellar dystrophy [3]. This condition has been attributed to (1) the diminished capacity of the nail plate to hold water as a result of a change in the ability of the protein structure to bind water, and to (2) the reduced water content between the corneocytes cells. It goes without saying that lamellar dystrophy can be prevented by increasing the hydration of the nail and improving the barrier function.

The use of nail care products and procedures in beautifying and grooming nails is very common. Unfortunately, when improperly used, nail cosmetics can lead to nail diseases [5]. Brittleness in the nail may be caused by trauma, such as repeated wetting and drying, repeated exposure to detergents and water, and excessive exposure to harsh solvents, such as those found in nail polish remover [6]. Acetone is a commonly used solvent for nails which is present in nail polish removers. Acetone is known to lead to nail brittleness and discolouration.

Water plays an important role in improving the condition of nails because of its effect on the many properties of human keratin tissues. Water diffusivity in wool, horn and the corneocyte phase of stratum corneum shows a marked increase as the water content in the tissue is raised [7]. Despite the fact that water sorption of wool is well documented [8], there are few data on water sorption of human nails.

C. Barba (✉) · M. Martí · A. Manich · J. Carilla · J. L. Parra · L. Coderch
Surfactant Technology Department, IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
e-mail: cbaesl@iqac.csic.es

A. Roddick-Lanzilotta
Agresearch, Private Bag 4749, Christchurch, New Zealand

The amount of water in a sample may be expressed in terms of either regain or moisture content. Regain is the mass of adsorbed water over the mass of dry sample, whereas moisture content is the same mass over the mass of the sample [9].

The determination of the water sorption isotherm by isothermally applying discrete, cumulative humidity changes involves dynamic and static aspects from which diffusion coefficients and equilibrium water contents are deduced [10]. Time/absorption isotherms provide a complete description of the absorption phenomenon under particular conditions such as initial regain of the sample, temperature and relative humidity (RH) [9]. The moisture sorption isotherm of keratins has been the subject of several studies and models specially developed for describing the shape of the moisture sorption and desorption. The Vrentas and Vrentas model emphasizes the role of the glass transition in generating the sigmoidal shape of the adsorption isotherm [11]. In another work, the uptake of water by polar polymers was described by the Flory–Huggins equation [12].

It is common knowledge that there is a good correlation between the number of water molecules that exist in a monolayer and the number of polar side chains using the classic Brunauer, Emmet and Teller (BET) sorption equation. This suggests that each polar group initially sorbs one molecule of water followed by multimolecular sorption at higher humidities [13]. The BET equation is used because of its simplicity and because it has the approval of the International Union of Pure and Applied Chemistry (IUPAC). However, the Guggenheim, Andersen and de Boer (GAB) sorption equation also provides monolayer sorption values and has become more popular because the range of relative vapour pressure interval is much wider than that of the BET equation [14]. The BET and the GAB isotherms are closely related since they are based on the same statistical model. The GAB is an improvement on the BET model and shares with it the two original BET constants: (a) the monolayer capacity W_m, and (b) the energy constant C_g.

There is a growing consumer trend toward the use of natural actives in personal care [15]. Wool proteins are mild, natural, biodegradable and sustainable with multiple functionalities and have considerable potential for use in the cosmetic and detergent markets. In this work the effect on nails of two keratin proteins isolated from wool were studied: an intact keratin intermediate filament protein extract (K-protein) and a low molecular weight keratin peptide from intermediate filament proteins (K-peptide). Given that the wool protein is isolated intact and in its natural state, the K-protein has the ability to form cohesive films which may have important implications for improving hair properties [16–18]. The cosmetic effectiveness of the wool peptide on skin has been previously demonstrated [19, 20]. Both keratins have cystine present in the active *S*-sulphonated form. This unique chemistry enables the keratin peptide and protein to reform disulphide bonds in damaged nails and directly affect nail properties (Fig. 1).

$$\text{Protein–S–SO}_3^- + \text{OH}^- + \text{Nail–SH} \leftrightarrow \text{Protein–S–S–Nail} + \text{SO}_3^{2-} + \text{H}_2\text{O}$$

Fig. 1 Chemical reaction of *S*-sulphonated form of the keratins

The main aim of this work is to study the reinforcement ability of two different *S*-sulfonated wool keratins, K-protein and K-peptide, on healthy human nails. Restoration of acetone-disturbed nails is also evaluated after the application of the wool keratin formulations. The effectiveness of these keratin ingredients in restoring the water sorption characteristics of the nails is determined.

Materials and methods

Materials

Acetone was supplied by Merck (Darmstadt, Germany) and Keratin peptide (MW <1000 D (SDS-PAGE)) and Keratin protein (MW of 55 kD (SDS-PAGE)) by Keratec Limited (New Zealand). Nail plates were obtained from several healthy volunteers who cut their own nails.

Subjects

Eleven healthy Caucasian volunteers (all females) with a mean age of 34 ± 7 years old participated in the studies. The subjects were advised to avoid topical drugs or moisturizers on the nails of both hands for a week prior to the experiments. The participants were given a detailed description of the study, and written consent was obtained.

Efficacy on healthy human nails

A long-term study was performed to test the effect of the keratin samples when applied repeatedly to healthy nails. Keratin treatments were performed on the nails of the dominant hand of the 11 volunteers: the nails of the index and ring fingers were subjected to topical treatment (2% keratin protein aqueous solution or the 2% keratin peptide aqueous solution) whereas the nail of the middle finger was untreated and used as a control. Keratin solutions were applied twice every day for 2 weeks with a total of 28 applications. Nail plates were obtained from the volunteers who cut their own nails on day 14, separately for each finger. Nail plates of the same finger for all volunteers were mixed together, therefore untreated (UT) nails, Kpep nails

and keratin protein extract (Kpro) nails samples underwent sorption experiments.

Restoration of acetone-disturbed human nails

A long-term study was performed to test the restoring capacity of the keratin samples when applied repeatedly to disturbed nails. First nails of the index, middle and ring fingers of the non-dominant hand of the 11 volunteers were treated with acetone twice a day for 1 week. Keratin solutions were applied on nails of the index (2% keratin protein aqueous solution) and ring fingers (2% keratin peptide aqueous solution). The nail of the middle finger was untreated and used as a control. Keratin solutions were applied twice every day for 2 weeks with a total of 28 applications. Nail plates were obtained from the volunteers who cut their own nails on day 7, after acetone treatment, and on day 21, after the keratin treatment. Nail plates of the same finger for all volunteers were mixed together, therefore UT nails, Ac nails, Ac-Kpep nails and Ac-Kpro nails samples underwent sorption experiments.

Sorption experiments

Absorption and desorption curves were obtained in a thermogravimetric balance equipped with a controlled humidity chamber, the Q5000SA Sorption Analyzer (TA Instruments, New Castle, USA). The weight of the nail samples analyzed ranged between 6 and 9 mg. All experiments were conducted at 25 °C with a total gas flow of 200 mL/min and followed the same measuring procedure:

1. *Initial drying*: temperature 60 °C and 0% RH overnight. The sample remains in this step until its mass reaches equilibrium (arbitrarily defined by a change in mass of less than 0.02% per minute for 10 min).
2. *Pre stabilization*: temperature 25 °C, 0% RH and then initial absorption kinetics at 5% RH.
3. *Absorption curve*: the sample previously stabilized at 5% RH is subjected to absorption tests progressively increasing in steps from 10% up to 95%, the sample being stabilized at 95% RH after the last step. The sample remains in each step until its mass reaches equilibrium (arbitrarily defined by a change in mass of less than 0.02% per minute for 10 min).
4. *Desorption curve*: the sample stabilized at 95% RH after the absorption process kinetics is subjected to desorption tests progressively decreasing in steps from 10% down to 5%, the sample being stabilized at 5% RH after the last step. The sample remains in each stage until its mass reaches equilibrium (arbitrarily defined by a change in mass of less than 0.02% per minute for 10 min).

The high reproducibility of these measurements was established in the validation study of this instrument in which three replicates of a single sample gave essential coincident sorption isotherms. For this reason, and given the long time needed for a measurement (4 days), only one measurement was performed for each sample.

Sorption isotherms are generally described by mathematical models based on empirical and/or theoretical criteria which can be found in the literature. One of the most commonly used equations is that of the Guggenheim–Anderson–de Boer (GAB) model. It has a theoretical background and its parameters provide a physical meaning to the sorption process when compared with empirical models. The GAB model is based on the monolayer moisture concept and gives the value of the monolayer moisture content of the material [21]. The GAB model has proved to be applicable in hydrophilic polymers [22, 23] and food [24] systems and has considerable theoretical justification [25]. Thus, in this work, sorption isotherm data were modelled according to the GAB model in line with other authors [26, 27]. In accordance with other authors [13] the GAB model was adjusted in the desorption isotherm process because the results were more reliable. Table 1 shows the sorption isotherm and the parameters used to fit the experimental sorption/desorption data. The goodness of the fit was evaluated by the determination coefficient (R^2).

Moisture sorption/desorption tests on hygroscopic samples take a very long time to reach equilibrium, although the most important interchange of water occurs in the initial steps. Therefore, some conditions are fixed to shorten the testing time. Although at this point the regain at the equilibrium was not reached, the regain at the equilibrium, R_∞, could be calculated by fitting the appropriate model. Therefore, the absorption/desorption curves of each step were fitted to the following kinetic model [9]:

$$R(t) = \frac{Bt^c}{A^c + t^c}$$

$R(t)$ is the regain of the sample at time t, B the regain at the equilibrium (R_∞), A coincides with the time of half absorption ($t1/2$) and c is the power coefficient of each step.

The application of the non-linear regression procedures obtained the best estimates of the model parameters yielding B, A and c, which enabled us to calculate the asymptotic regain at equilibrium R_∞ and the half absorption time $t1/2$ and rate $v1/2$. The non-linear regression required unbiased initial estimators of the model parameters that were provided by the linear regression between $t/R(t)$ and t through the straight line $t/R(t) = \alpha + \beta t$, where α/β and $1/\beta$ were, respectively, the initial estimators of A and B [9].

Table 1 GAB model and parameters used to fit the experimental sorption data

Model	Mathematical equation
GAB [28]	$W = W_m\ C_g\ Ka_w/[(1 - Ka_w + C_g\ Ka_w)]$
Parameter	**Definition**
a_w	Water activity expressed as vapour relative pressure p/p_0, where p_0 is the saturated vapour pressure
W	Equilibrium moisture content at a_w in g sorbed/100 g of sorbent on dry basis
W_m	Monolayer moisture content in g sorbed/100 g of sorbent on dry basis d.b
C_g	Energy constant related to the difference between the free enthalpy of the water molecules in the pure liquid state and in the monolayer. This is proportional to the rate between both the attachment and the escape rate constants of the primary sites
K	Ratio between the standard vapour pressure of the liquid and the vapour pressure of the sorbate in the secondary (upper) layers. This is proportional to the rate between the attachment and the escape rate constants for all higher layers

The diffusion coefficient was obtained using the method applied by Vickerstaff [29] to study the diffusion of dyes within the fibres. It appears that the diffusion is well fitted by an expression derived from Fick's equation applied to moisture diffusion. This expression yielded satisfactory results in the early stages of moisture absorption as in the case of those obtained in dye diffusion. If the fractional absorbed moisture is plotted against the square root of the absorption time, the points should lie on a straight line

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

The slope is considered to be the square root of the apparent diffusion coefficient, D_A, of the moisture. If the apparent diffusion coefficient is measured over sample mass instead of over sample surface, it is measured in min^{-1}.

Results and discussion

Efficacy on healthy human nails

Two kinds of keratin samples obtained from wool were tested on healthy human nails; a keratin protein extract (Kpro) and a low molecular weight keratin peptide (Kpep). Nail samples were collected from 11 volunteers before and after 28 applications of the wool keratin extracts (see 'Materials and methods' section). Temperature dependent isotherms for water sorption and desorption of human nails before and after keratin treatments were evaluated by the software provided by the TA Instrument and are shown in Fig. 2. A different behaviour is observed in the sorption isotherms for untreated nails and nails treated with either the keratin protein or the keratin peptide. The application of both keratin types leads to lower levels of moisture absorbed and desorbed across the range of RH studied when compared to the untreated nail sample. It is well known that the nail plate becomes soft and tends to

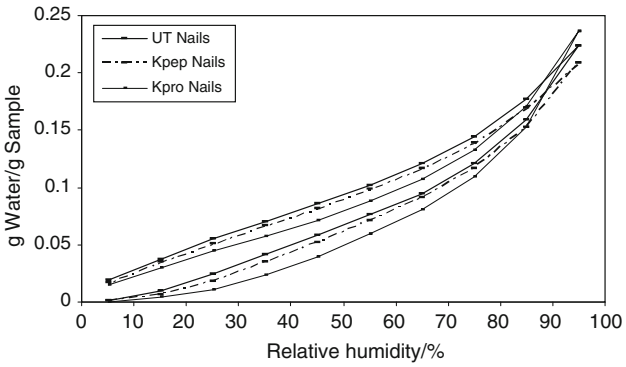

Fig. 2 Water sorption isotherms for nails untreated (UT) and treated with the keratin peptide (Kpep) and protein (Kpro)

be double layered when its water content exceeds 20% [30–32]. Furthermore, in an earlier work, nail water content was found to be increased when nails were subjected to a deterioration treatment [33]. Therefore, lower values of moisture uptake at different humidities for the nails treated with either wool keratins could indicate an improvement in nail structure integrity. This effect is more marked in the case of nails treated with keratin protein.

The time of half absorption and desorption in each step of RH was evaluated for the different nail samples (Fig. 3). After reaching equilibrium, a different behaviour of the nail samples was observed. As shown in Fig. 3, keratin-treated nails take longer to reach equilibrium than untreated nails. Consequently, the treated nails absorbed and desorbed more moisture at equilibrium than the untreated ones. This is more pronounced in the case of the nails treated with keratin protein.

The experimental desorption data for nails were also subjected to the GAB model, and values for the monolayer capacity (W_m) and the energy constant (C_g) are shown in Table 2. A good fit of the GAB model to the desorption data was achieved ($R^2 > 0.999$). The results given by the GAB model shows that nails treated with wool keratin

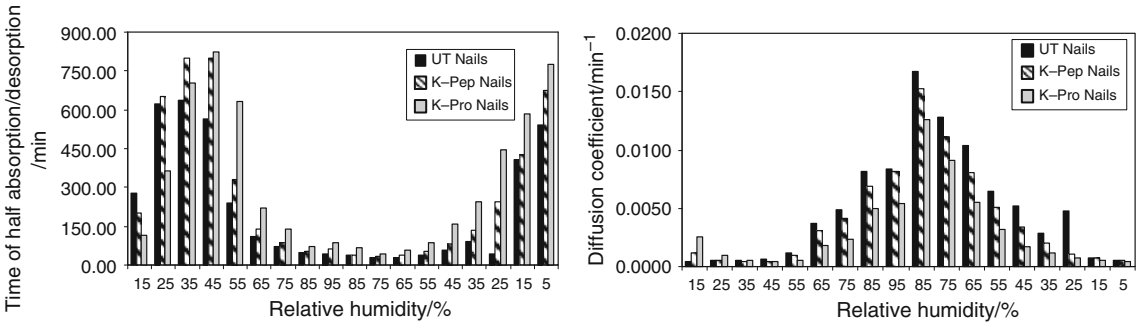

Fig. 3 Time of half absorption/desorption in min and diffusion coefficient in min^{-1} for nails untreated (UT) and treated with the keratin peptide (Kpep) and protein (Kpro)

Table 2 GAB monolayer capacity (W_m), GAB energy constant (C_g), GAB determination coefficient (R^2), total time to reach equilibrium (t_T) and apparent diffusion coefficient (D_A) (Mean value ± SD) for nails untreated (UT) and treated with the keratin peptide (Kpep) and protein (Kpro)

	W_m/%	C_g	R^2	t_T/min	D_A/min^{-1}
UT	7.001	7.9472	0.999	4806.34	0.0049 ± 0.005
Kpep	7.366	6.3494	0.999	5084.16	0.0041 ± 0.004
Kpro	5.625	6.6988	0.999	5577.66	0.0030 ± 0.003

protein undergo a decrease in the amount of water absorbed in the monolayer, with lower values of the energy constant. This reduction in the water content is consistent with the results derived from the sorption isotherms, where nails treated with the wool keratin protein led to a diminution in the nail water sorption and to a possible improvement in the nail structure.

Estimation of the kinetics of the moisture uptake and loss is a good strategy for obtaining more detailed information about the structural integrity of a given sample. The kinetics of moisture sorption/desorption was evaluated for all the nail samples. Nails treated with either the keratin peptide or the keratin protein take much longer to reach equilibrium compared with the untreated nails. Earlier studies have demonstrated that when the nail structure is damaged, the rate at which the nail reaches equilibrium is increased [33]. Therefore, the decrease in the rate at which the treated nails reach equilibrium could indicate an improvement in the nail structure due to keratin treatment. This decrease is more marked in the case of the keratin protein-treated nails.

The moisture diffusion kinetics through the nail structure was evaluated and the apparent diffusion coefficients (D_A) in each RH step were calculated as detailed in the experimental part. The results are summarized in Table 2 and plotted in Fig. 3. The apparent diffusion coefficient for each sample (Table 2) is the mean value of each diffusion coefficient obtained at each humidity (Fig. 3). There is an inverse relationship between the time parameter and the diffusion coefficient, i.e., longer time is needed to reach equilibrium for tissues with low water permeability and, therefore, a small diffusion coefficient. The evaluation of the total time to reach equilibrium shows a marked increase in the case of keratin-treated nails. This is more pronounced for the keratin protein-treated nails (Table 2). Furthermore, evaluation of the time of half absorption and desorption in each step of RH (Fig. 3) shows a clear upward trend of this parameter, in most of the RH steps in the case of keratin-treated nails. The relationship between the time parameter and the diffusion coefficient may be observed with a small diffusion coefficient for the keratin-treated nails with longer times to reach equilibrium. This marked diminution in the diffusion coefficient indicates a sharp decrease in water permeability, thus improving nail structure integrity. Again, this effect is much more important in the case of keratin protein-treated nails. Furthermore, as happened with the times at half sorption–desorption, the diffusion coefficient results indicate a large variation with the RH parameter being the evaluated effect much more noticeable for high values of RH.

Restoration of acetone-disturbed human nails

Another study was performed to evaluate the restoration capacity of wool keratin samples when applied to nails damaged by acetone. Acetone treatment was carried out because of the association of this product with nail brittleness and discoloration [34]. Nail samples were collected from 11 volunteers before the study after undergoing damage with acetone and after wool keratin treatments (see 'Materials and methods' section).

The water sorption isotherm of untreated (UT) nails, acetone-damaged nails (Ac) and acetone-damaged nails

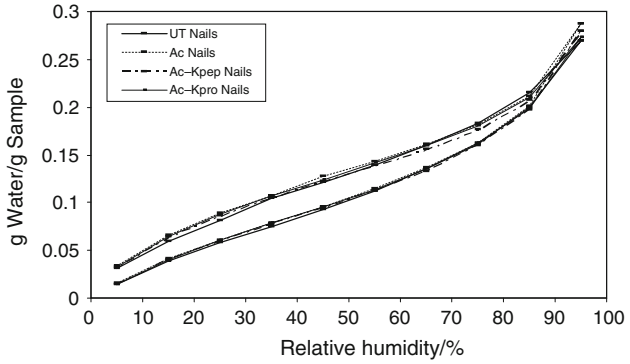

Fig. 4 Water sorption isotherms for nails untreated (UT), deteriorated with acetone (Ac) and treated with the keratin peptide (Ac-Kpep) and protein (Ac-Kpro) after acetone deterioration

Table 3 GAB monolayer capacity (W_m), GAB energy constant (C_g), GAB determination coefficient (R^2), total time to reach equilibrium (t_T) and apparent diffusion coefficient (D_A) (Mean value ± SD) for nails untreated (UT), deteriorated with acetone (Ac) and treated with the keratin peptide (Ac-Kpep) and protein (Ac-Kpro) after acetone deterioration

	W_m/%	C_g	R^2	t_T/min	D_A/min^{-1}
UT	7.024	8.1862	0.999	4885.24	0.0043 ± 0.004
Ac	7.098	7.6514	0.999	4894.98	0.0047 ± 0.005
Ac-Kpep	6.929	7.4160	0.999	5072.78	0.0042 ± 0.005
Ac-Kpro	7.177	6.1055	0.999	5322.98	0.0036 ± 0.004

treated with the keratin peptide (Ac-Kpep) or keratin proteins (Ac-Kpro) was determined and is depicted in Fig. 4. Comparison of the isotherms shows only minor differences, the most important ones being for the keratin protein treated nails which present a slight diminution in the moisture absorbed and desorbed in all the RH steps.

The experimental desorption data for nails were also subjected to the GAB model. Minimum differences in the monolayer capacity were found with only a diminution of the energy constant (C_g) for the protein treated-nails.

When acetone-damaged nails were subjected to wool keratin proteins or peptides the time to reach equilibrium is increased (Table 3). The nail keratin treatments significantly increase the time parameter, reaching values exceeding those for the untreated nails. These results demonstrate the effectiveness of keratin in restoring nails previously damaged by acetone.

The apparent diffusion coefficients of the nail samples in each RH step were also evaluated and results are plotted in Fig. 5 and summarized in Table 3. The results show that acetone treatment was detrimental to the nail structure, resulting in an increase in its diffusion coefficient and hence in its water permeability. In the case of keratin-treated nails, there is a marked fall in the values of the diffusion coefficient, indicating a decrease in the water permeability of the nails. Again, both effects, the damaging of the acetone treatment and the structure improvement of both keratin treatments are much more clear when evaluating the diffusion coefficients at high values of RH. Furthermore, in the evaluation of the time of half absorption and desorption in each step of RH it may be observed that acetone-damaged nails treated with either keratin needed longer times to reach equilibrium (Fig. 5). These results demonstrate an improvement in the integrity of the nail structure previously damaged by acetone, thereby highlighting the restoring capacity of both wool keratin samples.

A correlation between the GAB energy constant (C_g) and the diffusion coefficients was observed in both nail experiments, showing a decrease in the energy constant in the case of the samples with lower values of diffusion coefficients. As described in the experimental part, the GAB energy constant can be defined as the ratio between the attachment rate and the escape rate constants of the primary sites. Lower values of this energy parameter indicate that the escape rate is higher than the attachment rate, which accounts for the decrease in the water diffusion through the nail structure.

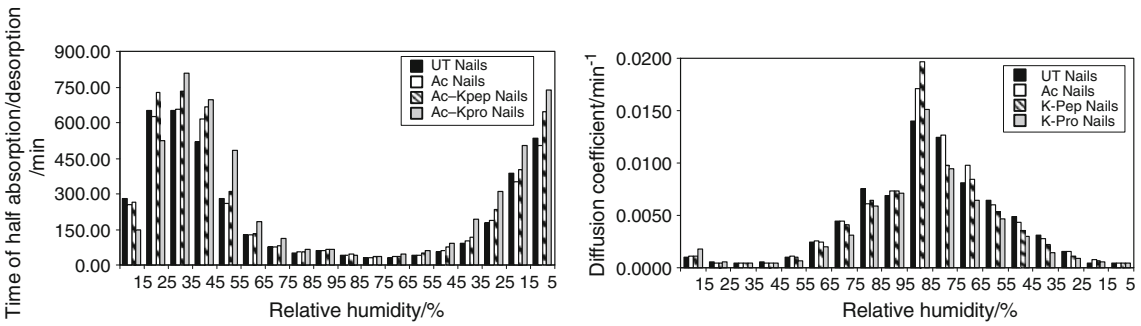

Fig. 5 Time of half absorption/desorption in min and diffussion coefficients in min^{-1} for nails untreated (UT) deteriorated with acetone (Ac) and treated with the keratin peptide (Ac-Kpep) and protein (Ac-Kpro) after acetone deterioration

Conclusions

Modification of nail water sorption properties following a commonly used acetone cosmetic treatment was demonstrated with an increase in nail permeability.

Keratin peptides and proteins proved effective in reinforcing healthy nails and in restoring the structural integrity of acetone-damaged nails by inducing a decrease in the nail diffusion coefficient. This indicates a diminution in nail permeability and an improvement in its keratinized structure. Wool keratin protein was much more effective in decreasing nail permeability in both studies. Its ability to form cohesive films could account for the protection and restoration of nails and hair.

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