Using solid-state 1H NMR relaxation methods to quantify small molecule interactions and damage in hair

Marsh, Jennifer M*[1]; Kang, Husen[2]; Whitaker, Shane[1]; Talley Allison[1]; Chen Tian[2]; Felts, Tim[1]; Molisso, Susannah[3]; Williams, Daryl R[4]; Ces, Oscar[3]; Rowlands, Lucy J[3]; Law, Robert V[3]

[1] The Procter & Gamble Company, Mason Business Center, 8700 Mason Montgomery Road, Mason, OH 45040, USA; [2] Procter & Gamble International Operation SA, 70 Biopolis Street, Singapore 138547; [3]Institute of Chemical Biology, Department of Chemistry, MSRH, Imperial College London, White City Campus, 80–92 Wood Lane, London W12 0BZ, UK; [4]Department of Chemical Engineering, Imperial College London, South Kensington Campus, London SW7 2AZ, UK

* Jennifer Marsh, The Procter & Gamble Company, 8700 Mason Montgomery Road, Mason, Ohio, 45040, Tel +1 513 410 5771, marsh.jm@pg.com

Abstract

Background:

How compounds penetrate into hair and impact its properties such as strength or bending stiffness is of high interest to the cosmetics industry. The purpose of these studies was to assess whether ^{1}H NMR and T_1 relaxation times could be used to measure these interactions and a selection of compounds was chosen for the work based on their molecular weight and Log P values - methanol, acetic acid, ethyl acetate, isoamyl acetate, benzyl acetate, panthenol.

Methods:

T_1 and T_2 relaxation measurements were measured using a solid-state Bruker Avance 200 MHz ^{1}H NMR spectrometer. Penetration of panthenol was measured with HPLC after a two-stage extraction with acetone (surface) and water/acetonitrile (penetrated). FT-IR imaging was used to visualize penetrated panthenol. Single fiber tensile strength measurements were made using a Dia-Stron MTT instrument.

Results:

Static ^{1}H T_1 relaxation times for a series of model small molecular compounds (methanol, acetic acid, ethyl acetate, isoamyl acetate and benzyl acetate) were measured in hair. Two populations of compound were identified with a rigid bound population identified with shorter relaxation times (T_1H_b) and a labile free population with longer relaxation times (T_1H_f). Comparison of solvent populations in hair to bulk solvent was used as a measure of interaction. For panthenol this method was not suitable but interaction with hair protein structures was inferred from penetration and tensile strength measurements.

Conclusion:

^{1}H NMR has been shown to be a method that can yield valuable information in the study of interactions between small molecules and hair but there are some limitations of the method depending on the compound chosen.

Keywords: NMR; Penetration; Tensile Strength; Protein Interactions

Introduction.

Understanding interactions of small molecules in hair is of great interest to the industry. Quantifying penetration of materials and measuring their impact on single fiber properties such as tensile strength has been studied but understanding specific locations and interactions that influence the measured fiber benefit has proved to be challenging. This work set out to determine if we can utilize ^{1}H NMR to study these keratin interactions with a series of small molecules and in a range of different hair damage levels. The ^{1}H NMR method has been used to study hair but its use to study small molecules and their interactions has been limited. These data can inform us whether small molecules behave in the same way through the hair fiber, how these interactions change in the presence of water. Penetration of materials into hair is important if we want to impact consumer relevant properties such as hair strength.

Materials and Methods.

Hair was chopped finely to lengths of approximately 0.5 cm and dried over several days over P_2O_5 to achieve a constant dry mass. Dried hair was then soaked for approximately 16 hrs in the chosen compound to reach full saturation. Rotors (4 mm, ceramic) were loaded

with damp fibers (axially). T_1 and T_2 relaxation measurements were measured using a solid-state Bruker Avance 200 MHz ^{1}H NMR spectrometer for a series of small molecules (methanol, acetic acid, ethyl acetate, isoamyl acetate and benzyl acetate) that vary in molecular weight and Log P values. As the solvents are present in great excess to protons present in hair, static NMR experiments were performed with the confidence that contribution from hair protons would be negligible in this work. Relaxation times as a function of humidity (0%, 33%, 75%, 95% RH) were measured to understand the role of water in these interactions. Changes in the relaxation times were also measured after hair had undergone a series of pretreatments – exposure to different pH buffers, heat, and oxidative damage.

Panthenol was measured on hair using a two-stage extraction process. Hair was first shaken in acetone to remove surface panthenol followed by a second extraction with water/acetonitrile to remove penetrated panthenol. Hair used was Caucasian and Asian hair that had been chemically treated with hydrogen peroxide/ammonia pH 10 system (equivalent oxidative damage to a consumer coloring regularly with a medium brown permanent level 3 colorant). Panthenol levels were measured on an Agilent 1200 series HPLC with UV detector. Panthenol penetration inside hair was visualized with FT-IR of hair cross-sections using two unique C-O and C-O-C panthenol peaks at 1071 and 1041 cm^{-1}. Both non treated (control) and D-Panthenol treated hair were cross sectioned at 5 µm thickness using Leica CM3050S cryo-microtome and placed on CaF2 crystal. The chemical imaging was done on multiple replicates of hair cross sections in transmission mode using Bruker Hyperion 3000 with 128x128 pixels focal plane array (FPA) detector and 36X objective lens with 1024 scans and 8cm-1 resolution. The spectrum processing was done in OPUS 7.2 and further analysis was done in ISys 5.0.

Single fiber measurements were made using a Dia-Stron MTT 686 instrument (Andover, Hampshire, UK) with an extension rate of 40mm/min. Fiber diameters were measured with a Dia-Stron Fiber Dimensional Analysis System (FDAS 770), which incorporates a Mitutoyo laser micrometer (LSM-6200) (Malborough, MA, USA). A minimum of 100 fibers were used for tensile measurements.

Results.

NMR has been used as a tool to understand small molecule interactions, with a focus on the spin-lattice (*T1*) and spin-spin (*T2*) relaxation times of water in proteins. The dependence of *T1* and *T2* relaxation processes on water mobility and local environment allows these measurements to be used to examine how water mobility changes in proteins, and what that can reveal about the protein structure itself. When a protein is in water, the relaxation of water is assumed to be a weighted average of molecules in bulk solution and those interacting with the protein [1]. The water molecules which interact with the protein can be further broken down to fast and slow relaxing regimes [2,3]. These two components relate to a relatively immobile 'bound' population and a more labile 'free' population [4-6] but both populations undergo some exchange [7]. In keratinous materials, the high heterogeneity of the fiber has led to multi-exponential fits being used to model the relaxation behavior of water [8,9].

In this work, ^{1}H T_1 relaxation measurements were used to examine the populations of a series of model small molecular compounds in hair - methanol, acetic acid, ethyl acetate, benzyl acetate, isoamyl acetate and panthenol (Figure 1).

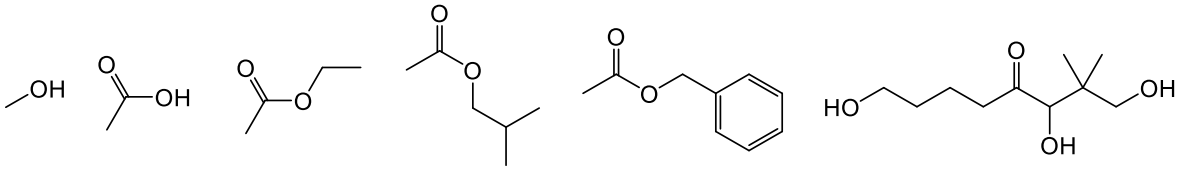

Figure (1) The structures of compounds investigated (a) methanol, (b) acetic acid, (c) ethyl acetate, (d) isoamyl acetate, (e) benzyl acetate and (f) panthenol

Hair was soaked in each solvent to achieve full saturation and then relaxation measurements made. The exception was panthenol which was too viscous to be used as a 100% material, so a 90% dilution in water was used. The relaxation behavior was fitted to the two component variant equation shown below. It was confirmed that a one component fit did not accurately fit the data generated.

$$A(\tau) = A_0 - B_b \exp\left(\frac{-\tau}{T_{1Hb}}\right) - B_f \exp\left(\frac{-\tau}{T_{1Hf}}\right)$$

Where B_b is a pre-exponential factor for the bound fraction and T_{1Hb} is the ^{1}H T_1 time for the bound fraction of the compound in hair. B_f and T_{1Hf} are the pre-exponential factor and ^{1}H T_1 time for the free population of compound in hair respectively.

Measurements of panthenol which is a viscous, self-associating "glassy" solvent proved problematic. In particular the intermolecular interactions meant that the relaxation behavior was difficult to interpret. Specifically, we plotted the viscosity (a measure of molecular motion) vs T1 response. For small molecules that have no or little intermolecular association, it is expected that as viscosity increases (and thus rotational correlation time increases) there would be a decrease in T1. With panthenol we saw as viscosity increased the T1 also increased indicating we are not in the fast motion limit/extreme narrowing regime and intermolecular interactions are dominating. This phenomenon has been observed with other high associating materials such as glycerol [10]

The T_1 recovery times for the compounds are shown in Table 1 along with T_1 time of the pure compounds. It is proposed that the observed shorter T_1 times seen for all the compounds are due to reduced mobility of the molecules inside hair. The source of this reduced mobility maybe due to possible fiber-compound interactions including hydrogen bonding, electrostatic interactions, and dipole interactions

	Compound only	Compound in hair	
	^{1}H T_{1Solv} (s)	^{1}H T_{1Hb} (s)	^{1}H T_{1Hf} (s)
Water	2.73	0.10	1.05
Methanol	3.12	0.45	1.36
Acetic Acid	2.03	0.23	0.78
Ethyl Acetate	3.20	0.51	2.95
Benzyl Acetate	2.43	0.47	2.28
Isoamyl Acetate	2.00	0.38	1.92

Table 1 – relaxation times for compound alone and in hair

As each compound investigated had a different T_{1Solv} time, the T_{1H} times were normalized for comparison as a ratio between T_{1Hb} or T_{1Hf} and T_{1Solv}. Table 2 shows ratios of ΔT_{1Hb} or ΔT_{1Hf} for compounds in hair.

	ΔT_{1Hb}	ΔT_{1Hf}
Water	0.10 ± 0.00	0.38 ± 0.03
Methanol	0.14 ± 0.02	0.43 ± 0.07
Acetic Acid	0.11 ± 0.02	0.38 ± 0.06
Ethyl Acetate	0.16 ± 0.01	0.92 ± 0.02
Benzyl Acetate	0.19 ± 0.02	0.94 ± 0.04
Isoamyl Acetate	0.19 ± 0.03	0.96 ± 0.05

Table 2 – Normalized relaxation times

The normalized relaxation changes for the different compounds allowed for comparison between compounds and were used to understand if there is any correlation of the relaxation time changes seen with properties of the compounds. A comparison was done with molecular weight and Log P (compound hydrophilicity/hydrophobicity). Figure 2 shows this comparison.

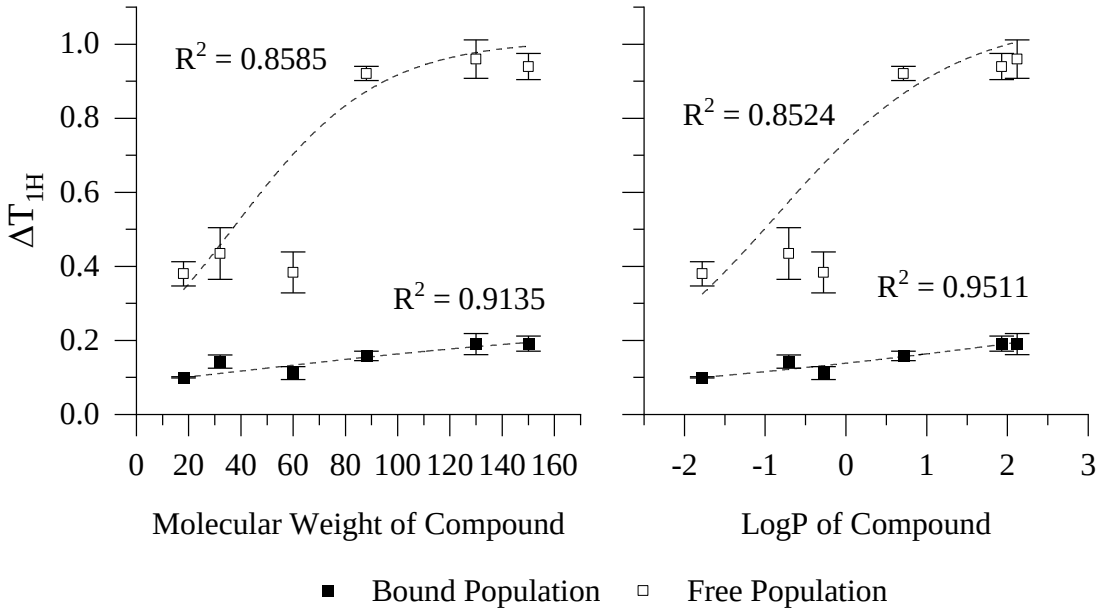

Figure 2 – Bound and free populations correlated with molecular weight and Log P

There appears to be a correlation of both molecular weight and Log P with relaxation time changes for both the bound and free population. As the molecular weight and Log P increases (higher hydrophobicity) the relaxation time increases indication that these compounds have higher mobility. This is not unexpected – water, methanol and acetic acid are all small molecules with the ability to form strong hydrogen bonds with hair and as a consequence would be expected to have reduced mobility (lower relaxation times). The larger more hydrophobic molecules, benzyl acetate and isoamyl acetate have less interaction with hair and have mobility closer to the compound alone. This is supported if relative population sizes of bound and free are calculated. For benzyl and isoamyacetate the free population makes up ~90% of the total amount of solvent in hair as compared to water and methanol which are closer to 70%

In the next set of experiments the relaxation times were investigated in the presence of humidity where before exposure to the compounds the hair was equilibrated at either 0%, 33%, 75% or 97% relative humidity. Measurement of the relaxation time changes vs relative humidity showed a reduction in relaxation time for all solvents indicating high mobility in the presence of moisture in hair. Interesting behavior was seen when relative populations of free and bound water were calculated for methanol and isoamyl acetate (Figure 3). For methanol the relative population of bound decreases with increasing humidity but increases for isoamyl acetate. We can speculate that the decreased bound population for methanol is due to competition with water for the same sites. For isoamyl acetate it maybe that increased moisture in hair is opening up parts of the fiber via swelling.

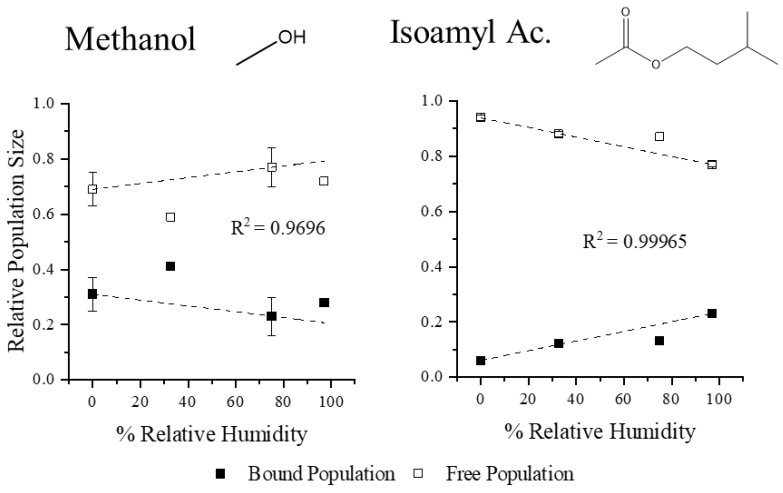

Figure 3 – Relative population vs %RH

As the 1H NMR studies were not successful with panthenol an alternate route was chosen to demonstrate the interaction of this compound with hair. The first stage was to demonstrate penetration of panthenol inside hair which was achieved with a two-stage extract, first with acetone to remove surface panthenol and second with water/acetonitrile to remove panthenol inside hair. Table 3 shows panthenol penetrates into Caucasian and Asian hair from a leave-on product containing 1% panthenol and >90% is inside hair vs on the hair surface. There is no clear difference between the two hair types, and both show accumulation between cycle 1 and 5 but not between cycles 5 and 10.

Hair Type	Surface (µg/g +/- std error)			Penetration (µg/g +/- std error)		
	1 cycle	5 cycles	10 cycles	1 cycle	5 cycles	10 cycles
Caucasian	16.5 (1.5)	79.6 (3.6)	103.9 (7.2)	514.2 (6.2)	1047 (17.7)	999.6 (13.2)
Asian	17.0 (3.0)	46.5 (4.2)	55.4 (1.3)	508.2 (3.1)	1120.4 (30.2)	1164.6 (16.2)

Table 3

Penetration inside hair was confirmed using FT-IR imaging of the unique panthenol peaks at 1071 and 1041 cm^{-1} (Figure 5).

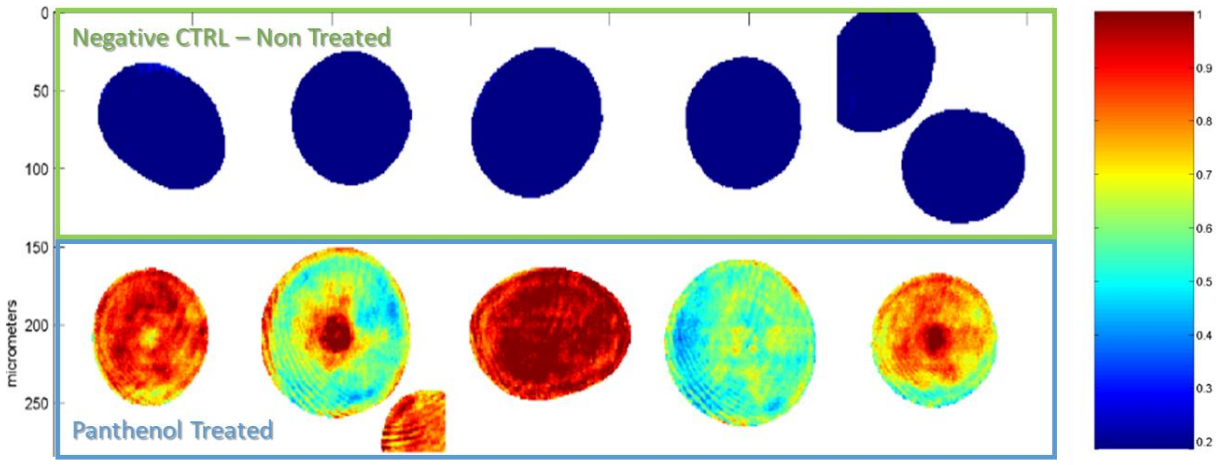

Figure 5 – FT-IR visualization of panthenol penetration

The images indicate penetration throughout the protein regions of hair as expected from the hydrophilicity of panthenol [11]. The influence on protein interactions is demonstrated by

its ability to influence the tensile strength which is dependent on protein interactions [12]. Tensile strength measurements show an increase in tensile strength with application of a panthenol-containing leave-on treatment at 1%.

Leg Details	Break Stress (gf/μm^2 +/- std error)
Control Leave on Treatment	0.01964 (0.00036)
Control + 1% Panthenol	0.02063 (0.00042)*

*Significant to >99% using student t-test

Table 4

Discussion.

These data show the utility of ^{1}H NMR studies to measure interactions of compounds that penetrate inside with hair proteins and that these interactions correlate to the physiochemical properties of the penetrated compounds. However, the method used was shown to not have universal utility to measure all compounds, especially ones that have strong intermolecular interactions. For these compounds such as panthenol a more indirect method to demonstrate protein interactions is required – utilizing penetration measurements and a method such as tensile strength which is known to be influenced by compounds that interact with hair proteins.

Conclusion.

^{1}H NMR has been shown to be a method that can yield valuable information in the study of interactions between small molecules and hair and how these interactions change as a function of humidity and hair damage. This interaction has been linked to changes to single fiber tensile strength.

Acknowledgments.

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Conflict of Interest Statement.

NONE.

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