J. Soc. Cosmet. Chem. 23 13–21 (1972) © *1972 Society of Cosmetic Chemists of Great Britain*

Rheology of stratum corneum—II: A physico-chemical investigation of factors influencing the water content of the corneum

A. C. PARK and C. B. BADDIEL*

Synopsis—ELASTIC MODULI have been obtained as a function of relative humidity in the range 30–100% for STRATUM CORNEUM samples that have been extracted successively with CHLOROFORM and water or with SODIUM DODECYL SULPHATE and water. These moduli differ significantly from the values obtained for untreated corneum and these changes have assisted in elucidating the mechanism of corneum hydration.

After extraction, a diminution in the water-retaining ability of the stratum corneum was observed by infra-red and thermogravimetric ANALYSES. The infra-red studies also established that LIPIDS were being removed from the corneum by the extracting media and that the principal protein component of the corneum was unchanged.

INTRODUCTION

The factors controlling absorption of water by stratum corneum and its subsequent effects on the mechanical properties of this material have been the subject of several studies (1–9). It has been shown that successive extractions of the corneum (1) with lipid solvents (e.g. chloroform/methanol, ether, detergent solutions), and (2) with water, reduces the water binding capacity of this substrate whilst either extraction alone has little effect. In addition, studies concerning changes in the stratum corneum after extraction demonstrated that it became less flexible, particularly at relative humidities between 60 and 90%. On the basis of these findings it has been

* Unilever Research, 455 London Road, Isleworth, Middlesex.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

established that the equilibrium water content and corresponding elasticity of stratum corneum depend on its efficiency in holding water-retaining substances. The way in which these hygroscopic materials are fixed in the corneum is not known but it is believed to be controlled by a lipid barrier as evidenced by their easy removal after extraction of the corneum with, for example, chloroform/methanol (2 : 1, v/v). Middleton (7) has proposed that this lipid barrier resides in the corneum cell membranes and that the keratinous contents of the cells are plasticized by water bound to the hygroscopic materials. However, it is difficult to envisage how the available water can hydrate the protein components and also satisfy the requirements of the hygroscopic compounds, and, in fact, Bulgin and Vinson (10) have demonstrated, using DTA methods on the isolated corneum components, that endothermic heat changes at 114° and 135°, attributable to water in the untreated corneum, were still present in the protein fractions. The above result suggests that bound water in the corneum is largely associated with the proteins. In an attempt to resolve this point and thus further elucidate the mechanism of corneum plasticization, this paper describes the measurement of the elastic modulus of extracted stratum corneum over the relative humidity range 30–100%. Infra-red spectroscopic and differential thermal analysis techniques provided information on structural and water binding changes in the corneum which helped to clarify the model arrived at by mechanical methods.

Experimental

Techniques and procedures employed in obtaining elastic moduli for stratum corneum have been previously described (9). A constant temperature of 25 $\pm$ 0.5° was maintained throughout, and relative humidity values were accurate to $\pm$2%.

High resolution ir spectra of stratum corneum were recorded in the range 4 000–400 cm^{-1} on a *Perkin-Elmer 521* double-beam spectrophotometer. The spectrometer was calibrated using water vapour, CO_2 and polystyrene. For normal instrumental conditions the scan rate was 1 cm^{-1} 4 s^{-1}, but when expansion was used (ordinate $\times$ 10; abscissa $\times$ 4) the scan rate was slower, 1 cm^{-1} 8 s^{-1}. All samples were dried for 24 h under vacuum before their spectra were recorded and the relative humidity within the sample cavity of the spectrometer was estimated to be $\approx$0%. Complete deuteration of untreated stratum corneum required 6 months in an atmosphere of D_2O vapour with a somewhat shorter time being necessary for solvent extracted samples.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

TGA runs were carried out on a Du Pont thermogravimetric analyser. The weight loss for approximately 5 mg samples of stratum corneum was recorded at a constant heating rate over the temperature range from room temperature to 200°. A number of heating rates were screened from zero (temperature held at specific values) to 10° min^{-1} in order to arrive at the optimum value for this substrate of 5° min^{-1}. Corneum samples were predried for 24 h under vacuum in an attempt to standardize initial environmental conditions.

Chloroform and solutions of sodium dodecyl sulphate (BDH, specially pure) were used as extractants for the stratum corneum. Sodium dodecyl sulphate solutions were made up to the required concentration by weight, and adjustments of pH were made with sulphuric acid and sodium hydroxide. Pieces of corneum ($\approx 2 \times 10^{-2}$ m square) were normally extracted for 2 days with chloroform ($\approx$200 ml) or sodium dodecyl sulphate ($\approx$200 ml) solutions, and then for a further day with water. Extraction was facilitated by stirring and the solvents were renewed several times during these periods.

RESULTS

The variation of elastic modulus with relative humidity, between 30 and 100%, was recorded for samples extracted with chloroform/water or 0.1 M SDS (pH 7 and 3)/water after conditioning (9). Within the limits of experimental error, the effects of the treatments were not graphically distinguishable and the normalized data (9) are combined in a single curve (a) illustrated in *Fig. 1.* Included in *Fig. 1,* for comparison, is the curve (b) previously obtained for untreated stratum corneum (9). Although these curves have the same general shape, the moduli of the extracted samples are higher than those for untreated corneum at every rh and they (i.e. the moduli of extracted samples) decrease at a much slower rate between 30 and 70% rh.

The ir spectrum of untreated stratum corneum is illustrated in *Fig. 2.* Frequencies, band assignments (11–13) and qualitative intensities are listed in *Table I.* Deuteration of the corneum was carried out to assist in establishing some of the assignments. Deuteration of the material also showed that even after attempts to remove water, by desiccation, some still remained, as evidenced in the normal spectrum by the weak shoulder on the high frequency side of the amide A (at 3 400 cm^{-1}) and by the broad band (800–500 cm^{-1}) in the amide IV, V and VI region. Removal of most of this

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

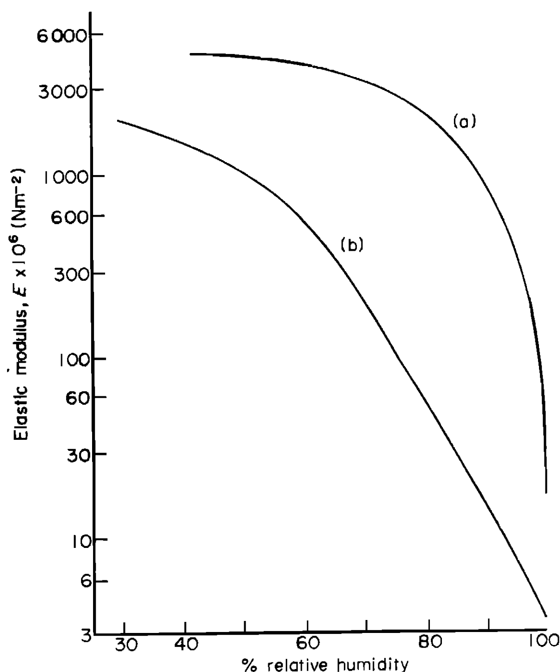

Figure 1. Normalized variation of elastic modulus of stratum corneum with relative humidity for (a) solvent extracted and (b) untreated material.

tenaciously bound water was only accomplished by a chloroform/water extraction followed by desiccation.

A striking feature of the spectra of treated and untreated stratum corneum was that the adsorption bands attributable to protein modes were not

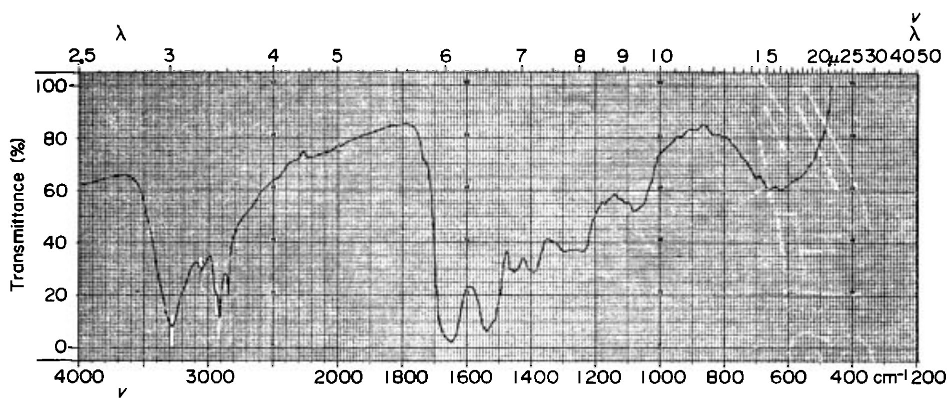

Figure 2. Infra-red spectrum of stratum corneum.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

Table I
Assignments of molecular modes in stratum corneum

Frequency (cm^{-1})	Assignment
3 289 (s)	Amide A N–H str from peptide groups
3 063 (w)	Amide B overtone of Amide II at 1 550 cm^{-1}
2 955 (w)	Antisym C–H str from CH_3 groups
2 919 (s–m)	Antisym C–H str from CH_2 groups
2 872 (w)	Sym C–H str from CH_3 groups
2 854 (w–m)	Sym C–H str from CH_2 groups
1 740 (w–sh)	C = O str probably from lipid esters
1 653 (vs)	Amide I, largely C = O str + a small contribution from NH bend
1 543 (s–vs)	Amide II, complex mode C–N str + N–Hb from peptide groups
1 518 (sh)	
1 464 (sh)	CH_3
1 454 (m–w)	CH_2
1 395 (m)	CH_2 (Wag)
1 340 (w–sh)*	CH_3 ?
1 295 (w)	?
1 270 (vm)*	Amide III complex mode similar to Amide II but includes
1 245 (m)	energy contributions from str C = O, and O = C–N.
1 171 (m)	?
1 126–1 100 (sh)	?
1 078 (w)	C–O or C–C str
970 (vw)*	
934 (vw)	C–C str
897 (vw)	?
826 (vw)	?
775 (vw)*	?
745 (sh)	Amide band possibly?
720 (shp–w)*	r CH_2 from lipids
700 (w)	Amide IV (probably) O = C–N + other } from peptide groups
665 (w)	Amide VI out of plane C = O str } from peptide groups
620 (w)	Amide V out of plane NH bend } from peptide groups
580 (vw)*	from lipids
425 (sh)	

vs, very strong; s, strong; m, medium; w, weak; vw, very weak; sh, shoulder; shp, sharp; str, stretch; b, bend; r, rock.

* Believed to be associated with lipid material.

affected by the extractions or by mechanical stress (9). However, bands arising from lipid material were modified by treatments. The adsorption intensities in the C–H stretching region of the spectrum contain components arising from the lipid content of untreated stratum corneum (14). After chloroform extraction, bands at 2 920^{-1} and 2 851 cm^{-1} were absent; these assignments are well established as the symmetric and antisymmetric

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

C–H stretching vibrations coming from CH_2 groups of the lipids. Confirmation of the removal of lipids from the corneum was observed by the reduction, and in some cases disappearance, of the C–H rocking mode at 720 cm^{-1} after treatment with chloroform.

The ir spectrum of stratum corneum after treatment with SDS showed no change in those absorptions assigned to the main peptide vibrations. It was found that extensive washing was necessary to remove the anionic detergent completely and that those samples treated at pH 3 required a significantly greater wash time than the ones treated at pH 7 (90 and 70 h, respectively). An interesting feature of these spectra was that the SDS (at both pH's) reduced the lipid content and water-retaining ability of the corneum, the effect being similar to that observed with $CHCl_3/H_2O$ extraction.

The conclusions from the TGA results confirmed the effects of these solvents in reducing the water-holding power of the stratum corneum and the results are given in *Table II.* Chloroform extraction alone had no effect

Table II
Percentage water loss in the temperature range 100°–140° for stratum corneum before and after extraction (average of three samples)

Extraction procedure	Weight loss (%)
None	2.43
$CHCl_3$	2.42
$CHCl_3/H_2O$	0.19
SDS(pH 7/H_2O)	0.33
SDS(pH 3/H_2O)	0.56

on water retention and the slightly higher values of percentage weight loss recorded for the samples extracted by SDS/water (compared with those extracted by chloroform/water) are probably due to incomplete wash out of the sulphate. Information could not be gained from TGA runs on SDS-treated corneum before wash out since a large weight loss occurred in the temperature region of interest (100–140°C) due to breakdown of the sulphate (SO_3 driven off).

Discussion

If stratum corneum is treated with chloroform or detergent solutions followed by aqueous extraction, the water-retaining ability of the substrate is reduced and this is reflected in a change in its elastic properties, i.e. after

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

treatment the elastic modulus increases significantly at a given relative humidity. The behaviour of stratum corneum described above contrasts with that observed if only lipid materials are removed, in which case there is no loss in the material's water-retaining capacity and no change in its elastic properties. A similar result, i.e. no change in elastic modulus, is obtained if the corneum is extracted only with water (lipid left intact). Based on findings of this type, Middleton (7), in particular, has proposed that water-soluble hygroscopic materials (e.g. urea, free amino-acids and carboxylic acid salts) are retained within the corneum by a semi-permeable membrane system which breaks down when lipids are removed. Water can pass freely through the membrane when it is intact but the water binding materials cannot be removed. This hygroscopically bound water is believed to act as corneum plasticizer. However, it is not at all clear how this hygroscopically bound water plasticizes the corneum proteins which are responsible for its mechanical integrity. One possible mechanism will now be suggested.

Due to their ionic or dipolar nature, the hygroscopic substances will be bound to the proteinaceous components of the stratum corneum. Location of these bulky molecules between protein chain segments will create a more open structure than would exist in their absence leading to enhanced protein hydration. Upon removal of the hygroscopic molecules the protein matrix collapses with the resultant formation of more protein-protein bonds (mainly hydrogen bonds and ionic interactions). An analogy can be drawn with, for example, the action of dilute phenol solutions on wool (15). When a wool fibre is stressed in such solutions it swells considerably and becomes much weaker as compared with its strength in water. This is believed to be due to the fact that phenol is much more effective in disrupting certain hydrogen bonds in the wool structure. In the case of the stratum corneum the naturally occurring hygroscopic substances act in a similar manner to phenol on wool. The higher modulus values obtained for the hygroscopic-free corneum samples (*Fig. 1*) result from the rigidity that the additional bonds formed (after removal of the hygroscopic substances) impart to the structure. Due to the reduction in swelling capacity of the corneum imposed by this structural collapse, water uptake will also be less as indicated by the TGA results presented here (*Table II*) and water-binding isotherms obtained by other authors (2, 4). The essential feature of this model is that aqueous plasticization of the corneum is due to direct protein hydration both in the presence or absence of hygroscopic substances. This is in accord with the DTA data of Bulgin *et al* (10) which indicates that bound water

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

in the stratum corneum is associated with proteins rather than with hygroscopic substances (or lipids).

Infra-red spectra of untreated and extracted stratum corneum were recorded to determine whether the molecular changes outlined above could be confirmed by this technique. Normal and polarized spectra failed to detect any change in the absorption band frequencies or intensities of the principal protein component (keratin). Consequently, any explanation that the differences in mechanical properties observed are due to a conformational change in the keratin or an alignment of the protein filaments can be ruled out. Attempts to force alignment or conformer change of the keratin by large extensions of the corneum ($>50\%$) also failed. This is not too surprising since the keratin filaments are completely contained within the corneum cells and the fibrils in contiguous cells are mechanically independent. The cell membrane/desmosomal system is, therefore, the only continuous phase pervading the entire cell structure of the corneum and, as such, is likely to be the load bearing component. Indirect evidence for the cell membrane system rather than the keratin being the rheologically active material in the stratum corneum, is provided by the fact that isolated keratin films swell considerably in water, almost to the point of dissolution, and display no detectable elasticity. This behaviour contrasts with that of untreated and extracted corneum (the extracted material should contain only keratin and cell membrane components) both of which have finite elastic modulus values in water (see 100% rh data in *Fig. 1*). The concept that the mechanical strength of the corneum resides in the cell membrane system is supported by the fact that the cell membrane protein (16) has a much higher cystine content ($\times$ 3–4) than the keratin filaments (17) leading to more permanent (with regard to disruption by water) disulphide cross-links in the membrane. Unfortunately, no independent experimental data can be obtained for the cell membranes in their native state since the ir spectrum of the whole corneum is principally that of the keratin filaments, owing to their preponderance in the structure ($\approx 60\%$ of the dry weight), and isolation of the cell membranes always involves their partial destruction.

CONCLUSIONS

(1) Elastic moduli can be used to detect changes in the stratum corneum.

(2) Extraction of the corneum with organic solvents or surface active agents followed by aqueous extraction reduces the water-retaining power of this substrate.

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)

(3) A mechanism of the effect of (2) on the stratum corneum is outlined. It is suggested that reduction of the water-retaining capacity of the stratum corneum, after extraction with the above solvents, is due to increased protein-protein interactions which reduce water binding, in the absence of hygroscopic substances, by creating a more compact structure.

(4) It is suggested that the cell membrane protein might be more important than the keratin in controlling the mechanical properties of the corneum.

(*Received: 26th July 1971*)

REFERENCES

(1) Blank, I.H. Factors which influence the water content of the stratum corneum. *J. Invest. Dermatol.* **18,** 433 (1952).
(2) Blank, I.H. Further observations on factors which influence the water content of the stratum corneum. *J. Invest. Dermatol.* **21,** 259 (1953).
(3) Blank, I.H. and Shappirio, E.B. The water content of the stratum corneum. III. Effect of previous contact with aqueous solutions of soaps and detergents. *J. Invest. Dermatol.* **25,** 391 (December, 1955).
(4) Singer, E.J. and Vinson, L.J. The water binding properties of skin. *Proc. Sci. Sect. Toilet Goods Ass.* **46,** 29 (December, 1966).
(5) Flesch, P. Chemical basis of emollient function in horny layers. *Proc. Sci. Sect. Toilet Goods Ass.* **40,** 12 (December, 1963).
(6) Jelenko, III, C. Purification of the water-holding lipid of intact skin and burn eschar. *Amer. Surg.* **35,** 864 (December, 1969).
(7) Middleton, J.D. The mechanism of water binding in stratum corneum. *Brit. J. Dermatol.* **80,** 437 (July, 1968).
(8) Laden, K. and Morrow, R. Torsional measurements on skin. *J. Soc. Cosmet. Chem.* **21,** 417 (1970).
(9) Park, A.C. and Baddiel, C.B. Rheology of stratum corneum. Part I. A molecular interpretation of the stress-strain curve. *J. Soc. Cosmet. Chem.* **23,** 3 (1972).
(10) Bulgin, J.J. and Vinson, L.J. The use of differential thermal analysis to study the bound water in stratum corneum membranes. *Biochim. Biophys. Acta.* **136,** 551 (April, 1967).
(11) Bendit, E.G. Infra-red absorption spectrum of keratin. 1. Spectra of α-, β-, and supercontracted keratin. *Biopolymers,* **4,** 539 (June, 1966).
(12) Susi, H. *Infra-red spectra of biological macromolecules and related systems.* In: Timasheff, S.N. and Fasman, G.D. *Structure and stability of biological macromolecules,* 575–663 (1949) (Dekker, New York).
(13) Krimm, S. Infra-red spectra and chain conformation of proteins. *J. Mol. Biol.* **4,** 528 (1962).
(14) Chapman, D. *The structure of lipids,* 52–128 (1965) (Methuen, London).
(15) Alexander, P. and Hudson, R.F. *Wool, its chemistry and physics,* 2nd edn, 63 (1963) (Chapman and Hall, London).
(16) Matoltsy, A.G. and Matoltsy, M.N. *J. Invest. Dermatol.* **46,** 127 (January, 1966).
(17) Crounse, R.G. *Nature (London),* **200,** 539 (November, 1963).

Purchased for the exclusive use of nofirst nolast (unknown)
From: SCC Media Library & Resource Center (library.scconline.org)