

Spectroscopic studies of skin *in situ* by attenuated total reflectance

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Synopsis—It has been shown that it is possible by the attenuated total reflectance technique to obtain ir spectra from skin *in situ*. The procedure involved the use of a 'V' shaped ATR crystal into which the side of the hand (the hypothenar eminence) was placed. The main features of such spectra agreed with those reported earlier from transmission studies through thin sections of various tissues, but there were relatively small differences in the ratios of the intensities of the absorptions in the wavelength range 1300-1000 cm^{-1} for different individuals, which were not further investigated.

The application of the ATR technique was extended to show that it was possible to detect the retention by the skin of relatively major components of a hand cream applied to the skin.

INTRODUCTION

Several reports have been published of the application of ir spectroscopy to study various tissues (1-3). These studies have been carried out by transmission through thin sections of the dried tissue, mounted onto silver chloride discs. Absorption studies of skin, in the wavelength range 2 to 15 microns, have however been rather limited (2, 4). The development of attenuated total reflectance (5), by which it is possible to obtain high contrast spectra from surfaces which are equivalent to those obtained by transmission through very thin films, suggested the possibility of obtaining spectroscopic information from skin *in situ*. Such a possibility has also been pointed out by Scheuplein (4), and more recently Hermann (6) has obtained ATR spectra from freshly removed organs, e.g. liver and kidney.

The purpose of the present communication is to show that ir spectra can be obtained from skin *in situ* by attenuated total reflectance, and

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further, that by this technique it is possible to study the retention, by the skin, of components present in products applied to the skin.

EXPERIMENTAL

The ATR spectra were recorded on a *Unicam SP 200* infrared spectrometer with increased slit width and gain. The ATR attachment, which is shown in *Fig. 1*, was equipped with a 'V' shaped analysing crystal supplied by *Research and Industrial Instruments Co.* This shape of analysing crystal,

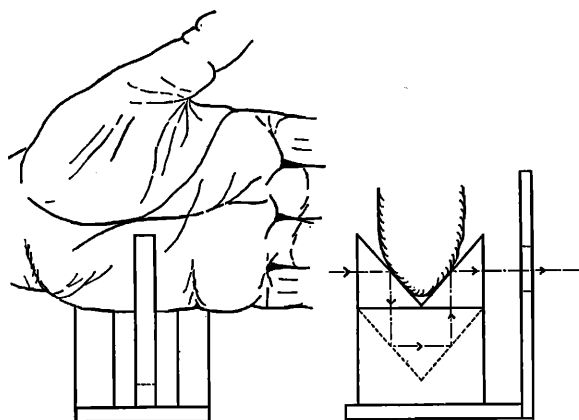


Figure 1 'V' shaped ATR unit.

which was originally suggested by Harrick (7), allowed two reflections from the skin with the incident radiation striking the crystal/skin interface at angles of 45° .

In order to record the ATR spectra, the ATR unit was placed in the sample compartment of the spectrometer and the reference beam attenuated to an absorbance value of approximately 0.1 at 1900 cm^{-1} , where the sample showed no absorption, since the transmission of the ATR crystal was only about 30%. The fleshy part of the right hand, the hypothenar eminence, was then placed into the 'V' of the ATR crystal using normal hand weight pressure, and the spectrum recorded over the wavelength range $650\text{--}5000\text{ cm}^{-1}$.

In *Fig. 1* is shown schematically the ATR unit, together with the position of the hand during the recording of the spectrum.

RESULTS AND DISCUSSION

The ATR spectrum shown in *Fig. 2(a)* was typical of those obtained from skin *in situ*. Its main features, which agreed with those reported by Blout and Mellors (1) for various tissues, are:— (i) the broad absorption centred at *ca.* 3400 cm^{-1} due to moisture, which probably obscured any absorptions due to NH groupings, (ii) the relatively weak C-H absorptions at 2950 and 2860 cm^{-1} , (iii) the very weak carbonyl absorption at 1740 cm^{-1} , due to an ester, (iv) the strong absorption at 1640 cm^{-1} , which was due to a combination of a moisture absorption and the polypeptide amide

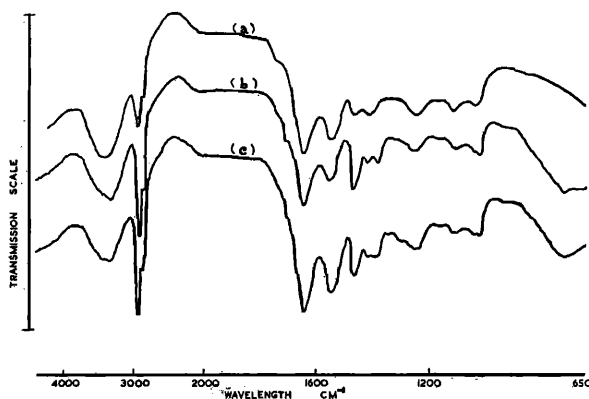


Figure 2 ATR spectra of skin.

I absorption, (v) the polypeptide amide II absorption at *ca.* 1540 cm^{-1} , and (vi) absorptions due to CH_2 and CH_3 groups at 1460 and 1380 cm^{-1} .

There were relatively small differences in the ratios of the intensities of the absorptions in the wavelength range 1300 to 1000 cm^{-1} for different individuals and a very significant difference in the intensity of the moisture absorption at *ca.* 3400 cm^{-1} . This latter effect was probably due to variations in the perspiration rate between individuals; the former variations have not been investigated further.

The spectrum shown in *Fig. 2(b)* is of the same hand after a hand cream, the major components of which were mineral oil, fatty alcohol, water and stearic acid, had been applied in the usual manner. Comparison of this spectrum with that shown in *Fig. 2(a)* shows a very significant increase in the intensities of the absorptions at 2950 , 2860 , 1460 and 1380 cm^{-1} . These absorptions are due to an aliphatic hydrocarbon grouping and the increased intensities indicated retention of the mineral oils and fatty alcohol by the

skin. There was also the appearance of a weak absorption at 1720 cm^{-1} in *Fig. 2(b)* which was due to the retention of the stearic acid. At the same time there was a marked decrease in the intensity of the moisture absorption at *ca* 3400 cm^{-1} . This indicated a fall in the moisture content of the surface of the skin, probably due to the cream exerting a barrier effect.

From *Fig. 2(c)* it can be seen that after the hand was washed with soap and water and dried, traces of the cream were still retained by the skin. This is shown by the persistence of the absorptions at 1720, 1460, 1380 and 1010 cm^{-1} .

These results show that the retention of relatively major components of a product by the skin can be demonstrated by this technique. The degree to which a component can be detected depends, of course, on the actual intensities of the component's infrared absorptions. It should be pointed out that when the retention of a component has been shown it is not known whether the component is actually on the surface of the skin or absorbed into the surface layers. From previous studies it is known, however, that the depth of sample from which ATR spectra are obtained is of the order of a few microns.

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REFERENCES

- (1) Blout, E. R. and Mellors, R. C. *Science* **110** 137 (1949).
- (2) May, L. and Grenell, R. G. *Ann. N. Y. Acad. Sci.*, **69** 171 (1957).
- (3) Schwarz, H. P., *Appl. Spectry.*, **6** 15 (1952).
- (4) Scheuplein, R. J., *J. Soc. Cosmetic Chemists*, **15** 111 (1964).
- (5) Fahrenfort, J., *Spectrochim. Acta*, **17** 698 (1961).
- (6) Hermann, T. S., *Anal. Biochem.*, **12** 406 (1965).
- (7) Harrick, N. J., *Anal. Chem.*, **36** 188 (1964).