

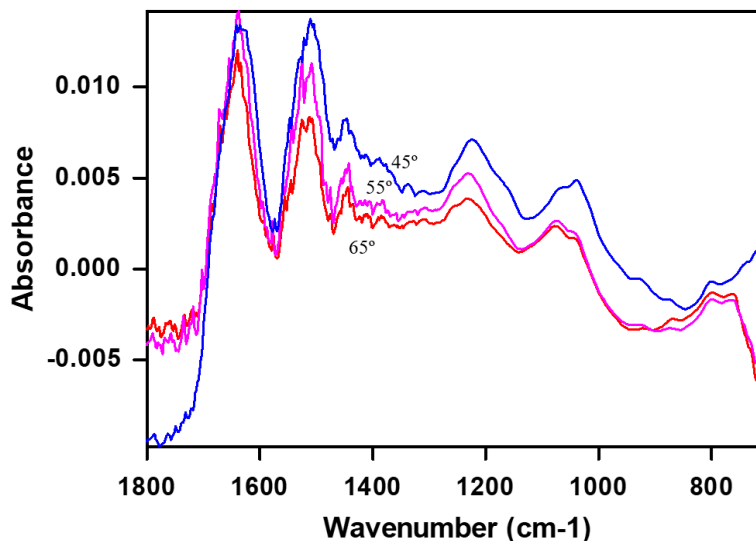
Figure 1. The Seagull™ Variable Angle Reflectance Accessory.

Analysis of Hair Using the Seagull™ Variable Angle Reflection Accessory

INTRODUCTION

The presence of breast cancer appears to alter the hair growth process, resulting in changes in the hair fiber structure.¹ A normal hair fiber is essentially dead tissue made up of alpha keratin, related proteins, and lipid materials.² The hair fiber is composed of an outer cuticle layer surrounding the central fiber core, or cortex. The cuticle is composed of flat, overlapping scales with an average cuticle layer thickness of about 2.5 μm . At the molecular level, the cuticle material is a heavily cross-linked, not highly organized, protein-lipid material that provides a chemical-resistant barrier to the cortex. The cortex (average diameter of 30 to 60 μm) is composed of a number of elongated, keratinized cells and intercellular membrane binding material, primarily protein and lipid acids and esters. The cells are symmetrically arranged so that their keratin macrofibrils are aligned

Figure 2. The ATR spectra of a sample of three human scalp hairs at incident light angles of 45° (showing cuticle and cortex material), 55° (showing primarily cuticle material) and 60° (showing only cuticle material).



along the hair shaft axis. The macrofibrils are aggregated structures of coiled-coil alpha keratin molecules. A gel-like proteinaceous matrix encapsulates these keratin filaments and macrofibrils.

Subtle changes have been observed in the synchrotron X-ray diffraction patterns of hair from breast cancer patients.¹ In the cancer hair, one or more rings of comparatively low intensity were superimposed at specific locations on the normal hair alpha keratin pattern. These changes suggest that compositional and/or conformational changes have occurred in the lipid and/or protein materials within the matrix associated with the intermediate filaments of the hair fiber. In order to demonstrate whether these changes could be seen on the infrared spectra of these hairs (without the necessity of physically removing the cuticle, which could alter the cortex), the effective penetration depth of the evanescent wave of the IR beam into the composite structure of the hair fiber had to be determined. The Seagull™ variable angle ATR cell³ was used to study whether a spectrum of the cortex portion of the hair fiber could be obtained without having to physically remove the overlying cuticle layer.⁴

EXPERIMENTAL

Spectra were obtained on a FTIR spectrometer equipped with a liquid nitrogen cooled MCT detector using the Seagull™ variable angle ATR cell (see Figure 1) with both germanium (Ge) and zinc selenide (ZnSe) IREs. Three scalp hair fibers from each individual were mounted on a sample card designed to hold the fibers in an alignment parallel to the IR beam. Spectra were collected from 4000 to 700 cm^{-1} using 128 scans at a resolution of 4 cm^{-1} (see Figure 2).

RESULTS AND DISCUSSION

Examination of the baseline corrected and smoothed (Grams 386 Savitsky-Golay program, 11 pts) spectra of adult hair fibers at 30° and 45° using a Ge IRE showed no significant differences in the spectra for the region above 1300 cm^{-1} . With the deeper penetration of the IR beam at an incident light angle of

30°, there were increased absorptions at 1180 (C-O ester stretch), 1077 (C-OH), 1043 (C-O ester stretch), and 950-850 cm^{-1} regions. This suggests that with a cuticle thickness of about 2.5 μm , the effective evanescent wave penetration is about $2d_p$. The effective depth of penetration (d_e) has been shown to increase as the ratio of the refractive indices of the sample/IRE approaches 1.0.⁵ For example, at a sample/IRE ratio of 0.25, d_e was found to approximate the calculated d_p and at a sample/IRE ratio of 0.66, d_e was shown to approximate $3d_p$.^{5,6} This is consistent with d_e for Ge being about $2d_p$.

For ZnSe (with a sample/IRE ratio of 0.64) d_e would be expected to be about $3d_p$. A comparison of a Ge 45° spectrum (cuticle material) with a ZnSe 45° spectrum (cuticle and cortex material) supports this.⁴ The cortex of the hair appears to contribute to some degree to the spectrum below 2400 cm^{-1} for a 45° incident light angle with the ZnSe IRE.

This study demonstrates that the FTIR-ATR spectrum of a hair fiber obtained with the Seagull™ variable angle reflection accessory with a ZnSe IRE at an incident light angle between 45° and 40° (the critical angle) appears to enable the detection of spectral changes in cortex materials in the 2000 to 700 cm^{-1} region without physically removing the cuticle from the hair fiber.

REFERENCES

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