SHIMADZU APPLICATION NEWS

LAAN-A-FT-E016

● SPECTROPHOTOMETRIC ANALYSIS

No.A389

Protein Thin Film Analysis by Reflection Absorption Spectroscopy and Single Reflection ATR

In Application News No. A362 and A377, we introduced the analysis of SiO_2 thin film on Si wafer and analysis of hydrogen-terminated silicon surface by transmittance measurement and single reflection ATR. Here, we introduce the results of analysis of protein thin film on gold and hydroxyapatite as examples of organic thin film analysis on metallic and inorganic surfaces, respectively, using reflection absorption spectroscopy and single reflection ATR.

■ Measurement of Fibrinogen Thin Film on Gold

Fibrinogen is a rod-shaped, water-soluble protein present in blood, having a molecular weight of about 340,000, a diameter of 9 nm and length of 45 nm. We conducted measurement of a monomolecular film of this fibrinogen on gold plating using 80-degree incident angle reflection absorption spectroscopy. The analytical results are shown in Fig.1. Amide Ⅰ and amide Ⅱ are clearly seen in the vicinity of 1666 and 1545 cm^{-1}, respectively, and the N-H expansion contraction vibration is evident near 3312 cm^{-1}.

Fig.2 shows the results of analysis of a laminate sample consisting of hydroxyapatite (thickness approx. 10 nm) and fibrinogen monomolecular film on gold plating (gold/hydroxyapatite/fibrinorgen). The hydroxyapatite (in vicinity of 1100 cm^{-1}) and fibrinogen peaks are plainly visible. In the measurement of this sample, the infrared light irradiated on the sample was reflected off both the surface of the fibrinogen as well as the fibrinogen-hydroxyapatite interface. However, as the total amount of reflected light from these surfaces is extremely small compared to that reflected from the gold, this can be considered to be a reflection absorption spectrum obtained due to reflection of the light off the gold surface after having been transmitted through the fibrinogen and hydroxyapatite layers.

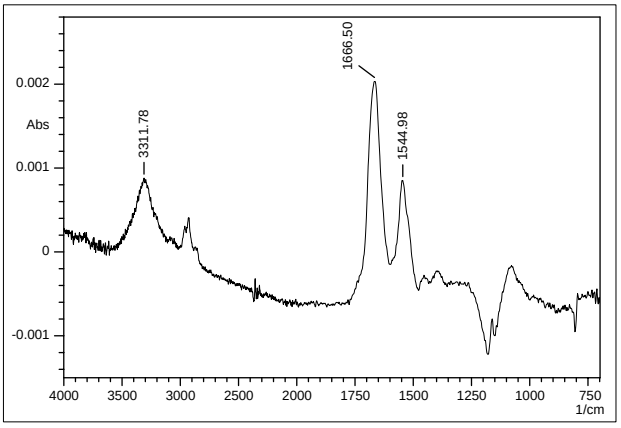

Fig.1 RA Spectrum of Fibrinogen Thin Film on Gold

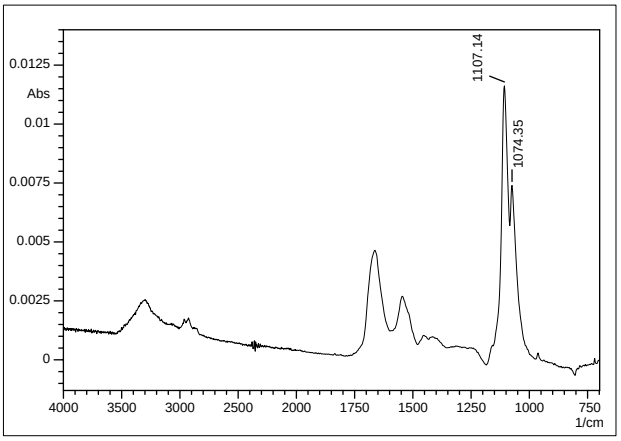

Fig.2 RA Spectrum of Hydroxyapatite & Fibrinogen Laminate Thin Film on Gold

■ Measurement of Fibrinogen Thin Film on Hydroxyapatite

Fig.3 shows the results of analysis of a fibrinogen monomolecular film on hydroxyapatite using the same method of 80-degree incident angle reflection absorption spectroscopy as mentioned above. The hydroxyapatite consisted of a mirror-polished sintered hydroxyapatite plate. The same type of hydroxyapatite was also used as the reference sample. Since the reflection coefficient of hydroxyapatite is lower than that of gold and other metals, the refection spectrum of film on hydroxyapatite consists not only of light reflected from the surface of the hydroxyapatite, but is affected by reflected light from the film surface. In Fig.3, the peak-like structures seen in the vicinity of 1250 to 1000 cm^{-1} are residues of the hydroxyapatite reflection spectrum. In the absorption region of hydroxyapatite, if the reflection spectra of BKG measurement and sample measurement were to match, they would offset each other, but they do not match due to the reflected light from the fibrinogen surface. The result is that it becomes a residue which appears in the spectrum.

A magnification of the region between 1900 to 1300 cm^{-1} is displayed with the spectrum in Fig.3. It is evident that the peaks of amide Ⅰ and amide Ⅱ are inverted. Since hydroxyapatite does not display much absorption in the region from 1800 to 1400 cm^{-1}, there are no great fluctuations in reflectivity. However, since fibrinogen displays considerable absorption, there is a great deal of reflectivity fluctuation in this region. The inversion of the amide Ⅰ and amide Ⅱ peaks can be attributed to this fluctuation.

Fig.4 shows the results of single reflection ATR measurement of the same sample, using a Ge prism. The peaks in the vicinity of 1084, 1016 and 962 cm^{-1} are peaks originating from the hydroxyapatite. The magnified section displayed in Fig.4 shows the region from 1900 to 1300 cm^{-1}. Here, the inverted amide Ⅰ and amide Ⅱ peaks obtained by reflection absorption are obtained in their normal orientation.

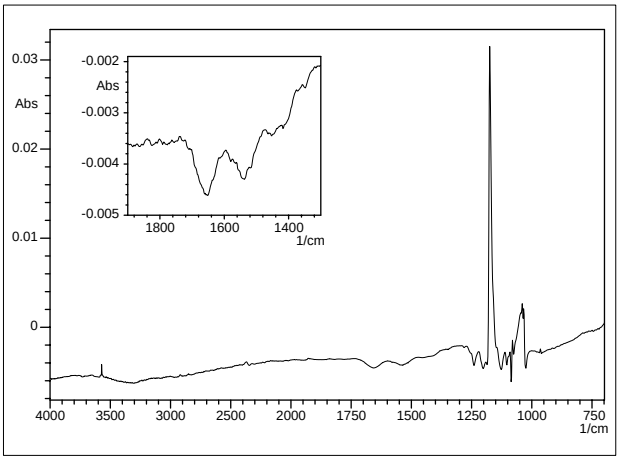

Fig.3 RA Spectrum of Fibrinogen Thin Film on Hydroxyapatite

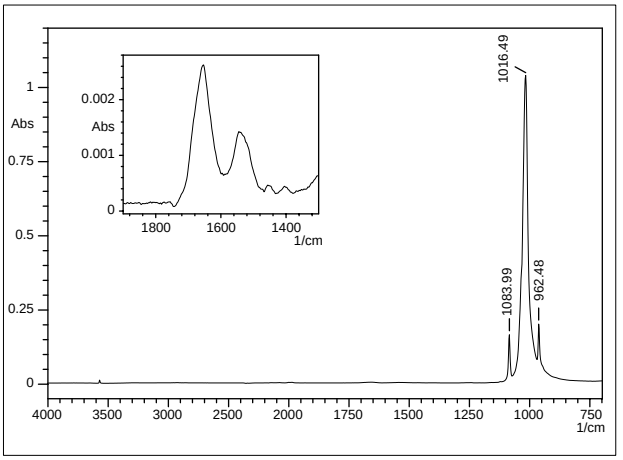

Fig.4 ATR Spectrum of Fibrinogen Thin Film on Hydroxyapatite

Reflection absorption spectroscopy is a very effective method for non-contact, simple measurement of metallic surface thin films. However, in cases where the substrate is a non-metallic material, various problems, such as weak peak intensity, peak distortion and inversion, are often encountered. Although single reflection ATR requires contact between the sample and prism, it can be used even for measurement of samples with non-metallic substrates.

Table 1 Analytical Conditions

Attachment	: VeeMAX (RAS), MIRacke-Ge (ATR)
Resolution	: 4 cm^{-1}
Accumulation	: 400 (RAS), 200 (ATR)
Detector	: MCT (RAS), DLATGS (ATR)

The measurement sample introduced here was kindly provided by Dr. Toshiyuki Ikoma and Dr. Akira Monkawa of the Biomaterials Center, National Institute for Material Science, Japan.

SHIMADZU

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