

FTIR TALK LETTER

vol. **6**

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Why does a spectrum baseline bend?

European Pharmacopoeia 5.0

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In Vol. 4 of FTIR Talk Letters, we discussed validation methods for infrared spectrophotometers. We introduced the European Pharmacopoeia 4.0 as one of the standards for the infrared of spectrophotometers. Content related to infrared spectrophotometers is described in the section “2.2.24 Absorption Spectrophotometry, Infrared”, and instrument validation methods are described under the items “Control of resolution performance” and “Verification of the wave-number scale”.

In 2005, the European Pharmacopoeia 5.0 was introduced, and these standards were revised. In this volume, we introduce the points of revision and how the Shimadzu FTIR series addresses these changes.

Revised Points

The items that were revised in revision relate to Fourier Transform infrared spectrometers (FTIR). There were no changes relating to dispersive infrared spectrophotometers.

Changes in Control of Resolution Performance

The description given in the European Pharmacopoeia 5.0 is as follows.

For Fourier-transform instruments, ... for example by recording the spectrum of a polystyrene film approximately 35 μm in thickness. The differences between the absorbances at the absorption minimum at 2870 cm^{-1} and the absorption maximum at 2849.5 cm^{-1} is greater than 0.33. The differences between the absorbances at the absorption minimum at 1589 cm^{-1} and the absorption maximum at 1583 cm^{-1} is greater than 0.08.

There was a change from the use of transmittance measurement of spectra in the European Pharmacopoeia 4.0 to the use of spectra obtained by absorbance measurement, and the resolution standard is now specified according to absorbance measurement exclusively.

Changes in Verification of Wave-number Scale

The verification of wavenumber scale in the European Pharmacopoeia 5.0 is as specified below.

3060.0 (± 1.0) cm^{-1}
2849.5 (± 1.0) cm^{-1}
1942.9 (± 1.0) cm^{-1}
1601.2 (± 1.0) cm^{-1}
1583.0 (± 1.0) cm^{-1}
1154.5 (± 1.0) cm^{-1}
1028.3 (± 1.0) cm^{-1}

The wavenumber verification criteria in European Pharmacopoeia 4.0 specified as ± 1.5 for the three wavenumbers 3060.0, 2849.5 and 1942.9 cm^{-1} was changed to an allowable variance of ± 1.0 in the new standard.

Validation Program Supporting European Pharmacopoeia 5.0

The validation program supporting the European Pharmacopoeia 5.0 is included in IRsolution Ver. 1.30.

In both the Japanese Pharmacopoeia and European Pharmacopoeia 4.0, instrument performance evaluation is based on the 5 test items: (1) power spectrum, (2) resolution, (3) wavenumber accuracy, (4) wavenumber repeatability and (5) absorbance reproducibility.

(1) Power Spectrum

Although not included in the European Pharmacopoeia 5.0, this test is used to evaluate the most basic performance of the FTIR. The test procedure is the same in the validation program for both the Japanese Pharmacopoeia and European Pharmacopoeia 4.0. This test compares the intensity of the power spectrum at a specified wavenumber to the criterion values. When the measured intensity is equal or larger than the criterion value, the test is passed.

(2) Resolution

This is as described above. A pass rating is assessed if each of the absorbance values is equal to or greater than the respective criterion value.



(3) Wavenumber Accuracy

This is as described above. A pass rating is assessed if each of the wavenumber measurement values is within the specified variance.

(4) Wavenumber Reproducibility

Although this test is not included in the European Pharmacopoeia 5.0, this is regarded as a test item due to standardization with the Japanese Pharmacopoeia. The test procedure is the same in the validation program for both the Japanese Pharmacopoeia and European Pharmacopoeia 4.0. Polystyrene film is measured twice, and the peak wavenumbers are compared at three specified points. The program assesses whether or not the difference between the two measurement results is within the permissible range. If the difference in peak wavenumbers is within the permissible range at all three specified points, a pass rating is assessed.

(5) Absorbance Reproducibility

Although this test is not included in the European Pharmacopoeia 5.0, this is regarded as a test item due to standardization with the Japanese Pharmacopoeia. The test procedure is the same in the validation program for both the Japanese Pharmacopoeia and European Pharmacopoeia 4.0. The permissible range according to the Japanese Pharmacopoeia is 0.5%, expressed in transmittance; however, in the validation program that supports the European Pharmacopoeia 5.0, transmittance values that fluctuate $\pm 5\%$ from the transmittance at the test wavenumber are converted to absorbance, and are then evaluated with respect to the permissible range. For example, the absorbance of the 2849.5 cm^{-1} peak is about 1 ABS (about 10% transmittance), so the absorbance fluctuation (± 0.03 ABS) corresponding to the $\pm 0.5\%$ transmittance fluctuation is considered the permissible range. Polystyrene film is measured twice, and the absorbance values are compared at three specified points. The program assesses whether or not the difference between the two measurement results is within the permissible range. If the difference in absorbances is within the permissible range at all three specified points, a pass rating is assessed.

Support for European Pharmacopoeia 5.0 in Shimadzu FTIR Series

The European Pharmacopoeia 5.0 is supported by IRsolution Ver. 1.30 and the included validation program. The supported instruments are the IRPrestige-21 and FTIR-8400S, 8300, 8400 (as of November 1, 2006).

To enable support for the European Pharmacopoeia 5.0 in IRPrestige-21 and FTIR-8400S instruments already in operation, please contact your Shimadzu representative to upgrade IRsolution to Ver. 1.30.

Standardizing Validation Standards of Japanese Pharmacopoeia and European Pharmacopoeia 5.0

The European Pharmacopoeia is, as the name indicates, the compilation of standards that apply to the European Union (EU). When products are shipped into the EU, they often require instrument validation complying with the European Pharmacopoeia 5.0. On the other hand, in Japan, compliance with the Japanese Pharmacopoeia is fundamental.

The Japanese Pharmacopoeia is periodically revised, often with a view to linking it with the pharmacopoeia of other countries. The Japanese Pharmacopoeia infrared absorption spectrum measurement method was linked to the European Pharmacopoeia 4.0 in Revision 14, Supplement 1. With the release of the European Pharmacopoeia 5.0, differences with the Japanese Pharmacopoeia again occurred; however, it is likely that re-linking of the standard will occur in the near future with another revision.

(However, as of November 1, 2006, there is no information regarding revision of the Japanese Pharmacopoeia infrared absorption spectrum measurement method.)

Microscope Measurement Techniques — Reflection and ATR Measurements —

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FTIR TALK LETTER Vol. 5 introduced transmission measurements with the infrared microscope. This FTIR TALK LETTER introduces both ATR and reflectance measurements with the same system.

IR Microscope Reflection Method Precautions

The IR microscope reflection technique is extremely simple, requiring almost little or no sample preparation. However, measurements are limited to certain forms of samples. One of the typical samples suited to microscope reflection measurement is foreign matter adhering to a highly reflective metal substrate.

Three precautions related to IR microscope reflection analysis of samples adhering to a metallic plate are described below.

(1) Analysis of a Thick Sample

Analysis of a thick object adhering to a metallic plate surface is illustrated in the schematic diagram of Fig. 1.

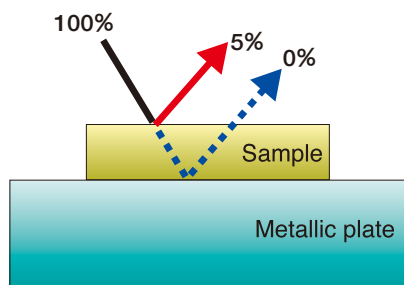


Fig. 1: Schematic Diagram of a Thick Sample Adhering to a Metallic Plate

The specular reflection from the sample surface, as indicated in Fig. 1, is about 5% of the total incident light. In Fig. 1, the sample is thick enough so that even if some light is reflected from the metal surface, almost zero light will be reflected from the sample surface. The specular reflectance spectrum obtained here displays a distortion in the first derivative spectrum due to a large change in the reflectance (refractive index abnormal diffusion) where strong absorption occurs. However, a spectrum similar to an absorption spectrum can be obtained by applying Kramers-Kronig (K-K) analysis.

Typically, less light will reach the detector during a reflectance measurement with an IR microscope as compared to a transmission measurement. Especially when analyzing a thick sample in reflection mode, the amount of light is markedly decreased, requiring considerable integration to improve the signal-to-noise ratio.

Fig. 2 shows a measurement example of a glossy coating sheet (about 1 mm thick) on a metallic plate. Measurement was conducted by focusing on the sample surface and setting the aperture to $30 \times 30 \mu\text{m}$.

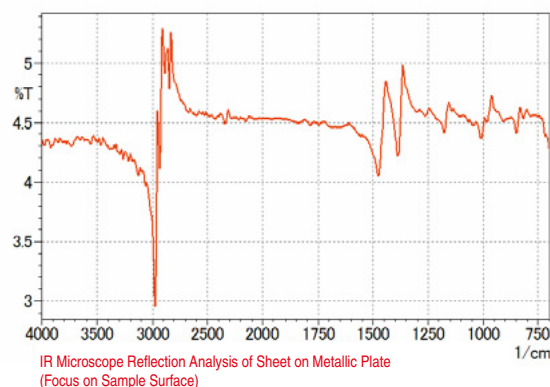


Fig. 2: Specular Reflectance Spectrum of Sheet on a Metallic Plate

In Fig. 2, the peak shows a distortion in the first derivative spectrum as described above, unlike that observed in a normal absorption spectrum. Fig. 3 shows the result of applying K-K analysis to the spectrum of Fig. 2. It is easily determined from Fig. 3 that the coating sheet is polypropylene.

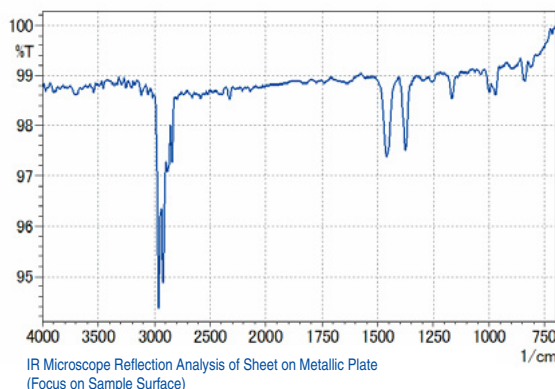


Fig. 3: Spectrum after K-K Analysis

Fig. 4 shows the results of a measurement when focusing on the surface of the metallic plate, rather than the sample surface.

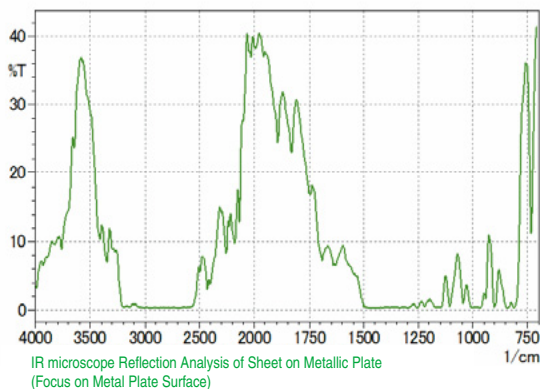


Fig. 4: Reflection Absorption Spectrum of a Sheet on a Metallic Plate

In Fig. 4, it is evident that peaks are saturated in the reflection absorption spectrum due to the thickness of the sample.

In this way, when the sample is thick, only the specular reflection spectrum can be obtained if the focus is on the sample surface, and qualitative analysis of the sample is possible by applying K-K analysis. However, with samples like powders and fibers, which have a rough surface, since most of the reflected light is diffused, it is difficult to detect only the specular reflection component from the sample surface. When sampling is possible, the IR microscope transmission method is suitable, and when the target object is protruding from the surface, the IR microscope ATR method is more applicable.

(2) Analysis of a Thin Sample

Analysis of a thin object adhering to a metallic plate surface is illustrated in the schematic diagram of Fig. 5.

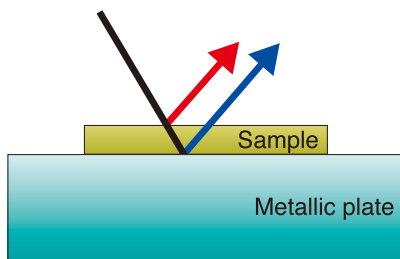


Fig. 5: Schematic Diagram of a Thin Sample Adhering to a Metallic Plate

When the sample is thin, as shown in Fig. 5, incident light is reflected from the surface of the metallic plate, and exits from the surface of the sample. The spectrum obtained in this case is a reflection absorption spectrum similar to that

obtained in transmission measurement when large amounts of light are reflected from the metal surface (blue line in Fig. 5). However, if only a small amount of light is reflected from the metal surface, a spectrum consisting of a mixture of pure specular reflection and reflection absorption spectrum is obtained. When such a mixed spectrum is obtained, positive peak distortion (increased transmission) occurs at the higher wavenumber side of peak position, and conversely, negative distortion (reduced transmission) occurs at the lower wavenumber side. This characteristic is the same as the Christiansen effect seen when using the KBr pellet and Nujol mul methods. (Christiansen effect: Phenomenon in which the amount of transmitted light changes in the vicinity of the peak position due to the relationship between the refractive index and reflective one. This often occurs with light-scattering samples.)

Fig. 6 shows a measurement example using a thin sample. Measurement was conducted using a $20 \times 20 \mu\text{m}$ aperture setting. Measurements 1 and 2 show results obtained at different positions.

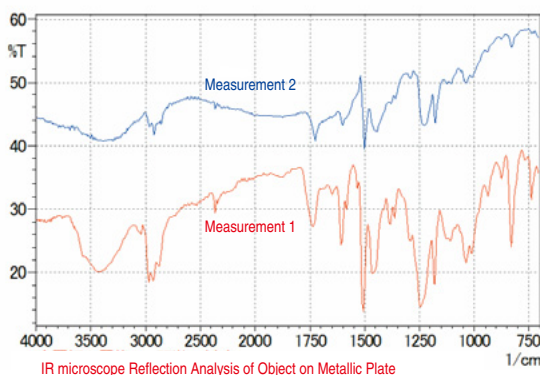


Fig. 6: Spectrum of an Object Adhering to a Metallic Plate Surface

In Measurement 1, a normal absorption spectrum is observed, but in Measurement 2, peak distortion is seen in the vicinity of 1500 cm^{-1} . When this type of spectrum is obtained, since the peak position and absorption intensity are different from the proper values, it is necessary to search for a position where a spectrum like that of Measurement 1 can be obtained. If the target object is extremely small and it is not possible to change the measurement position, use the IR microscope transmission or IR microscope ATR method to obtain an undistorted spectrum.

(3) Background(BKG) Measurement Position

As described in the precautions required when using the IR microscope transmission technique in Vol. 5, the BKG and sample measurement positions have an influence on the spectrum in the IR microscope reflection method as well. Fig. 7 and Fig. 8 show an example of analysis of an object adhering to a metal plate surface. Fig. 8 shows the spectra obtained by BKG measurements at the two positions (BKG 1 and BKG 2), with the sample position fixed on the foreign matter on the plate.

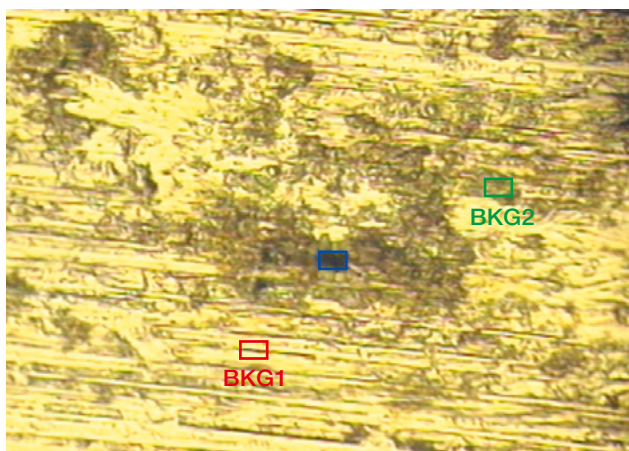


Fig. 7: Microscope Photograph of Foreign Matter on a Plate

In Fig. 8, peaks are not as distinguishable in BKG 2 as in BKG 1. It is thought that in BKG 2, a minute quantity of material identical to the foreign matter is stuck to the plate, and the BKG peaks are offset by the peak of the foreign matter.

This illustrates the importance of carefully selecting the BKG and sample measurement positions in reflection measurements, like in transmission measurements.

A spectrum search conducted for the red line spectrum in Fig. 8 (BKG 1) revealed that the substance is sodium lactate.

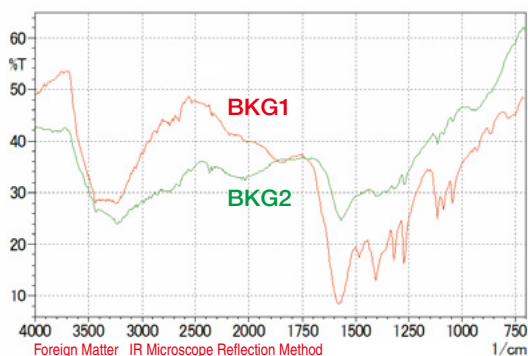


Fig. 8: Spectra of Foreign Matter on a Plate

Precautions in IR Microscope ATR Technique

The IR microscope ATR technique is extremely simple, requiring almost no sample pretreatment. However, there are several precautions to be kept in mind when using this method. Three important precautions are described below.

(1) Residual Substances on Prism

In analysis using the IR microscope ATR method, the sample is tightly pressed against the prism. Therefore, prior to analyzing another sample, it is necessary to check for any residual sample substance that may have been transferred to the prism, and to clean off this residual material. This operation flow is illustrated in Fig. 9.

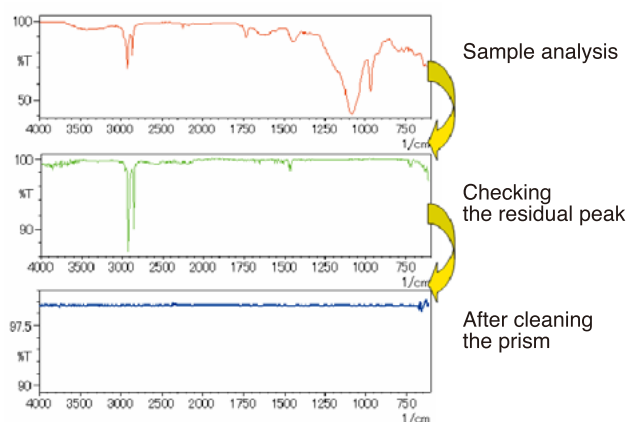


Fig. 9: Verification of Residual Matter on the Prism

After conducting BKG measurement, sample measurement is conducted with the sample pressed against the prism. After that, the sample and prism are separated, and the spectrum is checked using monitor-measurement. If no peaks are seen as shown in the bottom spectrum of Fig. 9, analysis of the next sample is possible because there is no residual substance on the prism. However, if peaks are seen as shown in the middle spectrum of Fig. 9, it indicates the presence of residual substance due to transfer of sample to the prism. In this case, the surface of the prism must be cleaned using, for example, a cotton-tipped applicator soaked in ethanol.

However, if the sample consists of a mixture of substances, it is highly possible that only one of the substances from the mixture is stuck to the prism, and by measuring the transferred spectrum, very detailed information might be obtained. For example, if the foreign matter includes oil, measurement of the sample in contact with the prism will provide a spectrum of both the foreign material and oil, but after separating the prism from the sample, only the oil spectrum will be obtained. Therefore, if a subtraction spectrum is calculated by subtracting the residue spectrum from the spectrum measured in the contact state, a spectrum of the foreign material alone will be obtained.

(2) Sample Surface Condition and Contact Position

As measurement using the IR microscope ATR technique requires that the sample and prism closely contact one another, depending on the condition of the sample surface and the contact position, a good spectrum may not be obtained. If the sample surface is irregular, as shown in Fig. 10, a spectrum may not appear depending on the position.

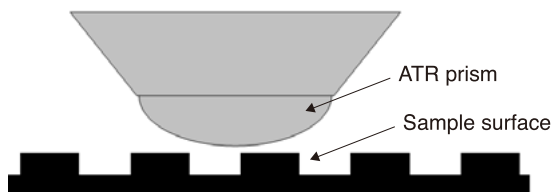


Fig. 10: Schematic Diagram of Sample and Prism Contact

Fig. 11 shows an example of IR microscope ATR analysis of dirt stuck to a metal surface.

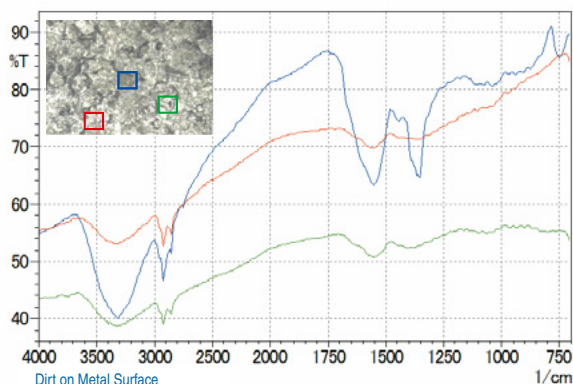


Fig. 11: Spectra of Dirt on a Metal Surface

In Fig. 11, peaks can be identified in the blue spectrum (blue-outlined area in the photo), but no distinct peaks are obtained in the red and green spectra (red- and green-outlined areas in the photo). This is probably because it is very difficult to obtain good contact with those areas, or because the adhering material in those areas is very thin. In this way, when no distinct peaks are obtained, it is necessary to change the measurement position.

(3) Pressing Force

When using the IR microscope ATR technique, the spectrum may change according to the force with which the sample is pressed against the prism. Care is especially necessary when the sample is soft. Consider the situation in which a multi-layer soft sample is measured, as illustrated in Fig. 12.

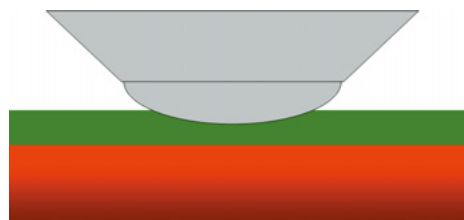


Fig. 12: Pressing the Prism Against a Multi-Layer Soft Sample

When lightly pressed, a spectrum is obtained only for the green layer in Fig. 12, but as the pressing force is gradually increased, the green layer is gradually stretched thin, and the spectrum of the red layer will be superimposed on that of the green layer.

An example of IR microscope ATR analysis of such a multi-layer soft sample according to the above description is shown in Fig. 13. The spectra correspond to pressing force in the order of $A < B < C$.

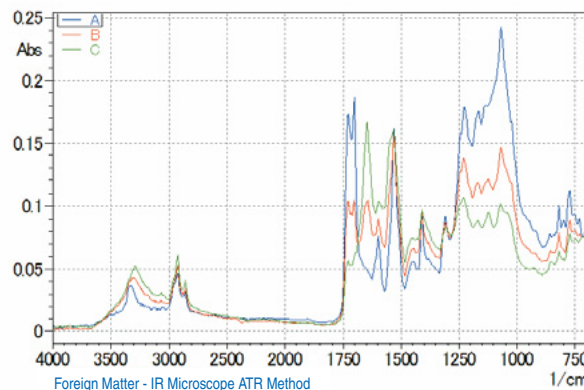


Fig. 13: Spectrum Change due to Changing Pressing Force

It is evident that as the pressing force increases, a peak becomes evident in the vicinity of 1650 cm^{-1} , and conversely, the peaks in the vicinity of 1700 cm^{-1} and between 1250 and 1000 cm^{-1} become weaker. The most superficial layer corresponds to spectrum A in Fig. 13, and the compounds in the lower layer can be obtained by calculating the subtraction spectrum between spectra C and A.

From the resulting subtraction spectrum, the most superficial layer was identified as urethane, and the inner layer as an amide compound.

This FTIR TALK LETTER introduced important precautions when using the IR microscope reflection technique and IR microscope ATR technique. Follow these precautions to more effectively utilize your IR microscope.

Q & A

Question

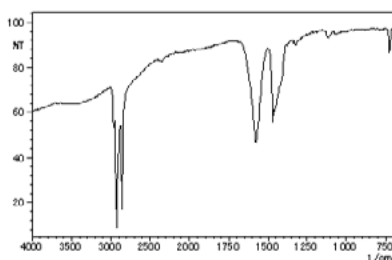
Why does a spectrum baseline bend?

Answer

The causes for bending of an infrared spectrum baseline are as follows.

1. Influence of scattering (in transmission, specular reflection and other methods)

Transmission spectra of samples with rough surfaces or samples containing powders of inorganic substances may show a baseline sloping down to the left (%T display). This is because the irradiated infrared light scatters on the sample surface or in its interior, and this effect increases with decreasing light wavelength. Therefore, infrared spectra influenced by scattering slope down toward the short wavelength's (high wavenumber) side (%T display).



Baseline Sloping Down to the Left due to Scattering
IR Microscope Transmission Spectrum of Magnesium Stearate

2. Influence of carbon black, etc. (ATR method)

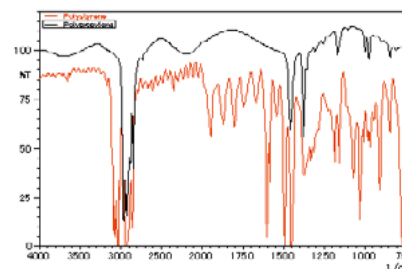
ATR spectra of black rubber and other samples containing carbon black show a baseline sloping down to the right (%T display). Carbon black displays absorption over the entire region from 4000 to 400 cm^{-1} , but when using the ATR technique, the influence of carbon black becomes greater at the long wavelength's (low wavenumber) side, where the baseline drops significantly. The result is a spectrum where the baseline descends on the right side (%T display).



ATR Spectrum of Butyl Rubber Containing Carbon Black

3. Influence of interference fringe (transmission, specular reflection and other methods)

When measuring the transmission spectrum of a sample with a smooth surface such as film, the baseline may appear as a regular sine curve pattern. These are interference fringes due to multiple reflections of light within the sample.



Transmission Spectra of Polystyrene and Polypropylene Affected by Interference Fringes
(Fringe length differs according to sample thickness and refractive index.)

4. Influence of background measurement

In specular reflection measurement of coatings on metals, if conditions differ between the surface of the reference sample and measurement target (measurement position), the baseline will be affected. For example, if the surface of the reference sample is rougher and more largely curved than that of the sample, the spectrum baseline shows a transmittance exceeding 100%T.

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