

Analysis of Amino Acids by KBr Tablet Method

In the transmission method, light is irradiated on a measurement sample, and the light that passes through the sample is detected. However, the adequate technique (accessory device) differs depending on the sample shape. Among techniques used with the transmission method, the KBr tablet method is mainly used to measure solids and powders. The KBr tablet method is a necessary and indispensable procedure which has been standardized not only in the Japanese Pharmacopoeia (JP), but also in the United States Pharmacopoeia (USP), standards of the American Society for Testing and Materials (ASTM).

Special care is necessary in order to obtain a satisfactory spectrum by the KBr tablet method, including securing a uniform powder size and maintaining a constant pressure during tablet forming (pelletizing). In particular, the effect of moisture requires special attention. In this experiment, we conducted an analysis of amino acids, which easily absorb moisture, by the KBr tablet method. This introduction also includes an example of failure.

S. Iwasaki

■ Pixie Portable Hydraulic Press for Pelletizing

A Pixie 2.5 t portable hydraulic press was used in pelletizing. Fig. 1 shows the appearance of the Pixie press. With a small footprint of W12.7 cm × D19.2 cm, Pixie can also be used in a glovebox or other environments with space limitations. The pelletizing form is fixed by tightening the blue knob on top to prevent movement, and pressure is applied by turning the hydraulic press knob on the front. Fine pressure adjustment is possible by checking the pressure gauge. Details of the Pixie press can be found in Application News No. A576 ⁽¹⁾.



Fig. 1 Pixie 2.5 t Portable Hydraulic Press

■ Analysis of L-Phenylalanine

Phenylalanine is an essential amino acid which is found in large quantities in proteins in milk, eggs, meat, and other foods ⁽²⁾. The official monograph for JP drugs in the 17th Edition of JP ⁽³⁾ describes the identification test for L-phenylalanine as follows: "Determine the infrared absorption spectrum of L-Phenylalanine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers".

The attenuated total reflectance (ATR) spectrum and transmission spectrum of L-phenylalanine by the KBr tablet method were obtained. Table 1 shows the measurement conditions. A QATR™ 10 single-reflection ATR accessory was used in the ATR measurement. Background measurement by the KBr tablet method was done using a tablet consisting of only KBr. Compensation for infrared scattering loss and water absorbed by KBr is possible.

Fig. 2 shows the measurement results. In comparison with the transmission spectrum, the relative peak intensity of the ATR spectrum is different, and the peak positions have shifted to the low wavelength side. However, it was possible to obtain a spectrum similar to the transmission spectrum, as shown in Fig. 3, by applying Shimadzu Advanced ATR correction processing feature to the ATR spectrum.

Table 1 Measurement Conditions

Instruments	: IRTracer™-100, Pixie, QATR 10 (diamond prism)
Resolution	: 2 cm ⁻¹
Accumulation	: 45 times
Apodization function	: Sqr-Triangle
Detector	: DLATGS

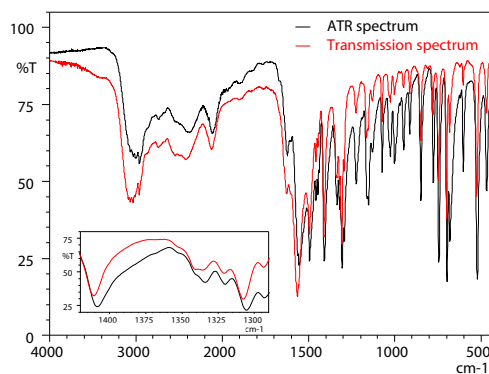


Fig. 2 Infrared Spectra of L-Phenylalanine
(Overlay of Transmission Spectrum and ATR Spectrum)

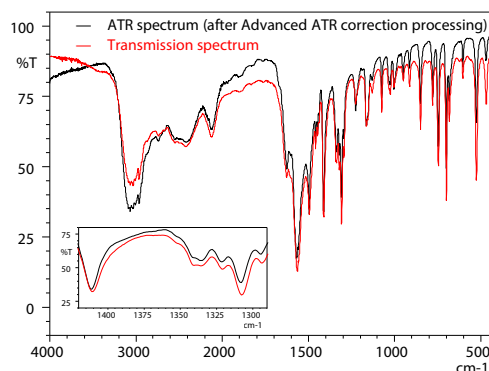


Fig. 3 Infrared Spectra of L-Phenylalanine
(Overlay of Transmission Spectrum and ATR Spectrum After
Advanced ATR Correction Processing)

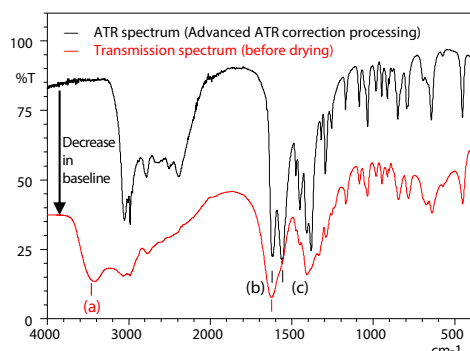
■ Analysis of L-Proline

Proline is the amino acid raw material for the collagen in skin and other tissues⁽²⁾. The description of the identification test in the monograph for JP drugs in the 17th Edition of the JP is essentially the same as the above-mentioned description for L-phenylalanine. However, L-proline absorbs moisture so easily that the JP describes it as “deliquescent.”

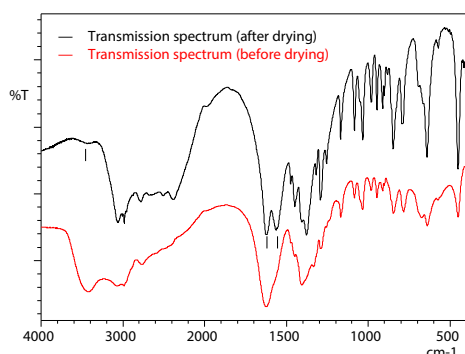
The ATR spectrum and the transmission spectrum of L-proline by the KBr tablet method were obtained. Fig. 4 shows the measurement results. Although the tablet was pelletized under the same humidity-controlled condition as the above-mentioned L-phenylalanine, a large peak appeared at 3,420 cm⁻¹ (a) in the transmission spectrum, and only one peak could be obtained for the two peaks that should inherently appear at 1,622 cm⁻¹ (b) and 1,563 cm⁻¹ (c). The baseline also decreased, presumably due to the poor transparency (cloudiness) of the tablet. All these problems are attributable to the effects of moisture. Even though the analysis was performed quickly, a good infrared spectrum could not be obtained.

Therefore, referring to Application News No. A376⁽⁴⁾, the tablet was dried using a drier. Fig. 5 shows the transmission spectrum after drying. The effects of moisture were almost completely eliminated, and the spectrum is similar to the ATR spectrum after Advanced ATR correction processing in Fig. 4.

As this example shows, when a sample has extremely high moisture absorption, it is difficult to obtain a good spectrum, even when the measurement is done with great care. As a solution to this problem, Shimadzu has also studied pelletizing in a dry box with the atmosphere replaced with dry air⁽⁵⁾.



**Fig. 4 Infrared Spectra of L-Proline
(Overlay of Transmission Spectrum before Drying and ATR
Spectrum After Advance ATR Correction Processing)**



**Fig. 5 Infrared Spectra of L-Proline
(Overlay of Transmission Spectra Before/After Drying)**

■ Tips for Analysis of Moisture-Absorbent Samples

The following summarizes the moisture-related precautions for analysis in measurements by the KBr tablet method.

- ① Store the sample and crystalline (or powder) KBr in a desiccator or a container with a desiccant such as silica gel.
- ② Carefully dry the tablet form device, agate mortar and pestle, and spatula.
- ③ Carry out powdering and pelletizing within a short time.

The actions and preventive measures in case of moisture absorption are as follows.

- ① Dry the tablets by using a drier or a drying machine. However, this method should only be used with stable samples, as the properties of the sample may be changed by heating.
- ② Although this method requires time, leave the tablets in a desiccator or a container with a desiccant for a sufficient time.
- ③ Conduct pelletizing in a dry box, which the atmosphere has been replaced with dry air.

■ Conclusion

This article introduced examples of analysis of amino acids by the KBr tablet method. In recent years, practical application of the ATR measurement method has progressed, and ATR has become a convenient method for analysis of solid samples. Nevertheless, the KBr tablet method is still an indispensable technique, as in the past. Smooth measurement by the KBr method is possible even with moisture-absorbent samples if certain simple precautions are applied to avoid moisture effects. For a more detailed explanation of the principle and measurement method, refer to FTIR TALK LETTER Vol. 14 “The ABCs of Measurement Methods – KBr Pellet Method”⁽⁶⁾.

References

- (1) Application News No. A576, “KBr Pellet Formation Using Pixie Mini Hydraulic Press for Pelletizing and Measurement by Compact FTIR”
- (2) Ajinomoto Co., Inc., Encyclopedia of Amino Acids
<https://www.ajinomoto.co.jp/amino/>
- (3) Ministry of Health, Labour and Welfare, Japanese Pharmacopoeia 17th Edition
- (4) Application News No. A376, “Considerations in KBr Pellet Method – Part 2 Samples that require attention when analyzed by KBr method (Amino Acids)”
- (5) Mitsuo Tasumi, Ed., “Infrared Spectroscopy – Fundamentals and Recent Techniques”
- (6) Shimadzu Corporation, FTIR TALK LETTER Vol. 14 “The ABCs of Measurement Methods – KBr Pellet Method –”

QATR and IRTracer are trademarks of Shimadzu Corporation in Japan and/or other countries.

Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol “TM” or “®”.