SHIMADZU APPLICATION NEWS

LAAN-A-FT-E009

● SPECTROPHOTOMETRIC ANALYSIS

No.A376

Considerations in KBr Pellet Method – Part 2 Samples those require attention when analyzed by KBr method (Amino Acids)

In previous Application News No. A370, we introduced hydrochloride as a typical sample that should not be analyzed by the KBr method due to the occurrence of ion exchange. Here we introduce an analysis of an amino acid by the KBr method, demonstrating the possibility that the spectrum shape could change using this method.

■ Measurement of L-Arginine

Fig.1 shows a spectrum of L-arginine measured by the KBr method. The analytical conditions are shown in Table 1.

Table 1 Analytical Conditions

Resolution	: 4cm^{-1}
Accumulation	: 45
Detector	: DLATGS

Fig.2 shows the measured spectrum of L-arginine using single-reflection total reflectance ATR (DuraSampl IR-Ⅱ, diamond/KRS-5 prism), which requires no pretreatment, overlaid with the spectrum of Fig.1. Here, the single-reflection total reflectance ATR data was subjected to ATR correction processing, in which the intensity was adjusted and displayed for comparison. The red profile is the spectrum obtained using the KBr method, and the blue profile is that using single-reflection total reflectance ATR.
In Fig.2, it is clear that the number of peaks in the vicinity of 1500 – 1750cm^{-1} is different between the two spectra. Moreover, even in the other regions, it is clear that the peak shapes are also different. Speculating that the different peak shapes may be caused by the presence of moisture in the KBr powder or the pressure applied during pellet formation, we attempted to verify these possibilities.

(1) Assumption that influence is due only to moisture in KBr powder
To verify that moisture in the KBr powder alone is responsible, eliminating the influence of the press, we performed measurement of L-arginine using the diffuse reflectance method (in the diffuse reflectance method, the sample is mixed with KBr powder, however, without pressing the mixture into a pellet, it is possible to verify the influence of moisture). Fig.3 shows the spectrum obtained using the diffuse reflectance method overlaid with that obtained by single-reflection total reflectance ATR. The green profile shows the spectrum obtained using diffuse reflectance, and the blue profile that obtained using total reflectance.
In Fig.3, there are slight differences in peak intensity, however, large differences are not seen. Therefore, the result indicates that moisture in the powder alone is probably not responsible for the changes.

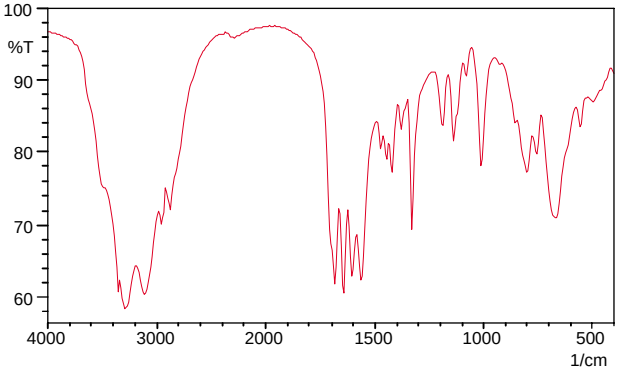

Fig.1 Spectrum of L-Arginine (KBr Method)

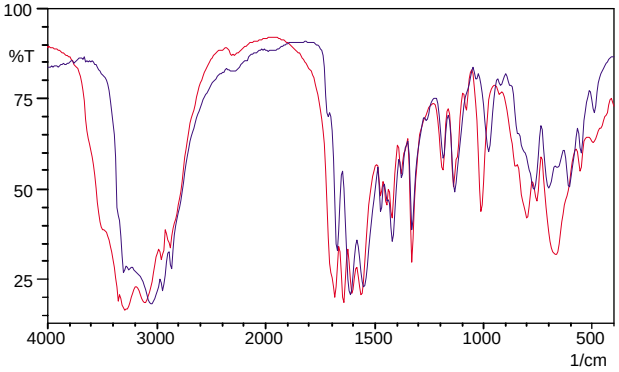

Fig.2 Spectra of L-Arginine (Comparison between KBr method and attenuated total reflectance method)

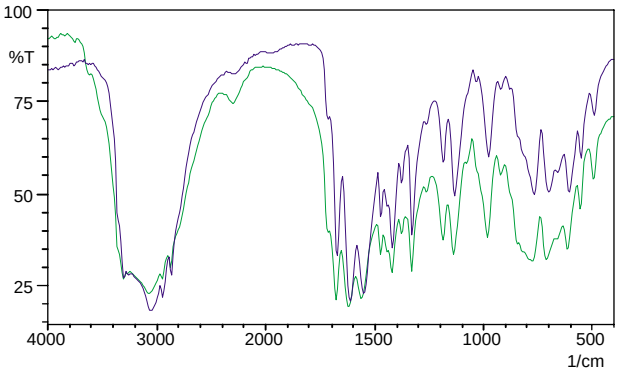

Fig.3 Spectra of L-Arginine (Comparison between diffuse reflectance method and attenuated total reflectance method)

(2) Assumption that influence is due only to pressure during pellet forming

To verify that only application of pressure during pellet formation is responsible for the changes, we formed a pellet using L-arginine alone, and conducted measurement by single-reflection total reflectance ATR. Fig.4 shows the spectrum obtained using the powdered form (red profile) overlaid with that obtained using the pellet (blue profile).

It is clear from Fig.4 that there are no large differences. Therefore, the result indicates that the pressure used in pellet formation alone is probably not responsible for the changes.

From the above results, since it is likely that neither the moisture in the KBr nor the pressure applied during pellet formation when using the KBr method are individually responsible for the changes, it is possible to speculate that the influences are due to both of these factors together. Then, to confirm whether or not moisture existing in the pellet influenced the spectrum, the formed pellet was heated using a drier. Fig.5 shows the spectra obtained after drying for different periods of time. The red-colored profile is the spectrum obtained just after pellet formation, and the blue, green and black profiles are the spectra obtained after heating for 1, 3 and 10 minutes, respectively. The inserted figure is an enlargement of the wavelength region between 1400 – 1800cm^{-1}.

As is clear from looking at Fig.5, along with a decrease in absorption by the O-H radical in the vicinity of 3500cm^{-1}, the number of peaks in the vicinity of 1500 – 1750cm^{-1} changes from 4 to 3. Then, after ten minutes, the spectrum becomes very similar to that measured by the single-reflection total reflectance ATR method.

From the above results, it was understood that when conducting measurement of L-arginine by the KBr pellet method, the moisture present in the KBr powder when forming the pellet influenced the spectrum shape.

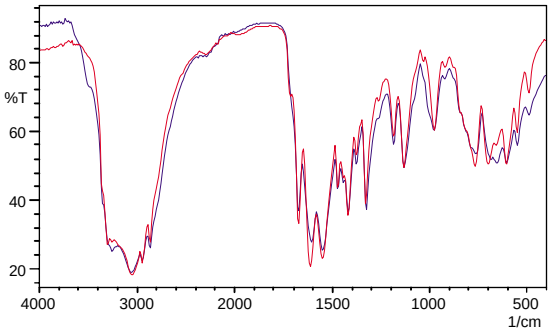

Fig.4 ATR Spectra of L-Arginine (Comparison between powder and pellet)

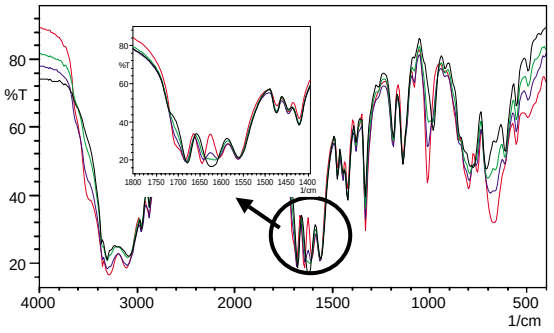

Fig.5 Change of L-Arginine Spectra with Drying Time (KBr Method)

■ Measurement of Citric Acid

Fig.6 shows a spectrum of citric acid measured using the KBr pellet method. Although not shown here, when compared with the spectrum published in the first supplement of the Japan Pharmacopoeia, Revision 14, the peak shapes are different (in the Japan Pharmacopoeia, the peak near 1700cm^{-1} is forked, and the peaks overall are larger)[1)]. Then, the spectrum was measured after drying the pellet with a drier (see Fig.7). The red profile is the spectrum measured just after pellet formation, and the overlaid blue and green profiles are those measured after drying for 3 and 10 minutes, respectively.

As is clear from Fig.7, the shape of the peak in the vicinity of 1700cm^{-1} changed along with drying using the drier. And, the spectrum obtained after drying for ten minutes changed to the same shape as that published in Japan Pharmacopoeia (the time differs depending on the drier wattage, etc.). Therefore, when conducting measurement of citric acid by the KBr pellet method, it is necessary to pay adequate attention to the moisture in the KBr powder itself.

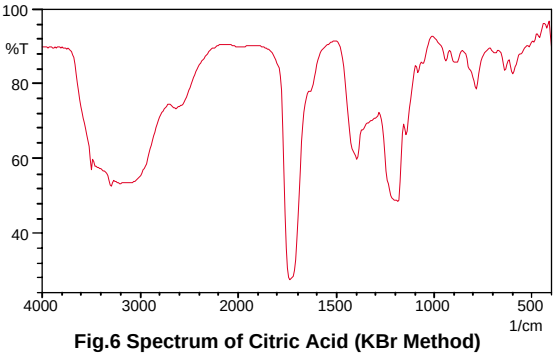

Fig.6 Spectrum of Citric Acid (KBr Method)

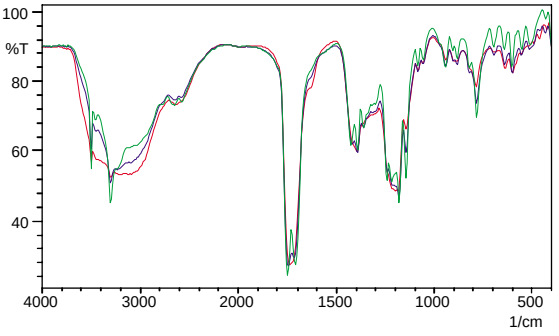

Fig.7 Change of Citric Acid Spectra with Drying Time (KBr Method)

(Bibliography)
1) http://jpdb.nihs.go.jp/jp14supp1/da1tuiho.pdf p.224

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