NONDESTRUCTIVE *IN SITU* FTIR MEASUREMENT BY DIFFUSE REFLECTANCE SPECTROMETRY -PENETRATION DEPTH, SURFACE MEASUREMENT AND INTERIOR ANALYSIS-

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Abstract - The penetration depth in diffuse reflection was found to be dependent on the design of optics, i.e. incidental angle, reflection collecting angle, and azimuthal angle which is the directional angle between the incidental and reflection collecting axis projected on the surface plane. The possibility for quantitative application was examined in respect of matrix choice and KMC linearity with concentration. Surface and interior analysis of pharmaceutical granules for an anticholesterol tablet was performed with microspectroscopy in diffuse reflection mode, and the increase of drug concentration on the surface relative to that in the interior was clarified.

Key words FTIR, diffuse reflectance, penetration depth, surface measurement, FTIR microscopy

Diffuse reflectance FTIR spectrometry is a sensitive method for trace analysis, especially when the testing sample is concentrated around the surface. For this reason many experiments have been carried out to clarify the diffuse reflection mechanisms. One problem in this physical phenomenon is that we still don't know how deeply the light penetrates and returns back to the detector.

The effect of the optics has been examined by Griffiths et al.¹⁻³⁾ on the spectrum and intensity linearity. The contribution of specular reflection toward diffuse reflection has been an issue toward ideal optics for diffuse reflection accessories. Knowledge of the penetration depth is needed to conduct surface analysis in light of the observed thickness of the surface. Penetration depth in diffuse reflectance spectrometry was investigated in this report using sample cups of various bottom depth, and the penetration depth obtained experimentally was less than the value reported earlier.⁴⁾

Many data have not been available in the application of diffuse reflectance spectrometry for quantitative analyses. The choice of matrix and the irregularity of the surface of the sample or matrix, if any, may affect the sensitivity. The penetration depth may also affect the quantitation when diffuse reflection is attempted to be employed for HPLC/FTIR.

Diffuse reflection measurement has been performable with modern microscopes. The distribution of the prescriptive drug in formulated granules has been pharmaceutically important in view of its stability and biological availability. An *in situ* measurement of the surface and the interior was successfully attempted to find the distribution deviation of the drug in the pharmaceutical granules for tablet.

EXPERIMENTAL

The diffuse reflectance accessories used for this penetration study were commercial ones. "Collector" from Spectra Tec Co.(Stanford, CT, USA) has 50° for both incidental and reflection collecting angles with no azimuthal offset.

The diffuse reflectance accessory from Harrick Scientific Co.(Ossining, NY, USA) has 41° for the incidental angle and has a collecting mirror offset out of plane with a 41° collecting angle. The sample cups, made of copper with various bottom length from 0.27 mm to 2.7 mm were 8.4 mm in diameter and were painted inside with black lacquer. The sample used in this study was aminopyrine (Sigma, USA). an antipyretics, mostly at 0.05% unless otherwise stated.

The microscopic measurements of the pharmaceutical granules have been performed with "IR-Plan" from Spectra Tec Co. equipped to a Nicolet 740 FTIR spectrometer. Mapping of the entire granule was possible at the granule which has a large flat surface. The interior of the granule was observed by cutting it with a sharp razor approximately through the center portion.

RESULTS AND DISCUSSION

Dependence of penetration depth on optics

The plot of the KMC value of aminopyrine at 1661 cm^{-1} shows dramatically different characteristics with the change of sample cup depth. The most shallow penetration was obtained with an offset collecting mirror (Harrick) and the deepest penetration was observed with in-plane optics from Spectra Tec. In Griffiths et al.'s optics, which offsets its collecting angle from incidental angle but rather widely collects almost every reflection, an intermediate penetration depth was reported.⁴ Since the analyses were based on KMC values at rather low concentration, and furthermore penetration depth was derived from relative values, Fresnel reflectance may not be affect these penetration experiments.

Fig.1. Dependence of KMC values of aminopyrine on the depth of sample cups. a: Spectra Tec Co. b: Harrick Co.

These results could simply be understood in the figure that a deeply penetrated light contribution is higher with an in-plane detector. In other words, horizontally traveling diffused light hits the offset detector, and deeply penetrating, vertically traveling diffused light can hit the in-plane detector.

These results indicate a different implication for the application of diffuse reflectance spectrometry with different optics. For the purpose of observing only surface, offset optics has some advantages. The sample volume could be minimized with a shallow cup depth. This is another advantage when the sample amount is limited, such as the case that HPLC eluents should be identified by diffuse reflectance spectrometry. An advantage of the in-plane detector is the strong intensity of collecting light. For strongly absorbing substances it will give a fairly good spectrum with a small accumulation. It is recommended, however, to check any specular reflection involved.

Quantitativeness in diffuse reflectance spectrometry

Use of diffuse reflectance spectrometry for a quantitative analysis appears preferable to a translucent mull measurement since there are no complex pellet making procedures.

Fig.2. Linearity of KMC values

The linearity of KMC values with the concentration shown in Fig. 2 assures the applicability of diffuse reflectance to quantitative analyses.

HPLC is now widely used in modern analytical chemistry not only as a qualitative method but also for quantitation. Many papers have been published on the interfacing for HPLC/FTIR and commercialized instruments have not gained yet any popularity for HPLC using a conventional (4.6 mm in diameter) column. The reason for this unpopularity is due to the limited adaptability of the interface to versatile chromatographic conditions. Diffuse reflectance spectrometry might be a convenient technique of larger flexibility toward various mobile phases.

For HPLC/FTIR application, dropwise sampling is more conventional than the mobile phase elimination technique and flow-through ATR technique. Dropwise deposition on the matrix may change the physical characteristics of the surface and the matrix inside. Figure 3 shows the change of the single beam output due to the disturbances of the surface caused by dropping 70 μ l acetonitrile. The degree of the disturbance was large in CaF_2 and small in KBr which has the largest crystalline specific gravity. The surface invariance of KBr and its large refractive index, leading to high measurement sensitivity, suggested KBr as the favorable matrix for this purpose.

Fig.3. Fluctuation of single beam output (ten independent experiments)

In situ surface analysis and interior analysis with microscope

Diffuse reflectance spectrometry without any highly refractive matrices explores primarily the surface or the surface including the layer of several tens of microns. A microspectrophotometer is an excellent tool for *in situ* observation of the small area, such as integrated circuits, computer memories, and even cells. Pharmaceutical granule is an inevitable process intermediate for therapeutic tablets and *in situ* analyses of its surface and interior have been needed.

Fig.4. Diffuse reflectance spectrum of ground pharmaceutical granules in KBr matrix. a: 4000-800 cm^{-1} . b: 1800-1400 cm^{-1}

The instability of CS-514, anti-cholesterol drug developed in Sankyo, by UV light irradiation of the granules for tablet manufacturing suggested the increased concentration of this drug on the surface of pharmaceutical granules. The granule has many ingredients and the ground powder spectrum diluted with KBr matrix is shown in Fig. 4. The bands at 1724 cm^{-1} and 1502 cm^{-1} were attributed to CS-514, and the band at 1657 cm^{-1} to lactose as an ingredient.

Microscopic diffuse reflectance spectra at the surface, and in the interior along a cross plane are shown in Fig. 5. Despite that the fluctuation of the intensity due to the constituents was evident, the averaged spectrum showed a difference of the relative peak intensity of the drug to lactose between the two sites. It is clear that the concentration of the drug is higher at the surface than the interior

Fig.5. Diffuse reflectance spectra of one granule at a: surface, and b: interior

Table 1 Relative concentration (KMC ratios) of CS-514 at surface and in interior in pharmaceutical granules (relative to lactose)

homogeneous matrix	surface		interior	
	S-1	0.908	I-1	1.158
	S-2	1.434	I-2	1.406
	S-3	2.356	I-3	0.829
	S-4	1.029	I-4	1.273
	S-5	1.537	I-5	0.581
	S-6	2.026	I-6	0.923
	S-7	1.904		
AVERAGE	1.424	1.613	1.028	

The KMC values of CS-514 at 1724cm^{-1} are ratioed to 1657cm^{-1} of the lactose band. The spectra were measured in a $200 \times 200\text{ }\mu\text{m}$ area at 4000 accumulations. All surface and cross plane areas are covered by these microscopic mappings.

This fact might be speculated to be caused by drying process, in which the solvent carried the drug toward the surface of the granules. The information obtained with microscopic diffuse reflectance measurements turned out to be very valuable in order to improve the formulation process.

It is concluded that *in situ* diffuse reflectance microscopic measurement of pharmaceutical granules is now established since the ingredients for any granules generally have a similar composition. The formulation ingredients have been proved to be a particle or porous matrix, which makes the IR measurement of granules possible.

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