Using Terahertz Reflectance Spectroscopy to Quantify Drug Substance in Tablets

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The purpose of this research is to investigate the applicability of terahertz (THz) reflectance spectroscopy for quantification of drug substance in tablets, so as to demonstrate the feasibility for applying this technique to tableting process monitoring. In order to acquire a suitable absorbance spectrum for this purpose, it was necessary to enhance the reflection intensity. By using an aluminum plate as a mirror at the opposite surface of the tablet, a reasonable absorbance spectrum could be acquired to reflect the bulk information of the tablet. To assess the limit of tablet thickness, linearity between the tablet thickness and the absorbance value was investigated using lactose and mannitol tablets. Since linearity was found within 0.75–5.0 mm for both tablets using 0.4 and 0.8 THz region, it was confirmed that THz reflectance spectroscopy is applicable to tablets within at least 5.0 mm thickness. Mannitol tablets containing sodium salicylate as the model drug substance were used to investigate the quantitative performance of this technique. It was confirmed that the established calibration model was acceptable for this quantification because of the root-mean-squared error of cross-validation (RMSECV) being 1.95%. In order to evaluate the applicability of this technique, content quantitative performance in double layered tablets having active and placebo layers were assessed. Since this calibration model achieved the root-mean-squared error of prediction (RMSEP) of 1.42% for the double layered tablets, this technique was considered feasible even if the drug substance is localized in the tablets.

Key words terahertz; reflection; in-line monitoring; process analytical technology; tablet; sodium salicylate

The tablet is one of the popular dosage forms in pharmaceuticals, and quantification of drug substance in drug product, including the content uniformity test, is a critical and mandatory quality assurance requirement in tablet manufacturing. Although a conventional HPLC method is the most popular technique for quantification, there are growing interests to examine alternative analytical techniques that are possible to measure drug products in-process with a nondestructive manner. These interests are also in line with the United States Food and Drug Administration (FDA) Process Analytical Technology (PAT) initiative and guidelines.¹⁾ As a well-known example of the alternative techniques, near infrared (NIR) spectroscopy is being extensively used for the quantification of drug substance as a PAT tool.^{2–4)}

Typically, only 10–30 tablets are generally employed as the representative for batch manufacturing of several million tablets. In-line monitoring during the tableting process is expected to attain higher quality assurance than the traditional end-product testing, because quantification for each and every unit dose would be expected. A reflectance spectroscopy is usually employed as the in-line monitoring tool, because a detector of a transmission spectrometer cannot be installed into a rotary tableting machine to detect the transmitted beam through a tablet. Some monitoring techniques in the tableting process with diffuse reflectance NIR spectroscopy have actually been reported.^{5,6)}

Variations in the physical properties of sample generally affect the spectra when diffuse transmission or reflectance NIR spectroscopy is used.^{7,8)} Especially, the difference in tablet density is shown as a baseline shift in the spectra,⁹⁾ leading to an

issue that should be solved because this effect complicates the quantification. There is another important aspect in the quantification, which is a penetration depth of an incident beam into a tablet. A disadvantage of diffuse reflectance NIR spectroscopy is the extremely shallow penetration depth into a tablet (0.1–0.2 mm¹⁰⁾ or ≤ 0.5 mm¹¹⁾). This technique can detect information only near the surface of the tablet, therefore it is not applicable when the drug substance is localized in the tablets.

Terahertz (THz) spectroscopy has become popular as a novel technique for the quantitative analysis of pharmaceuticals; drug substances in tablets have been demonstrated as quantifiable.¹²⁾ By using THz pulsed imaging in reflectance mode, it was possible to qualitatively analyze the chemical distribution of lactose and sucrose pellets on the surface of a tablet.^{13,14)} Quantitative calibration models for the prediction of content in theophylline tablets have also been developed using surface reflection information.¹⁵⁾ In THz reflectance spectroscopy, however, a precise quantitative analysis for assessing bulk information of the tablet has not been studied. Applicability of this technique to the quantification of localized drug substance in the tablets has also not been studied.

In this study, we performed four experiments to investigate the applicability of THz reflectance spectroscopy for the quantification so as to demonstrate the feasibility for applying this technique to tableting process monitoring; (1) determining the appropriate analytical conditions to obtain the suitable absorbance spectrum; (2) assessing the impact of tablet thickness and porosity on the absorbance value; (3) developing the quantitative calibration model for tablets containing sodium salicylate (SS) as the model drug substance; (4) evaluating the applicability to quantification of the localized drug substance in tablets.

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Experimental

Materials Sodium salicylate (SS) (Kanto Chemical Co., Inc., Japan) and polyethylene powder (Sigma-Aldrich Co., U.S.A.) were of reagent grade. SS was passed through 300- μ m mesh screen before use. Lactose (Dilactose S, Freund Corporation, Japan) and mannitol (Parteck M200, Merck KGaA, Germany) were employed as diluents because they were the most common excipients for pharmaceutical tablets. Polyethylene powder, lactose, mannitol, and magnesium stearate (NF Non-Bovine HyQual, Mallinckrodt Inc., U.S.A.) were used as received.

Preparation of Lactose and Mannitol Tablets To determine the analytical conditions and to evaluate the impact of tablet thickness and porosity on the absorbance spectrum, lactose and mannitol tablets were prepared. Lactose (99% (w/w)) or mannitol (99%) with magnesium stearate (1%) was mixed using an agate mortar and pestle. The blends were compressed with a flat-faced punch and die with a 7.0-mm diameter, using a hydraulic oil compactor (Riken Power P-1B, Riken Seiki Co., Ltd., Japan). The lactose and mannitol tablets having the thickness of *ca.* 0.75–5.0 mm were prepared by changing the weight (Table 1). True densities were measured using a helium pycnometer (Ultra pycnometer 1000, Quantachrome Instruments, U.S.A.) at room temperature. Tablet thicknesses were measured using a caliper (SM-112, Teclock Corporation, Japan), and tablet weights were measured using an analytical scale (AX504, Mettler Toledo International Inc., Switzerland). Tablet porosity (F (%)) was calculated based on the following

equation.

$$F = 1 - \rho / \rho_0 = 1 - (m \cdot v^{-1}) / \rho_0$$

where ρ (mg/mm³) is the tablet density, ρ_0 (mg/mm³) is the true density of the blend, m (mg) is the tablet weight, and v (mm³) is the tablet volume.

Preparation of SS Tablets Prior to examining the THz measurement condition for tablets containing SS, the SS specific peak was assessed considering the discrimination against the other component peaks in the absorbance spectra. The formulae of polyethylene tablets for assessing the specificity are shown in Table 2. The blends were prepared by mixing each component with polyethylene which has no absorbance in the THz region, and compressed into tablet forms prior to measurements.

Calibration tablets were prepared to develop a quantitative calibration model, and double layered tablets were manufactured to assess the applicability of this technique to quantification of the localized drug substance in tablets. The labeled

Table 2. Formulae of Polyethylene Tablets for Assessing the Specificity (mg/tablet)

Sodium salicylate	10.8	—	—
Mannitol	—	139.68	—
Magnesium stearate	—	—	1.52
Polyethylene	141.2	12.32	150.48
Unit weight	152	152	152

Table 1. Physical Properties of Lactose and Mannitol Tablets

Lactose tablet			Mannitol tablet		
Thickness (mm)	Weight (mg)	Porosity (%)	Thickness (mm)	Weight (mg)	Porosity (%)
0.76	38.98	12.75	0.75	36.93	13.94
1.00	52.17	11.43	1.02	50.10	14.22
1.51	79.69	10.35	1.49	74.53	12.70
2.03	107.00	10.46	2.00	101.08	11.79
2.98	159.49	9.04	2.98	152.15	10.89
4.99	269.68	8.15	4.98	254.96	10.69

Table 3. Formulae of Calibration Tablets (mg/tablet)

Content (% of labeled claim)	70%	85%	100%	115%	130%
Sodium salicylate	7.56	9.18	10.8	12.42	14.04
Mannitol	142.92	141.3	139.68	138.06	136.44
Magnesium stearate	1.52	1.52	1.52	1.52	1.52
Unit weight	152	152	152	152	152

Table 4. Formulae of Double Layered Tablet (mg/tablet)

Content (% of labeled claim)		75%	90%	100%	110%	125%
Placebo layer	Mannitol	94.05	82.764	75.24	67.716	56.43
	Magnesium stearate	0.95	0.836	0.76	0.684	0.57
SS layer	Sodium salicylate	8.1	9.72	10.8	11.88	13.5
	Mannitol	48.33	57.996	64.44	70.884	80.55
	Magnesium stearate	0.57	0.684	0.76	0.836	0.95
Unit weight		152	152	152	152	152

Fig. 1. Time-Domain Spectra of Mannitol Tablet Having 1.49mm Thickness with and without an Aluminum Plate

claim for the SS tablet was 10.8 mg, and the content range was 70–130% for the calibration tablets and 75–125% for the double layered tablets. The formulae for the SS tablets are shown in Tables 3 and 4. The blends were prepared by mixing each component, and compressed into the SS tablets. The double layered tablets were prepared as follows; after the blends of placebo layer were compressed at 2 kN, the blends of SS layer were added on the placebo layer and compressed at 10 kN. The unit weight of the calibration tablets and the double layered tablets was 152 mg. The thicknesses and porosities of the tablets were 2.95–3.02 mm and 10.10–12.09%, respectively.

Content Quantification of SS Tablet Each individual tablet was diluted with distilled water in a 200-mL volumetric flask. The flask was then shaken and ultrasonicated for 10 min to ensure complete disintegration of the tablet. Aliquots (50 mL aliquot per sample) were subjected to centrifugation at 3000 rpm for 10 min, and the resultant supernatants were analyzed for SS content using a UV spectrophotometer (UV-2550, Shimadzu Corporation, Japan) at 296 nm.

THz Reflectance Spectroscopy A time-domain spectrum was acquired to investigate which reflected beam (on the incident or opposite surface) is suitable for analyzing the bulk information of the tablets, using a THz time-domain spectroscopy apparatus (TAS-7500, Advantest Corporation, Japan) in reflectance mode. An absorbance spectrum was obtained by Fourier-transforming the time-domain spectrum. The absorbance spectral range was 0–2.0 THz with a resolution of 0.0076 THz; background corrections were performed based on blank tests. An absorption peak of the environmentally absorbed water was offset by the peak of the environmental moisture in the blank test. Spectral pre-treatment was not performed on the absorbance spectra.

Multivariate Data Analysis Multivariate data analyses were performed on the absorbance spectra using OPUS software (version 6.5, Bruker Optics Inc., U.S.A.). The absorbance spectra were converted to their first derivative. The calibration model was calculated using a partial least squares regression algorithm with cross-validation (1 sample removed per cycle). The root-mean-squared errors of cross-validation (RMSECV) were determined for each rank, and the number of rank used to develop the model was defined based on the lowest RMSECV.

Results and Discussion

Measurement Conditions for THz Reflectance Spectroscopy In order to attain in-line monitoring for tableting process by a spectroscopy, the detector should be presented on the same side of the incident beam (not opposite direction). Therefore, a conventional THz reflectance method shown in Fig. 1 (without plate) was assessed at first with the mannitol tablet having 1.49 mm thickness. In this figure, the *x*-axis represents the time when the detector detects the reflected beam, and *y*-axis indicates the intensity of the reflected beam. The relatively strong peak denoted as ‘a’ was the first reflectance on the surface of the tablet, and the weak peak of ‘b’ was the reflectance from the opposite surface. Peak ‘b’ includes not only the surface but also the bulk information in the tablet, and this peak can also contain the chemical information of mannitol. Although the absorbance spectrum was obtained by Fourier-transforming peak ‘b’ to acquire the information throughout the tablet (data not shown), chemically characteristic absorbance peaks of mannitol could not be detected in the absorbance spectrum due to the low intensity of peak ‘b.’

In order to enhance the reflection intensity by detecting the transmitted beam denoted as ‘c,’ an aluminum plate was used as a mirror on the opposite side of the tablet as also shown in Fig. 1. The significantly strong peak denoted as ‘e’ was the reflectance from the opposite surface, and the weak peaks of ‘f’ and ‘g’ were the multi-reflected beams. Since peak ‘e’ showed the highest intensity in the spectrum, it was used to acquire an absorbance spectrum.

To measure each and every unit dose in the tableting process, rapid measurement for each tablet is preferable. Since the measurement time is proportional to the cumulative number, the relationship between the standard deviation (S.D.) and the cumulative number of a measurement was assessed to define the appropriate cumulative number. The mannitol tablet having 1.49 mm thickness was scanned 1–16384 times under a ‘with plate’ condition, and the absorbance spectra were obtained from the reflected beam, which was indicated as ‘e’ in Fig. 1. Figure 2 shows the absorbance spectra with the cumulative number of 16 for each measurement as a typical example (the measurement was repeated 5 times). A specific peak derived from mannitol was observed at 1.095 THz, and the S.D. was calculated from the absorbance values of the peaks. Figure 3 shows the relationship between the S.D. and the cumulative number of measurements. It was demonstrated

Fig. 2. Absorbance Spectra of Mannitol Tablet Having 1.49 mm Thickness

Fig. 3. Standard Deviation of Absorbance Value versus the Cumulative Number of Measurement Using a Mannitol Tablet Having 1.49 mm Thickness

Fig. 4. Absorbance Spectra of Lactose and Mannitol Tablets

that the S.D. decreased as increase in the cumulative number. From this result, 256 was set as the cumulative number since the S.D. reached a plateau. This result also indicated that the measurement time can be decreased by reducing the cumulative number, considering the required level of S.D. for each experiment.

Since the cumulative number of 256 corresponds to approximately 2 s, it is difficult to apply this technique right now for the in-line monitoring of each and every unit dose. This technique would be expected to be applied to the tableting process monitoring, when the measurement time could be decreased by improving the detection accuracy of the apparatus.

Limits of Tablet Thickness for THz Reflectance Spectroscopy Measurements To evaluate the impact of tablet thickness and porosity on the absorbance spectrum, lactose and mannitol tablets having the thickness of *ca.* 0.75–5.0 mm were used.

Figure 4 shows the absorbance spectra of the lactose and mannitol tablets. The reflected beam that passed once through the tablet was used for absorbance spectra. Therefore, the larger the tablet thickness, the higher the absorbance value would be expected based on the Beer–Lambert law. Linearity between the absorbance value and the tablet thickness was investigated to assess the limit of tablet thickness for the measurements (Fig. 5). The absorbance values ranging from 0.4 to 1.2 THz were used for this evaluation because many THz spectroscopic studies with drug substances and

pharmaceutical excipients have been conducted by using this region. A good linearity ($R^2 > 0.94$) was obtained over the whole range of thicknesses at both 0.4 and 0.8 THz, and the limit of tablet thickness was confirmed to be at least 5.0 mm for both lactose and mannitol tablets. At 1.2 THz, the limit was 1.5 and 3.0 mm for the lactose and mannitol tablets, respectively. Therefore, this technique can be applied to a majority of commercial pharmaceutical tablets at below 0.8 THz, because of their general thicknesses being less than 5.0 mm.

Figure 6 shows the relationship between the tablet porosity and the absorbance value normalized for tablet thickness. It was confirmed that no or low correlation was observed ($R^2 < 0.48$), and the absorbance value was not substantially affected by tablet density in the porosity range of this study.

Quantitative Analysis of SS in the Tablet In order to investigate the applicability of this technique for the monitoring of drug content in tablets, the quantitative performance was assessed using SS tablets.

Prior to examining the quantitative performance with SS tablets, the specificity of the SS peak was assessed using the polyethylene tablets. Figure 7 shows the absorbance spectra for each component in SS tablets. A single specific absorption peak was observed at 0.4 THz for SS without the interference of mannitol or magnesium stearate. The absorbance spectra of the calibration tablets are shown in Fig. 8a. Since the intensity of the 0.4 THz peak corresponded to the SS content in the tablet, this peak was considered suitable for the SS content

Fig. 5. Relationship between Absorbance Values of Absorbance Spectra and Tablet Thicknesses

The symbols ●, ◆, and ■ indicate 1.2, 0.8, and 0.4THz, respectively. Each symbol represents the mean value of three measurements (one tablet) and the error bar represents S.D.

Fig. 6. Plot of Absorbance Normalized for Tablet Thickness against Tablet Porosity

The symbols ●, ◆, and ■ indicate 1.2, 0.8, and 0.4THz, respectively. Each symbol represents the mean value of three measurements (one tablet) and the error bar represents S.D. (the error bars are too small to see).

quantification with high specificity.

Multivariate data analysis was performed to correlate the absorbance spectra of the calibration tablets with the SS content (Table 5). The result indicated that the calibration model was a reasonable quantitative model for the content quantification of the conventional SS tablets.

By using the aluminum plate as a mirror, it was demonstrated that the suitable absorbance spectrum for the quantitative analysis could be acquired from the tablets. Although the aluminum plate cannot be set to the tablet in the tableting machine, the surface of the lower punch can fulfill the role as a mirror in place of the aluminum plate when this technique is applied to the in-line monitoring of the tableting process. Steel is usually used as material for the punch of the tableting machine. There are no significant differences in the reflectance of iron (*ca.* 85%), which is the main component of steel, and aluminum (*ca.* 98%) in the THz region.¹⁶⁾ In addition, the absorbance spectra for comparing the aluminum plate with the steel one using the SS calibration tablet with 100% content are shown in Fig. 9. Since there were no significant differences in the absorbance value of the peak at 0.4THz, the performance of the content quantification was suggested to be equivalent in the both cases. From these results, this technique was judged feasible for in-line monitoring of the tableting process, because the lower punch could play a role as a mirror.

Fig. 7. Absorbance Spectra of Each Component in SS Tablets

Quantitative Analysis of Double Layered Tablet Considering the applicability of this technique, the double layered tablet was used for the quantitative analysis using the established calibration model. In the double layered tablet composed of SS and placebo layers, SS was localized in the SS layer. The double layered tablet was considered difficult to quantify with NIR reflectance spectroscopy due to the shallow penetration depth of the incident beam into the tablet. Even if the SS concentration (weight/weight) in the SS layer can

Fig. 8. Absorbance Spectra of the Calibration Tablets
Three tablets for each SS content were measured.

Fig. 9. Absorbance Spectra for Comparing the Aluminum Plate with the Steel Plate
One tablet having 100% content was measured three times with each plate.

Fig. 10. Time-Domain Spectra of the SS Tablets Having 100% Content

Table 5. Performance Parameters of the Multivariate Calibration Model for Correlating the Content of the Calibration Tablets with the Absorbance Spectra

Frequency (THz)	0.3281–0.3815
	0.5188–0.6180
Rank	1
RMSECV	1.95%
Slope	0.9891
R^2	0.9897

be acquired using the reflected beam from the SS layer, the weight of SS layer cannot be estimated correctly.

Figure 10 shows the time-domain spectra of the calibration tablet and the double layered tablet having 100% content. The peak denoted ‘a’ was the first reflectance on the surface of the tablet, the weak peak ‘b’ was the reflectance on the intermediate between placebo and SS layers, ‘c’ was the reflectance from the opposite surface, and ‘d’ was the multi-reflected beam. In this study, since the peak ‘c’ reflected from the opposite surface was only used to acquire the absorbance spectra, those of the double layered tablets showed almost the

Table 6. SS Content in the Double Layered Tablets ($n=3$ for Each Tablet with a Given Theoretical Content)

Content (%) (Theoretical value)	Measured content (%)	Predicted content (%)	Residue (%)
75	74.1	76.9	–2.8
	76.1	78.3	–2.2
	75.2	76.9	–1.7
90	91.0	89.9	1.1
	90.0	90.2	–0.2
	90.1	86.7	3.4
100	99.7	100.0	–0.3
	98.3	95.9	2.4
	100.9	100.5	0.4
110	107.0	109.6	–2.6
	109.1	110.6	–1.5
	109.5	110.2	–0.7
125	121.0	120.0	1.0
	120.9	120.0	0.9
	122.6	122.6	0.0

same peak intensity and shape as the calibration tablets (Fig. 8). The absorbance spectra of the double layered tablets were

confirmed not to be influenced by the reflectance on the intermediate of two layers.

With THz reflectance spectroscopy, the absorbance spectra were collected from the placebo layer to confirm that the incident beam reached the SS layer. The predicted contents of the tablets were calculated from the absorbance spectra using the established calibration model. The summary of the predicted and measured contents are shown in Table 6. The root-mean-squared error of prediction (RMSEP) was 1.42%. These results indicated that the incident beam from the placebo layer reached to the SS layer and the SS content in the double layered tablet could be predicted precisely by this technique.

Conclusion

By using the aluminum plate as a mirror, the suitable absorbance spectra could be acquired. Since the surface of the lower punch can fulfill the role as a mirror in place of the aluminum plate, this technique was judged feasible for in-line monitoring of the tableting process.

It was confirmed that THz reflectance spectroscopy is applicable to tablets within at least 5.0 mm thickness and there was no impact of tablet porosity on the spectra.

The reasonable quantitative model for the content quantification of the conventional (single layered) SS tablets could be developed with THz reflectance spectroscopy. It was also confirmed that this calibration model could be applied to predict the content of double layered tablets. This means that the quantification of localized drug substance in the tablet can be conducted with this technique.

THz reflectance spectroscopy was confirmed to be applicable to the content quantification of tablets, and the feasibility

for applying this technique to the in-line monitoring each and every unit dose during the tableting process could be demonstrated.

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