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Chemometric Evaluation of Pharmaceutical Properties of Antipyrine Granules by Near-Infrared Spectroscopy

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ABSTRACT

The purpose of this research was to apply near-infrared (NIR) spectroscopy with chemometrics to predict the change of pharmaceutical properties of antipyrine granules during granulation by regulation of the amount of water added. The various kinds of granules (mean particle size, 70-750 μm) were obtained from the powder mixture (1 g of antipyrine, 6 g of hydroxypropylcellulose, 140 g of lactose, and 60 g of potato starch) by regulation of the added water amount (11-19 wt/wt%) in a high-speed mixer. The granules were characterized by mean particle size, angle of repose, compressibility, tablet porosity, and tablet hardness as parameters of pharmaceutical properties. To predict the pharmaceutical properties, NIR spectra of the granules were measured and analyzed by principal component regression (PCR) analysis. The mean particle size of the granules increased from 81 μm to 650 μm with an increase in the amount of water, and it was possible to make larger spherical granules with narrow particle size distribution using a high-speed mixer. Angle of repose, compressibility, and porosity of the tablets decreased with an increase of added water, but tablet hardness increased. The independent calibration models to evaluate particle size, angle of repose, and tablet porosity and hardness were established by using PCR based on NIR spectra of granules, respectively. The correlation coefficient constants of calibration curves for prediction of mean particle size, angle of repose, tablet porosity, and tablet hardness were 0.9109, 0.8912, 0.7437, and 0.8064, respectively. It is possible that the pharmaceutical properties of the granule, such as mean particle size, angle of repose, tablet porosity, and tablet hardness, could be predicted by an NIR-chemometric method.

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INTRODUCTION

Preparation of high-quality granules offers a number of potential advantages to the pharmaceutical industry in the production of beads or granules as both finished and intermediate products. Therefore, various kinds of formulations, techniques, and types of equipment have been developed to obtain high-quality granular materials.[1-11] However, physical properties of the granules, such as granule size, hardness, and porosity, are affected by granulation formulations and methods, and the properties influence reproducibility of the preparations by affecting granular flowability, tablet compressibility, disintegration time, and dissolution rate. Therefore, to ensure the manufacturing of safe and efficacious pharmaceutical products and meet regulatory requirements, production process validation is required.

Using the near-infrared (NIR) spectroscopic method, the spectra can be measured directly on the surface of nondestructed samples without any pretreatment.[12] Consequently, NIR spectroscopy is fast becoming an important analytic technique for use in validation of pharmaceuticals. Additionally, chemometrics provides an ideal method of extracting quantitative information from samples through NIR spectra of multicomponent samples.[13] A number of chemoinfometric and statistical techniques are employed in NIR quantitative and qualitative analysis because these approaches have been proven to be successful in extracting the required information from unprocessed NIR spectra. Calibration methods such as multiple linear regression, principal component analysis/principal component regression (PCA/PCR), and partial least-squares regression are commonly used.[13] Chemoinfometric NIR spectroscopic methods were reported to determine tablet hardness,[14] drug stability,[15] tablet coating,[16] polymorphic contents of pharmaceuticals,[17-20] and particle size of powders.[21-

[25] However, there are few reports evaluating pharmaceutical properties of actual formulations for the solid-dosage forms. Therefore, the purpose of this study was to develop a quick and accurate way to determination the pharmaceutical properties of granules and tablets in the formulation of pharmaceuticals by applying chemoinfometric NIR spectroscopy.

MATERIALS AND METHODS

Materials

The bulk powder of antipyrine of Japanese Pharmacopoeia XIII grade was obtained from Hoeiyakukou Co (Tokyo Japan). Crystalline alpha-lactose monohydrate (Pharmatose 200M, DeMelkindustrie Veghel Co, Veghel, The Netherlands) and potato starch (Matsuya Chem Co, Osaka, Japan) were used as diluents and disintegrator, respectively. Hydroxypropylcellulose (HPC) (HPC-L, Nihon Soda Co, Tokyo, Japan) and magnesium stearate (Kishida Chem Co, Osaka, Japan) were used as a binder and lubricant, respectively. All other chemicals were of analytical grade.

Granulation Process

The manufacturing process for antipyrine granules and tablets is as follows[4]: 1 g of antipyrine bulk powder, 6 g of HPC powder, 140 g of lactose, and 60 g of potato starch were mixed in a high-speed mixer (Model LFG-GS-1J, Fukae Industrial Co, Ltd, Osaka, Japan) for 10 minutes at 300 rpm for the main blade and 0 rpm for the chopper. The mixture was kneaded at 300 rpm main blade and 3000 rpm chopper for 10 minutes. The purified water (11-19 wt/wt%) was added to the mixture by pipette divided 4 times within the initial 2 minutes during kneading. The granules thus obtained were dried with a fluid bed dryer (Midget-dryer, Fuji Powdal Co, Osaka, Japan) for 20 minutes and passed through a 1700-μm sieve to remove coarse lumps. The sample granules were stored in desiccators with silica gel at room temperature for 1 week.

Tableting Compression Process

Sample granules were mixed with 1.0% magnesium stearate in a twin-shell mixer (Model V-1, capacity 2 L, mixing speed 28 rpm, Tokujyu Ind Co, Tokyo, Japan) for 10 minutes. A compression/tension tester (Autograph, model IS-5000, Shimadzu Co, Kyoto, Japan) was used at 25 ± 1°C. An 8-mm diameter die and punch compressed samples of 200 mg at 73.5 MPa (maximum upper punch pressure) with a flat surface compression speed of 15 mm/min.

Tablet Hardness Test

The tablet was compressed at 5 mm/min by a hardness tester (Toyama Co, Osaka, Japan). The tablet hardness was measured 5 times.

Micrometric Characterization

The true densities of the powders were determined using an air comparison pyknometer (model 930, Beckman-Toshiba Co, Tokyo, Japan). The sample powders were measured 5 times.

Angle of Repose

The poured angle of repose was measured 5 times using a disk 80 mm in diameter.

Measurement of Mean Particle Size

Particle distribution was determined by measuring 50 g of granule samples by the sieve method (Ro-tap Shaker, Iida Ind Co, Ltd, Tokyo, Japan). Screens with openings of 44, 125, 350, 420, and 500 μm were used for separation of each fraction. The mean particle size (D50) of the samples was estimated as a median diameter from weight of the fractions based on percentage cumulative curves.

Measurement of Compressibility Weight

The compressibility of the granules was measured as follows: 7 g of sample granules was put into a graduated cylinder (1 cm in diameter and 25 mL in volume) and tapped by a tapping instrument (RHK-type, Konishi Co, Osaka, Japan). Compressibility was estimated by Equation 1:

$$C = \frac{\rho_f - \rho_0}{\rho_f} \times 100 \qquad (1)$$

where ρ_f is the bulk density of the sample granules at a tapping number of infinity, and ρ_0 is the bulk density of the sample granules.

Tablet Porosity

The thickness and diameter of tablets were measured by micrometers. The volume of the tablet was calculated from the tablet's geometry. The tablet porosity (ε) was evaluated based on the volume of the tablet and the true density of the bulk powders:

$$\varepsilon = \frac{V_T - (V_A + V_L + V_S + V_{HPC})}{V_T} \times 100 \qquad (2)$$

where V_T is the apparent volume of the tablet, V_A is the true volume of antipyrine, V_L is the true volume of lactose, V_S is the true volume of potato starch, and V_{HPC} is the true volume of HPC.

Fourier Transform NIR Spectroscopy

Fourier transform NIR (FT-NIR) spectra were taken using an NIR spectrometer (InfraProver, Bran+Luebbe Co, Norderstedt, Germany). Briefly, a fiber-optic probe was inserted into the sample powder (2 g) in a 20-mL glass bottle. Five scans per sample were recorded in the wavenumber range of 4500 to 10 000 cm^{-1}. A ceramic (Coor's Standard) reference scan was taken for each set of samples for calibration purposes. FT-NIR spectra of 9 calibration samples were recorded 5 times with the NIR spectrometer. For each sample, 45 spectral data were analyzed by various methods, and chemometric analysis was performed using the PCR program associated with the Pirouette software (InfoMetrix Co, Woodinville, Washington).

An NIR spectrum including n spectral data can be seen as a point in an n-dimensional space. In multivariate data analysis, PCA/PCR of a spectral data matrix X is a basic tool. PCA/PCR decomposes X into a score matrix T, times a loading matrix P, plus a residual matrix E.

$$X = t_1 p'_1 + t_2 p'_2 + \cdots + E = TP' + E \qquad (3)$$

This decomposition is particularly useful for converting X to a few information plots (score plots and loading plots) and for modeling the systematic structure in X.

In this study, the NIR spectra consisted of 459 data points between 4500 and 10 000 cm^{-1} at intervals of 12 cm^{-1}. Even batches of standard samples with various values of pharmaceutical properties, such as granular size, angle of repose, tablet porosity, and tablet hardness, were prepared; 4 spectra were collected per batch. A total of 36 spectra were selected for the calibration (calibration set) to predict individual pharmaceutical properties, and 9 spectra were used for prediction of calibration (prediction set), respectively. The best conditions were determined to minimize the standard error of cross-validation (SEV).

RESULTS AND DISCUSSION

Effect of Added Water Amount on Physical Properties of Granules Obtained by High-Speed Mixer

Figure 1 shows particle size distribution of granules obtained by high-speed mixer. The log-log plots of cumulative weight percentage versus mean particle size of all of the granules showed a linear relationship. The particle size distribution followed Equation 4, and the degree of particle size distribution (the slope of the plots) and mean particle size were estimated from the plots by the least-squares method.

$$\log y = a \bullet \log x + C \qquad (4)$$

where y is the cumulative weight percentage, x is the particle diameter, a is the slope of the plot, and C is a constant.

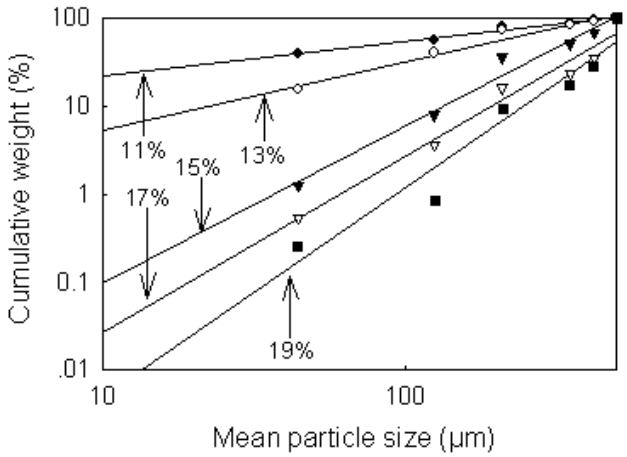

Figure 1. Cumulative weight percent of antipyrine granules obtained by addition of 11 to 19 v/wt% water. □ 11%, ○ 13%, ▼ 15%, □ 17%, ■ 19%.

Figure 2A shows the effect of amount of water on mean particle size and particle size distribution of the granules. The mean particle size of the granules increased with an increase in the amount of water.[5] The slope of the regression line in **Figure 1**, which was an index of particle size distribution, also increased with an increase in the amount of water added. This result suggested that the amount of kneading liquid affected the formation of granules.

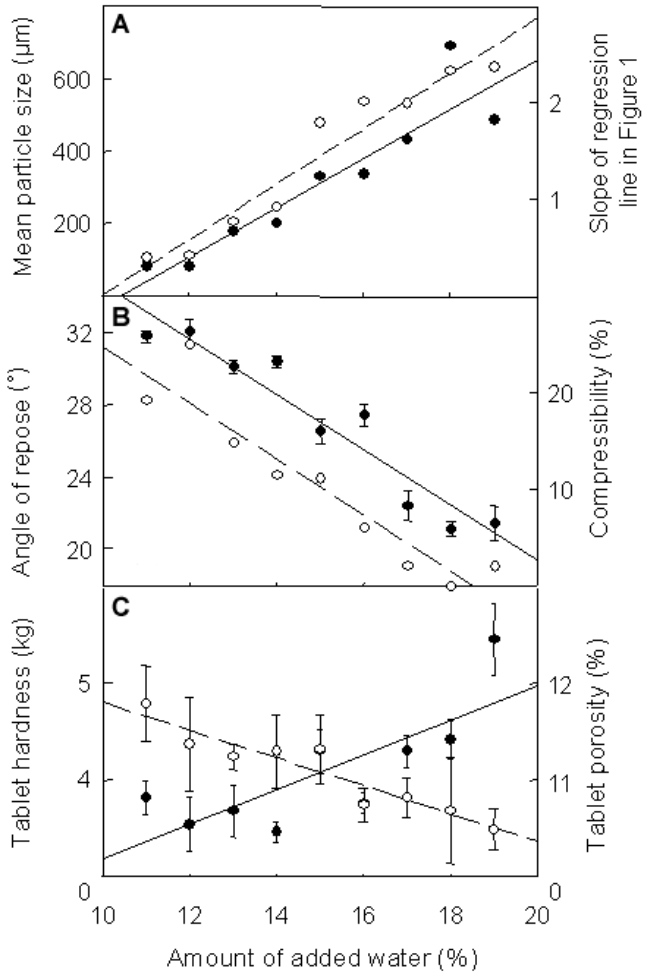

Figure 2. Effect of added water amount on pharmaceutical properties of antipyrine granules. (A) The open and closed symbols represent particle size and particle distribution. (B) The open and closed symbols represent angle of repose and compressibility. (C) The open and closed symbols represent tablet porosity and hardness. The open and closed symbols represent left and right axis, respectively.

SEM observation of granules obtained by various added water amounts indicated that the granules obtained with an 18% added water amount were spherical and 300 to 500 μm in diameter, but those of 11% were less than 200 μm in diameter and contained a lot of fine powders. These results mean that it is possible to make larger spherical granules with narrow particle size distribution using a high-speed mixer and an efficient amount of water, but mean particle size and distribution are affected by the added water amount in the formulation of the granules.

Effect of Added Water Amount on Pharmaceutical Properties of Granules

Figure 2B shows the effect of added water amount on angle of repose and compressibility of granules. The angle of repose and compressibility decreased with an increase in the amount of water added. It is well known that angle of repose decreases with an increase of powder flowability, and so it was used as an index of the powder flowability. Since higher-flowability powder that is a larger particle size has higher apparent powder density before the tapping test, the compressibility by tapping test decrease increasing of particle size. Therefore, it seems that the angle of repose and compressibility were affected through an increase in mean particle size with an increase in added water.

Figure 2C shows the effect of added water amount on tablet porosity and hardness. The added water amount during granulation affected the porosity and hardness of the tablet obtained from the granules. The tablet hardness slightly decreased between 11% and 16% of added water amount, but after 16% water, the hardness increased with an increase of added water amount. In contrast, the tablet porosity decreased with an increase of added water. Since powder flowability was improved with an increase in added water, as shown in **Figure 2B**, the powder flow resistance during tableting decreased with an increase in the amount of added water. However, since the tablet hardness corelated with both particle size and powder flow resistance, the result suggested that the relationship between the geometric structure of the tablet and the tablet hardness was not so simple.

Prediction of Pharmaceutical Properties of Granules by Chemometric NIR Method

Figure 3 shows the NIR spectra of the sample granules obtained by various amounts of added water. The NIR absorption peaks of antipyrine and additives were identified.[12] The absorption peaks at 4560, 4668, 5940, 6036, and 8750 cm^{-1} are associated with antipyrine, and those at 4776, 5172, and 6500 cm^{-1} are associated with a mixture of excipient powder. All NIR spectrum patterns were almost the same, but a baseline of the spectrum increased with an increased amount of added water, indicating that it reflected increasing particle size of the sample granules.

Since chemometrics can be used to analyze raw data and help researchers understand the significant contributions of some process variables toward the variability in the raw data profiles the quantitative relationship

between the objective parameters, such as the pharmaceutical properties of granules and the loadings of the principal components in NIR spectra, was obtained from the NIR spectral results. Therefore, PCR could be applied to predict the pharmaceutical properties of the tablets and granules obtained by various pharmaceutical techniques.

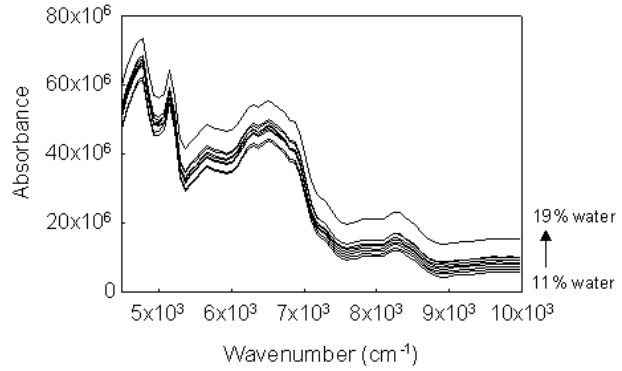

Figure 3. Near-infrared spectra of antipyrine granules with various particle sizes obtained by various amounts of added water.

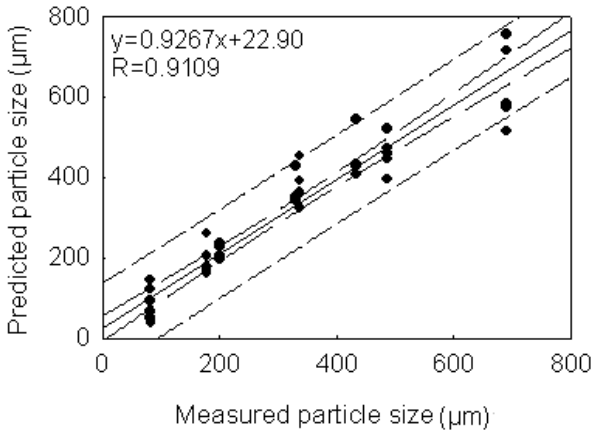

Figure 4. Prediction of mean particles size of granules obtained using various amounts of added water. The solid line, short-dash line, and long-dash line represent a regression line, 95% predicted interval, and 95% confidence interval, respectively.

Figure 4 shows the relationship between actual and predicted granule size of antipyrine by PCR. The predicted values were reproducible and had a reasonable standard deviation. The linear plot shows a slope of 0.9267, an intercept of 22.90, and a correlation coefficient constant, γ, of 0.9109. The relationship between SEV and the number of principal components (PCs) for evaluation of the particle size of granules based on NIR absorption suggested the following: the SEV decreased with an increase in the number of PCs, but it was almost constant after Factor 6. As a result, the minimum SEV value could be realized by using a 6-principal component model for the analysis in NIR spectra.

Figure 5 shows loading vectors corresponded to the PCs. The loading vector of PC1 was a plateau around –0.05, but there were positive peaks at 4765, 5150, and 6500 cm^{-1}, attributable to a mixture of excipient powder on loading vector of PC2. The peaks at 5110, 5220, and 7070 cm^{-1} were attributable to antipyrine of PC3. It is well known that spectral background is related to particle size of samples.[22-25] The level of spectral background is related with light scattering of small particles. Therefore, the results suggested that PC1 was attributable to the particle size of the sample, and PC2 and PC3 might be related with antipyrine and excipient mixture, respectively. Therefore, the obtained calibration line represents a satisfactory correlation between the actual and the predicted particle size values of the granules, meaning that the mean particle size of the granules could be evaluated from the NIR spectra.

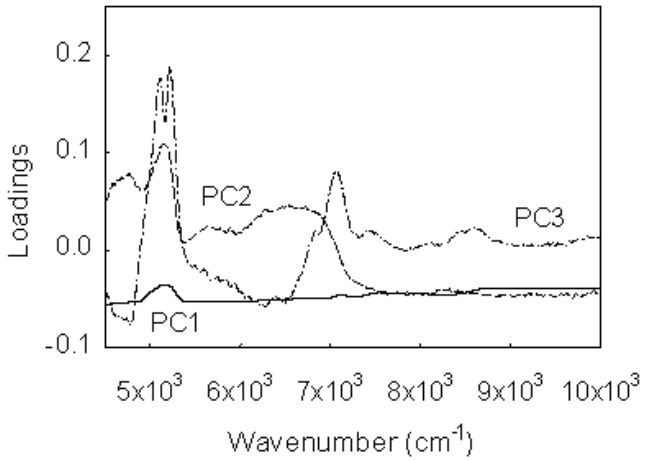

Figure 5. Loading vectors of PCR analysis for prediction of mean particle size of granules based on NIR spectra.

On the other hand, the angle of repose of the granules is an index of powder flowability—an important factor for production of high-quality pharmaceuticals. Prediction of the characteristics of granules is useful for the pharmaceutical industry.

Figure 6 shows the relationship between actual and predicted angles of repose of antipyrine granules by PCR. The linear plot shows a slope of 0.9057, an inter-

cept of 2.521, and a correlation coefficient constant, γ, of 0.8912. The relationship between SEV and the number of factors for evaluation of angle of repose of the granules suggested the following: since the SEV value was almost constant after Factor 5, the calibration model was calculated based on a 5-principal component model. The loading vectors of PC1, PC2, and PC3 were almost the same as those of **Figure 5**, suggesting that angle of repose is related to particle sizes, and that an index of powder flowability of the granules could be predicted from the NIR spectra.

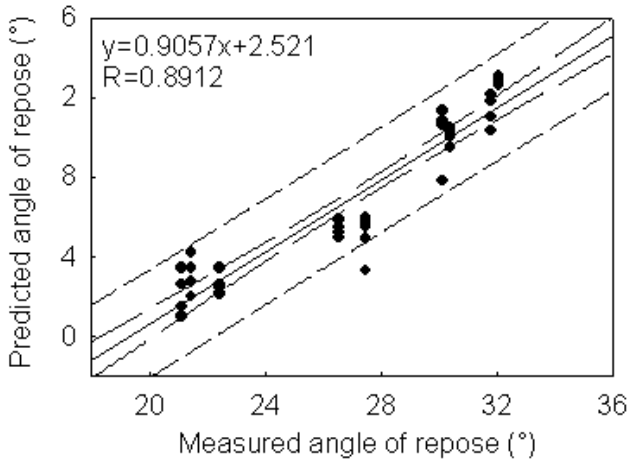

Figure 6. Prediction of angle of repose of granules obtained using various amounts of added water. The solid line, short-dash line, and long-dash line represent a regression line, 95% predicted interval, and 95% confidence interval, respectively.

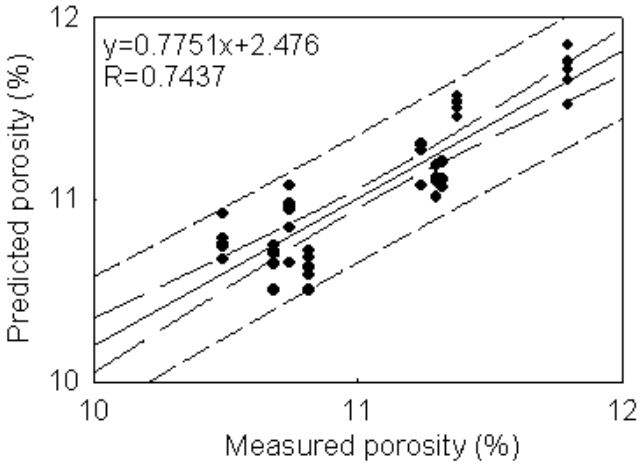

Figure 7. Prediction of porosity of tablets obtained from various kinds of granules. The solid line, short-dash line, and long-dash line represent a regression line, 95% predicted interval, and 95% confidence interval, respectively.

To maximize efficient production for high-quality pharmaceuticals, perdition of pharmaceutical properties of final products based on characteristics of row granules is required. So we investigated prediction of tablet characteristics by using an NIR chemoinfometric method.

Figure 7 shows the relationship between the actual and the predicted porosity of tablets obtained from antipyrine granules by PCR. The linear plot shows a slope of 0.7751, an intercept of 2.476, and a correlation coefficient constant, γ, of 0.7437. The relationship between SEV and the number of factors for evaluation of tablet porosity suggested the following: the SEV value was almost constant after Factor 7, so the calibration model was trained based on a 7-principal component model.

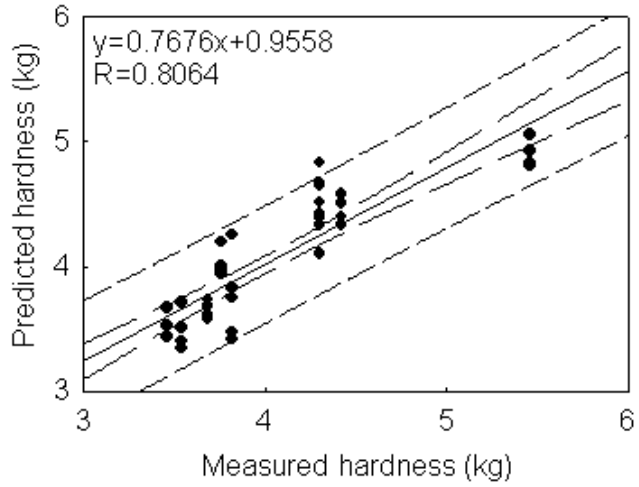

Figure 8. Prediction of hardness of tablet obtained from various kinds of granules. The solid line, short-dash line, and long-dash line represent a regression line, 95% predicted interval, and 95% confidence interval, respectively.

Figure 8 shows the relationship between actual and predicted tablet hardness obtained from the granules. The linear plot shows a slope of 0.7676, an intercept of 0.9558, and a correlation coefficient constant, γ, of 0.8064. The relationship between SEV and the number of factors for evaluation of tablet hardness was as follows: the SEV value was almost constant after Factor 3, so the calibration model was calculated for NIR absorption spectra based on a 3-principal component model. The loading vectors of PC1, PC2, and PC3 of analysis of porosity and tablet hardness were almost the same as the result of particle size prediction. Since it seems that the particle size of granules affected compaction behavior, the mechanical strength of the tablet

depended on powder flowability and/or particle size. The results suggest that the characteristics of tablets, such as porosity and mechanical strength were possibly predicted from the NIR spectra by using PCR, becaused the values were evaluated based on particle size of the granules.

CONCLUSION

Using a high-speed mixer and a sufficient amount of water allowed control of mean particle size and distribution of spherical granules. The physical and pharmaceutical properties, such as particle size, particle size distribution, angle of repose, and compressibility of granules, were affected by the added water amount. The calibration models to evaluate granule particle size and angle of repose, and tablet porosity and hardness, were obtained by decomposed NIR absorption spectra using PCR. The PCR results might indicate that pharmaceutical properties of final pharmaceutical products could be predicted from the NIR spectra of raw material (granules) by using PCR.

The NIR chemometric method is expected to provide a rapid quantitative analysis of pharmaceutical properties, as characterized by the simple, nondestructive, and highly sensitive nature of the method. The chemometric method is possible to apply to evaluate pharmaceutical properties in the industry, so it could be call "pharmainfometrics."

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