Self-Organizing Map Analysis Using Multivariate Data from Theophylline Tablets Predicted by a Thin-Plate Spline Interpolation

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The "quality by design" concept in pharmaceutical formulation development requires the establishment of a science-based rationale and a design space. We integrated thin-plate spline (TPS) interpolation and Kohonen's self-organizing map (SOM) to visualize the latent structure underlying causal factors and pharmaceutical responses. As a model pharmaceutical product, theophylline tablets were prepared based on a standard formulation. The tensile strength, disintegration time, and stability of these variables were measured as response variables. These responses were predicted quantitatively based on nonlinear TPS. A large amount of data on these tablets was generated and classified into several clusters using an SOM. The experimental values of the responses were predicted with high accuracy, and the data generated for the tablets were classified into several distinct clusters. The SOM feature map allowed us to analyze the global and local correlations between causal factors and tablet characteristics. The results of this study suggest that increasing the proportion of microcrystalline cellulose (MCC) improved the tensile strength and the stability of tensile strength of these theophylline tablets. In addition, the proportion of MCC has an optimum value for disintegration time and stability of disintegration. Increasing the proportion of magnesium stearate extended disintegration time. Increasing the compression force improved tensile strength, but degraded the stability of disintegration. This technique provides a better understanding of the relationships between causal factors and pharmaceutical responses in theophylline tablet formulations.

Key words tablet formulation; simulation; multivariate analysis; physical characterization; thin-plate spline; self-organizing map

Many pharmaceutical formulations are composed of several formulation factors and response variables, with complicated relationships between these causal factors and responses. In addition, the formulator's expertise and experience are essential for designing an acceptable product formulation. The empirical approach requires a prolonged development time and significant resources. The "quality by design" concept was proposed in the International Conference on Harmonization (ICH) Q8 guidance,[1] and the need to apply scientific formulation development has been recognized.[2] To gain insight into pharmaceutical formulations, a quantitative visualization technique is needed to understand the relationships between the causal factors and the pharmaceutical responses.

The response surface method (RSM) is widely used to visualize the relationships between causal factors and responses. However, this method is limited to the three-dimensional space and includes basic information about the relationship between only two limited factors and one response. The self-organizing map (SOM) proposed by Kohonen allows one to map multidimensional data onto a two-dimensional surface.[3] Applications of the SOM have been reported across a range of pharmaceutical and medical fields.[4–8] In this study, we used thin-plate spline (TPS) interpolation to generate a large amount of predictive data for a model formulation,[9] and we applied the SOM to classify the data into several distinct clusters. TPS can predict the nonlinear relationship between multivariate data with high accuracy.[10] This method does not require any complicated procedures such as an artificial neural network. Using TPS, one can easily estimate the nonlinear relationships between causal factors and response variables, and thereby simulate a large number of untested pharmaceutical responses instead of conducting experiments. We previously developed a quantitative approach, correlation analysis, and latent structure analysis using a hybrid TPS and SOM method.[11–13]

In this study, we investigated the possibility of using TPS-SOM analysis to understand the comprehensive relationships between multivariate data observed in the case of the tableting process. Tablet formulation is more complicated than powder formulation because it contains many process factors. As the model experiment, theophylline tablets based on the standard formulation were prepared and analyzed.

Experimental

Materials Theophylline (JP grade) was purchased from Hachidai Pharmaceutical Co. (Osaka, Japan). Microcrystalline cellulose (MCC; Ceolus PH-101, Asahi Kasei Chemicals Co., Tokyo, Japan), lactose (200-mesh grade, DMV International, Veghel, the Netherlands), and cornstarch (Nihon Shokuhin Kako Co., Tokyo, Japan) were gifts from Daiichi Sankyo Co. (Tokyo, Japan). Magnesium stearate (Mg-St) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Experimental Design and Preparation of Theophylline Tablets The model formulations for theophylline tablets are summarized in Table 1. The proportion of MCC added (X_1) was selected as a formulation variable, and the proportion of Mg-St (X_2) and the compression force (X_3) were selected as process variables. The formulation variables and process variables were assigned according to a completely randomized four-level design. Thus, 64 variations of model formulations were prepared. Theophylline tablets were prepared using a typical dry process.

Preparation of Theophylline Tablets All ingredients were dried at 75°C for 24 h and sieved through a 20-mesh

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Table 1. Formulation and Process Variables of Theophylline Tablets

Formulation variables	
Theophylline	100 mg
Lactose:Corn starch = 7:3 mixture / Microcrystalline cellulose (MCC) *X_1	150 mg
Process variables	
Magnesium stearate (Mg-St), X_2	0.5, 1.0, 1.5, 2.0%
Compression force, X_3	6, 8, 10, 12 kN

*X_1: Percentage of MCC 10, 30, 50, 70%.

screen. The sieved ingredients were accurately weighed according to the experimental formulations and all ingredients except Mg-St were blended in a polyethylene bag for 1 min. Mg-St was then added to the mixture, which was blended for 3 min using a drum mixer. The final blend was compressed at a predetermined force according to the experimental design into a round tablet, 8 mm in diameter, using a Handtab 100 hydraulic press (Ichihashi-Seiki Co., Kyoto, Japan).

Measurement of the Response Variables of the Model Formulations Tensile strength (TS) and disintegration time (DT) of the tablets were evaluated before (TSB and DTB, respectively) and after (TSA and DTA, respectively) an accelerated test. TS was determined using a diametric compression tester (Monsanto tablet hardness tester) to accurately measure the maximum diametric crushing force. TS was calculated as:

$$\mathrm{TS} = \frac{2F}{\pi dt} \tag{1}$$

where F is the maximum diametric crushing force, and d and t are the diameter and thickness of the tablet, respectively. The disintegration test was performed according to the JP15 disintegration test for tablets, namely using a disintegration tester (NT-20H; Toyama Sangyo Co., Osaka, Japan) and water as a solvent at 37°C. DT was defined as the interval required for a tablet or its particles to disappear completely from the tester net. Stability of TS and DT were estimated as:

$$S = \frac{y_i' - y_i}{y_i} \tag{2}$$

where y_i is TSB or DTB, and y_i' is TSA or DTA, respectively.

The TSB (Y_1), DTB (Y_2), stability of TS (Y_3), and stability of DT (Y_4) were selected as the response variables of theophylline tablets.

Accelerated Test To evaluate the effect of stress conditions on TS and DT, the tablets were stored in a dish at 40°C and 75% relative humidity for 30 d in a stability chamber (CSH-110; ESPEC Co., Osaka, Japan).

Data Generation from Theophylline Tablets Using TPS TPS is based on the concept of the transformation problems of elastic beams. Green functions are used for the minimum curvature interpolation of multidimensional data points. TPS estimates multidimensional data using the sum of interpolations made with a green function and a linear polynomial equation. This can naturally incorporate multivariate observational data, including experimental errors.

A large number of untested tablet formulations were generated sequentially. Formulation and process variables, namely the proportion of MCC added (X_1), the proportion of Mg-St (X_2), and compression force (X_3), were used to generate 16 data points each. In total, 4096 untested formulations were prepared. The tablet characteristics as the response variables for these untested formulations were predicted with TPS.

SOM Clustering of Theophylline Tablets The SOM is a feedforward-type neural network model. The SOM comprises one input layer and one output layer, with the array of nodes located in the output layer. The SOM algorithm is based on unsupervised, competitive learning. The network ultimately associates the output nodes with groups or patterns of input vectors by repeating the learning.

An SOM was used to make the feature maps and clustering of theophylline tablet formulations. To make the maps, the weight (w) of each tablet characteristic (Y_1–Y_4) was assigned a value of 1 (w=1), but no weight (w=0) was adopted for causal factors (X_1–X_3). For the graphical display and feature mapping, the four-dimensional characteristic spaces of tablets were projected onto an output layer comprising 6×7 nodes (neurons).

SOM software offers several clustering techniques: the SOM-Ward method, Ward method, and SOM-Single-Linkage method; SOM-Ward was used for clustering in this study because it is generally considered the most efficient. In the SOM-Ward method, the local distance of each node as an output of SOM analysis is classified based on the Ward method.[14]

Evaluation of the Desirability of the Response Variables To compare each of the tablet formulations quantitatively, desirability (d_i) was calculated as:

$$d_i = \frac{|y_i| - |y_{\mathrm{worst}}|}{|y_{\mathrm{best}}| - |y_{\mathrm{worst}}|} \tag{3}$$

where Y_{worst} represents the worst value (for Y_1, the lowest value; for Y_2, the highest value; for Y_3 and Y_4, the value that is the farthest from 0), Y_{best} represents the best value (for Y_1, the highest value; for Y_2, the lowest value; for Y_3 and Y_4, 0 or the value that is the nearest to 0), and Y_i indicates the relevant experimental value. In addition, if Y_i is equal to Y_{best}, then d_i=1 (best score). If Y_i is equal to Y_{worst}, then d_i=0 (worst score).

The global desirability (D), which is the geometric mean of desirability d_i, was defined as:

$$D = (d_1 \times d_2 \times d_3 \times \cdots d_r)^{1/r} = \left[\prod_{i=1}^{r} d_i\right]^{1/r} \tag{4}$$

where r is the number of responses.

An SOM feature map of the global desirability was generated and used to compare the formulation, process, and response variable feature maps.

Software dataNESIA version 3.2 (Yamatake Corp., Tokyo, Japan) was used for TPS interpolation and for generating the prediction data. Viscovery SOMine version 5.0 (Eudaptics Software, Vienna, Austria) was used for SOM clustering of the theophylline tablet data.

Results

Prediction of Response Variables Using TPS Each response variable was estimated by TPS based on the original dataset. The accuracy of the predictive ability was evaluated by leave-one-out cross-validation. The results are shown in Fig. 1. The correlation coefficients for the response variables were high in the cases of TSB (Y_1: 0.995) and DTB (Y_2: 0.984), whereas the TPS predictions of the stability of TS (Y_3: 0.957) and stability of DT (Y_4: 0.924) were lower than these

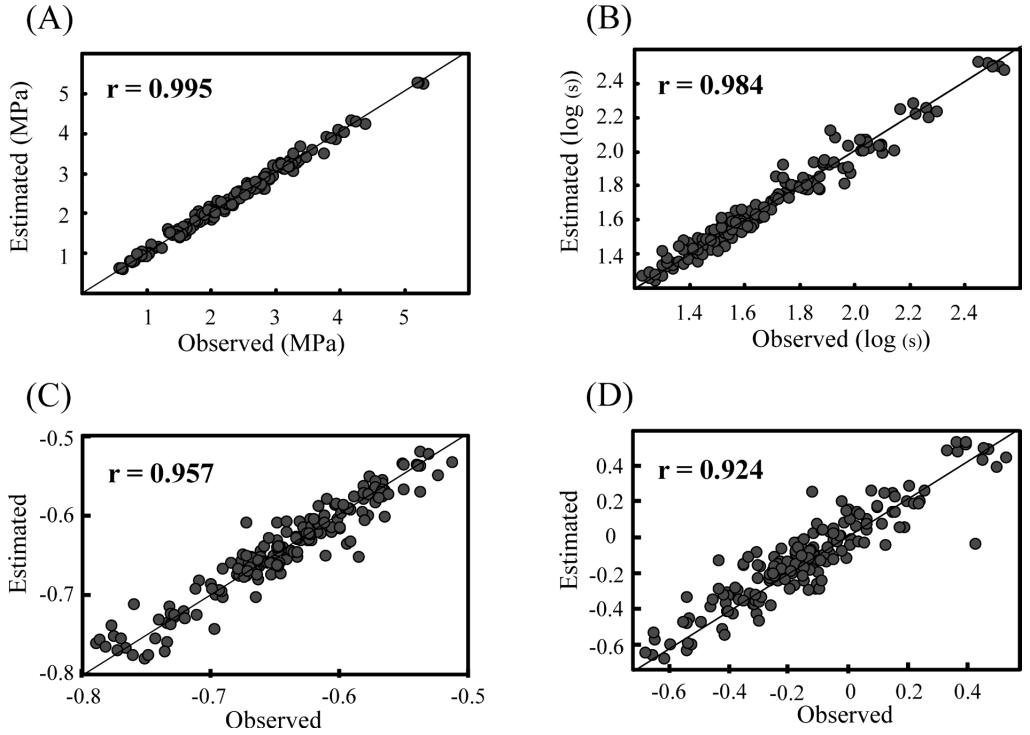

Fig. 1. Leave-One-Out Cross-Validated Predictive Accuracy of TPS for (A) Tensile Strength, (B) Disintegration Time, (C) Stability of Tensile Strength, and (D) Stability of Disintegration

responses. Because the values of Y_3 and Y_4 were calculated using two experimental values (before and after the accelerated test), the predictive ability of TPS for these two responses (Y_3 and Y_4) is more limited than that for the other two responses (Y_1 and Y_2).

Visualization of the Causal Relationships Using SOM To visualize the causal relationships between the formulation factors and responses, feature maps were estimated using an SOM. The results are shown in Fig. 2. Each feature map shows the values of one variable in each map unit. The feature maps make it possible to analyze the global and local correlations between variables.

TSB (Y_1 in Fig. 2A) is higher on the left side and is linked to a high proportion of MCC (X_1 in Fig. 2E) and a strong compression force (X_3 in Fig. 2G). In the lower node of DTB (Y_2 in Fig. 2B), the proportion of Mg-St (X_3 in Fig. 2F) is low. The map of stability of TS (Y_3 in Fig. 2C) shows that stability of TS was inversely proportional to the proportion of MCC (X_1 in Fig. 2E). The stability of DT (Y_4 in Fig. 2D) increased with increasing compression force (X_3 in Fig. 2G) and decreased with increasing or decreasing proportion of MCC (X_1 in Fig. 2E).

The feature map of global desirability (overall score), which was estimated using the overall tablet characteristics, is shown in Fig. 3. A higher score indicates a better overall performance of the tablet formulation (1 is the best and 0 is the worst). Tablet formulations with better disintegration or better tensile strength are clustered in the high-score nodes (Figs. 3A, B). In these nodes, tablet formulations are designed with a high proportion of MCC and a low proportion of Mg-St. In particular, the proportion of Mg-St was remarkably low in better disintegration nodes, and the proportion of MCC was remarkably high in better tensile strength nodes. Conversely, the formulations with the worst characteristics converged in the low-score nodes (Fig. 3C). Tablet formulations in these nodes are designed with a low proportion of MCC, a high proportion of Mg-St, and weak compression force.

Comprehensive Visualization of the Causal Associations Using SOM Clustering To analyze the relationships between the formulation, process, and response variables in theophylline tablets, SOM clustering of the response variables was applied. The results are shown in Fig. 4. The theophylline tablet formulations generated (n=4096) were classified into five distinct clusters with similar tablet characteristics. The response variables (Y_1–Y_4) of the theophylline tablets were used as the input vectors, and the number of nodes in the output was set at 42 (97.5 formulations per node). The values of responses in each cluster are shown in Table 2. The formulations in cluster 1 included tablets with high disintegration and high stability of disintegration (the lowest value of Y_2 and the value of Y_4 that is nearest to 0). Cluster 2 included tablet formulations with high tensile strength, high disintegration, and high stability of tensile strength (high value of Y_1, low value of Y_2, and a value of Y_3 near 0) but low stability of disintegration (the highest value of Y_4; DT was extended by an accelerated test). The formulations in cluster 3 had low tensile strength and low stability of tensile strength and disintegration (the lowest values of Y_1, the values of Y_3 and Y_4 that are farthest from 0). Cluster 4 included formulations with average tablet characteristics. Cluster 5 included tablet formulations with high tensile strength and high stability of tensile strength (the highest values of Y_1 and the value of Y_3 nearest to 0), whereas the disintegration and the stability of disintegration were low (the highest value of Y_2 and the value of Y_4 farther from 0).

Based on the reference vectors of the clusters, the three-dimensional diagrams for tablet formulations (X_1–X_3) can be represented as shown in Fig. 5. The formulations in cluster 1 contained mainly a small proportion of Mg-St (X_2), and had

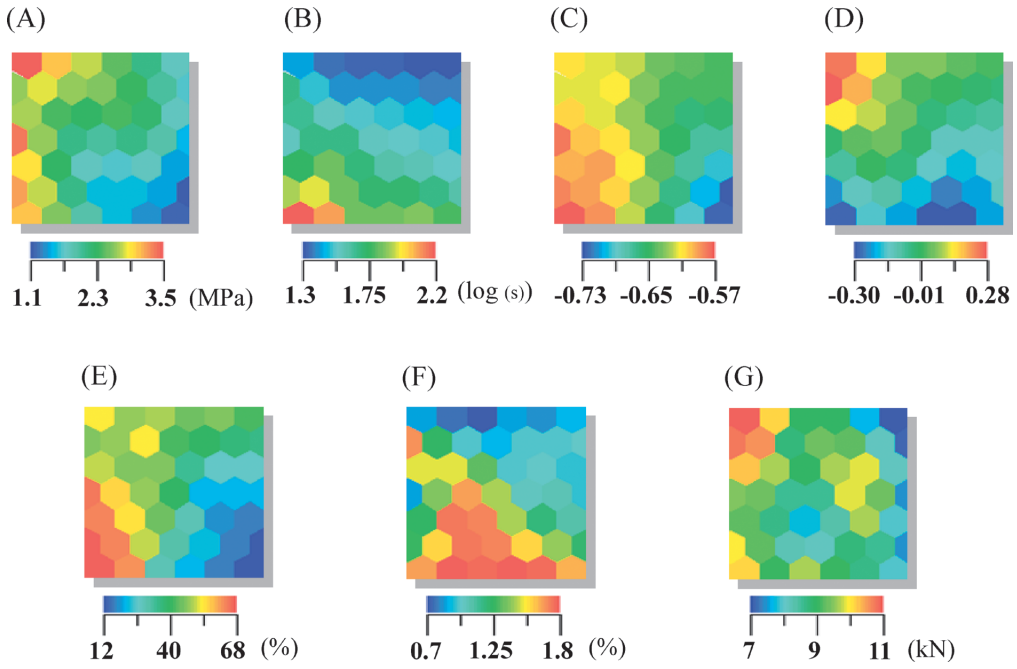

Fig. 2. SOM Feature Maps of Response (A–D), Formulation, and Process (E–G) Variables

The SOM was constructed based on four tablet characteristics. The four upper plots depict the distribution of tablet characteristics: (A) tensile strength, (B) disintegration time, (C) stability of tensile strength, and (D) stability of disintegration. The three lower plots depict the distribution of formulation and process variables: (E) proportion of MCC, (F) proportion of Mg-St, and (G) compression force.

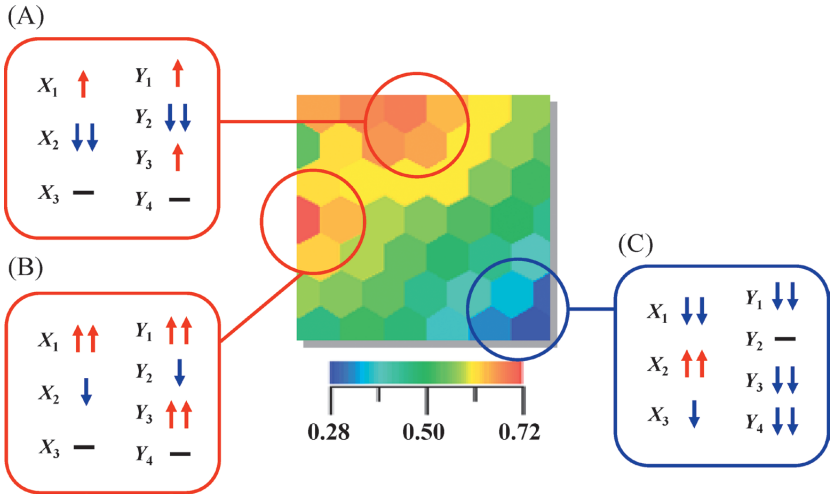

Fig. 3. SOM Feature Map of the Overall Score (Calculated as the Desirability Function)

The overall score was associated with no weight in the SOM analysis. (A) Region comprising tablets with better disintegration, (B) region comprising tablets with better tensile strength, and (C) region comprising low-quality tablets.

about 30–50% MCC (X_1) for a low compression force (X_3) and about 10–30% MCC (X_1) for a high compression force (X_3). The formulations in cluster 2 contained mainly a large proportion of MCC (X_1) and a small proportion of Mg-St (X_2), and had a high compression force (X_3), whereas the formulations in cluster 3 contained mainly a small proportion of MCC (X_1) and a large proportion of Mg-St (X_2), and had a low compression force. Most of the formulations in cluster 4 contained large proportions of MCC (X_1) and Mg-St (X_2) and had a low compression force (X_3). The formulations in cluster 5 contained large proportions of MCC (X_1) and Mg-St (X_2) and had a high compression force (X_3).

Discussion

A pharmaceutical product comprises several formulation factors and process variables. The relationships between the causal factors and the pharmaceutical responses are often complicated, and visualizing techniques are imperative for understanding the causal relationships. The RSM is increasingly applied to visualize the input–output relationships. However, this method is not sufficient because it is limited to a three-dimensional diagram, and it cannot visualize data in four dimensions.

The SOM algorithm differs from linear mapping methods such as principal component analysis because the SOM preserves the original multivariate data comprising several causal factors and response variables, and summarizes the data *via*

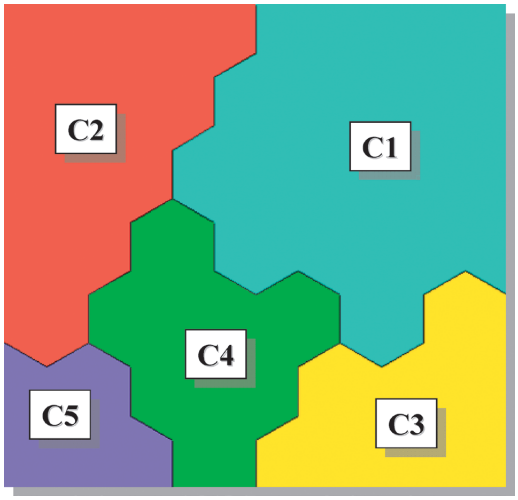

Fig. 4. SOM Clusters of the Simultaneous Predicted Data Estimated by TPS

Each cluster comprises similar tablet characteristics. The number of nodes was fixed at 72.

We then applied this method for optimizing pharmaceutical product formulations and our findings to date suggest the usefulness of TPS.[15–17] In addition to generating response surfaces, TPS can predict untested variables with high accuracy using reading points on the response surfaces. Taking advantage of this predictive ability, we were able to predict the characteristics of a large number of untested tablets (4096) instead of conducting experiments.

We first estimated the causal association between the formulation, process, and response variables using the SOM feature maps. Based on the SOM feature maps, response variables as the input vector were mapped distinctly into each map, together with the formulation and process variables, which were not used as input vectors (Fig. 2). A strong connection was seen between the design variables and tablet characteristics. TSB (Y_1) is higher on the left side and lower on the right side. In the higher TSB node, (Y_1) is linked to a high proportion of MCC (X_1) and a strong compression force (X_3) (Figs. 2A, E, G). This result suggests that the TSB (Y_1) had a strong positive correlation with the proportion of MCC

Table 2. Tablet Characteristics of Each Cluster Estimated by TPS and SOM Clustering (Data are Mean Values)

Cluster	Y_1 (MPa)	Y_2 (log (s))	Y_3	Y_4
C1	2.040	1.466	−0.627	−0.050
C2	2.940	1.537	−0.617	0.103
C3	1.290	1.719	−0.706	−0.223
C4	1.868	1.747	−0.621	−0.078
C5	2.974	2.049	−0.593	−0.222

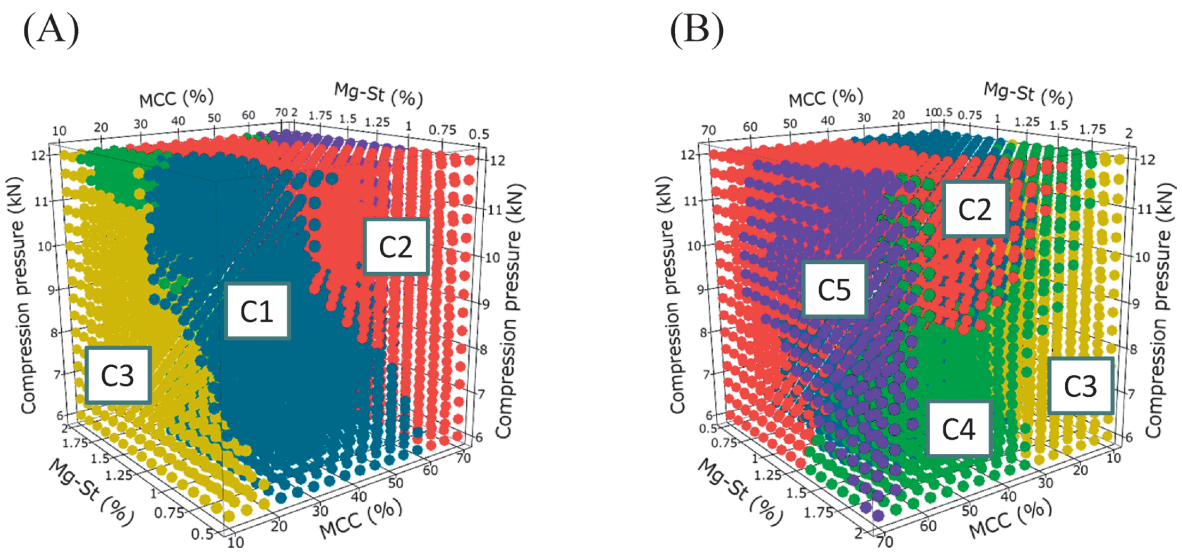

Fig. 5. Three-Dimensional Diagrams of the Proportions of MCC (X_1) and Mg-St (X_2) and the Compression Force (X_3)

The diagram on the left is viewed from the front (A), and that on the right is viewed from the back (B).

a small number of nodes in the output layer, which can be related directly to the original variables. The SOM provides a better illustration of the relationships when nonlinear structures exist in the dataset.

Because a large amount of data are generally needed for SOM visualization, we applied TPS to make up for the lack of experimental data. We estimated the nonlinear relationships between the factors and characteristics with high accuracy. The leave-one-out cross-validation results showed sufficiently high correlation coefficients for tablet characteristics (Fig. 1).

(X_1) and the compression force (X_3). DTB (Y_2) is higher on the lower-left side and lower on the upper-right side. This result suggests that the DTB (Y_2) is strongly and positively correlated with the proportion of Mg-St (X_2). In addition, the highest node of DTB (Y_2) is linked to the highest node of the proportion of MCC (X_1). The stability of TS (Y_3) is higher on the lower-left side and lower on the lower-right side. This finding suggests that the stability of TS (Y_3) is strongly and positively correlated with the proportion of MCC (X_1). The stability of DT (Y_4) is higher on the upper-left side, an area that is linked

to the higher nodes of the compression force (X_3). This finding suggests that the stability of DT (Y_4) increases with increasing compression force (X_3). The stability of DT (Y_4) is lower on both the lower-left and lower-right sides. This finding suggests that the stability of DT (Y_4) decreases with an increasing or decreasing proportion of MCC (X_1). That is, the proportion of MCC (X_1) has optimal value for the best stability of DT (Y_4).

We next performed a comprehensive analysis of the tablet characteristics. Using the desirability function, which can place all the characteristics into the unity response, we defined an overall score range of 0 to 1 and analyzed the score using an SOM feature map (Fig. 3). Although the overall score defined in Eq. 4 was associated with no weight in the SOM analysis, high-quality (better disintegration or better tensile strength) and low-quality tablets were identified clearly. In addition, each feature map indicated the same positional relationship in every map, and this overall score map could be compared with the other feature maps. These results suggest that high-quality tablets are designed with a high proportion of MCC and a low proportion of Mg-St. In particular, disintegrative performance (Y_2 and Y_4) is strongly affected by the proportion of Mg-St, and tensile strength (Y_1 and Y_3) is strongly affected by the proportion of MCC. As described above, the SOM feature map analysis indicated that the method is useful for understanding the multivariate data.

Finally, SOM clustering was applied to the tablet formulations to classify them into clusters. Because the clustering was constructed using only the tablet characteristics (Fig. 4), each cluster exhibits clear differences in the tablet characteristics (Table 2). Consequently, the three-dimensional diagrams indicate clearly that theophylline tablet formulations distributed into five clusters (Fig. 5). Formulations in cluster 1 had fast disintegration and good DT stability (a value near 0) with a low proportion of Mg-St (X_2) and an adequate amount of MCC (X_1); the proportion of MCC was about 30–50% for a low compression force and about 10–30% for a high compression force. Formulations in cluster 2 had high TSB and DT stability (DT was extended by an accelerated test) with a high proportion of MCC (X_1) and a low proportion of Mg-St (X_2); the proportion of MCC (X_1) was about 40–60% when there was a high proportion of Mg-St (X_2) and a high compression force (X_3). Formulations in cluster 3 had low TSB and abysmal TS and DT stability (TS fell to a lower value, DT was hastened by an accelerated test) with a low proportion of MCC (X_1) and high proportion of Mg-St (X_2). Formulations in cluster 4 had average properties with high proportions of both MCC (X_1) and Mg-St (X_2) and a low compression force. Formulations in cluster 5 had high TSB, good TS stability (the highest value among the clusters), slow disintegration, and poor DT stability (DT was hastened by an accelerated test) with high proportions of MCC (X_1) and Mg-St (X_2) and a high compression force. These results suggest that increasing the proportion of MCC improved the TSB and stability of TS as shown in clusters 2 and 5. The proportion of MCC has an optimum value for DTB and stability of DT, as shown in cluster 1. Increasing the proportion of Mg-St extended DTB, as shown in clusters 3, 4, and 5. Increasing the compression force improved TSB and degraded DT stability (DT was extended by an accelerated test), as shown in cluster 2.

Our model shows that the combined use of TPS and SOM makes it possible to visualize quantitatively the causal association between the design variables and tablet characteristics. Our results indicate that the combination of TPS and SOM is useful for instinctive understanding of the relationships between causal factors and pharmaceutical responses.

Conclusion

We successfully classified the design variables of theophylline tablet formulations into five clusters with similar characteristics. The three-dimensional diagrams cover a wide range of formulations, allowing us to understand fully the behavior of a formulation and the process variables of the responses. As described above, this method using TPS and SOM offers a useful tool for the visualization and quantitative study of complicated causal relationships. It might also be possible to analyze more complicated or realistic cases, such as a tablet containing many formulation factors, different types of excipients, and different tableting process variables.

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