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Reliability assessment for the optimal formulations of pharmaceutical products predicted by a nonlinear response surface method

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ABSTRACT

A novel method of evaluating the reliability of the optimal formulations of pharmaceutical products was developed based on statistical techniques. Hydrogel ointments, PEGylated emulsions, and solid dispersions were chosen as the model data for the pharmaceutical products, and the formulations of these models were optimized using a nonlinear response surface method incorporating multivariate spline interpolation. A bootstrap resampling method combined with a Kohonen's self-organizing map, was used to estimate the confidence intervals of the optimal formulations. To understand the factors significantly affecting the optimal formulations, a leave-one-factor-out (LOFO) method and a random number technique were introduced as sensitivity analyses. Our results suggest that the random number technique is a better approach than the LOFO method. To determine the design space and control space based on a scientific rationale that can satisfy a number of specifications of the pharmaceutical responses, a novel approach that takes advantage of the random number technique was investigated. The control space was successfully defined as a super-cubic area inscribed in a super-spherical area in the design space of the factors.

1. Introduction

In many pharmaceutical formulations, there are complicated relationships between the formulation factors and the response variables relating to the effectiveness, usefulness, stability, and safety of the product. Therefore, the causal relationships must be understood in designing a pharmaceutical formulation. In recent years, ICH Q8 guidance (Yu, 2008) has propounded the establishment of a science-based rationale. The concept of quality by design described in the ICH Q8 guidance states that "quality cannot be tested into products, i.e., quality should be built in by design."

Previously, we developed an ingenious response surface method incorporating multivariate spline interpolation (RSM-S), which has been used to determine acceptable formulations of pharmaceuticals (Takayama et al., 2004). The basic concept of multivariate spline interpolation (MSI) involves a boundary element method. Green functions are used for the minimum curvature interpolation of multidimensional data points (Sandwell, 1987). MSI estimates multidimensional data using a thin-plate spline that represents the sum of the interpolations made with a Green function and a linear polynomial equation (Wahba, 1990). This can naturally interpolate observational data, including experimental errors. Using RSM-S, we can easily understand nonlinear relationships between causal factors and response variables, and estimate a stable and reproducible simultaneous optimal solution. Furthermore, this method does not require any complicated procedures, such as an artificial neural network, and it has been applied to pragmatic cases to optimize pharmaceutical formulations (Onuki et al., 2004, 2005). However, no proper method of evaluating the effects of individual factors on the response variables has yet been established for RSM-S. The aim of this study was to evaluate the factors affecting the optimal solutions estimated by RSM-S, using a bootstrap (BS) resampling method, Kohonen's self-organizing map (SOM), and a leave-one-factor-out (LOFO) method or a random number technique.

The ICH Q8 guideline suggests that the design space must be identified in a scientific sense in the development of a pharmaceutical formulation. The design space is defined as the multidimensional combination and interaction of factors that have been demonstrated to provide an assurance of quality. However, few tangible methods have been suggested with which to establish a science-based design space and control space (ICH Draft Consensus Guideline, Pharmaceutical Development, Annex to Q8, 2007; MacGregor and Bruwer, 2008). In addition to the reliability assessment presented in this study, a novel method of determining the design space and control space is proposed that can satisfy various specifications.

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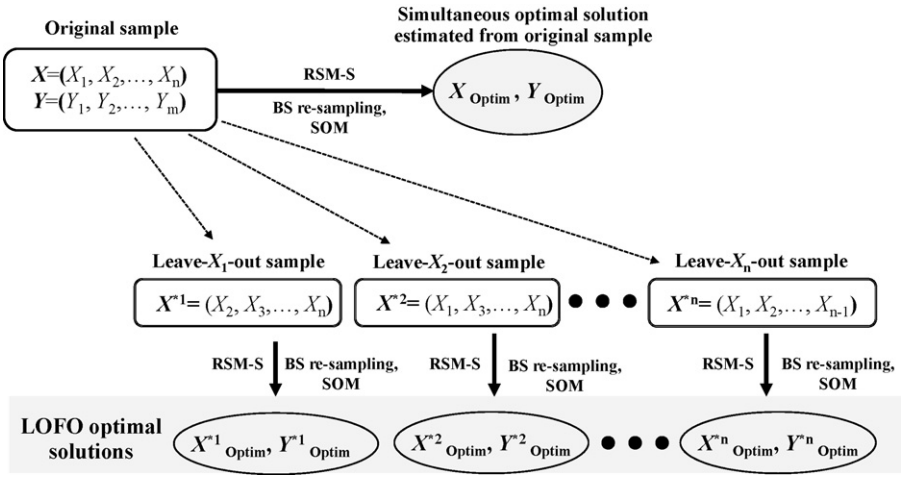

Fig. 1. Schematic representation of the sensitivity analysis of the formulation factors using a leave-one-factor-out (LOFO) method. A number of duplicated samples with replacement (BS-LOFO samples) were generated using the BS resampling method. RSM-S was applied to the BS-LOFO samples, and then the cluster of BS-LOFO optimal solutions (X^*_{optim}, Y^*_{optim}) was estimated using SOM. The difference between the BS-LOFO and BS-original optimal solutions (X_{optim}, Y_{optim}) was evaluated using statistical quantities.

2. Theory

2.1. Sensitivity analysis based on a leave-one-factor-out method

A leave-one-factor-out method was newly developed to understand the causal factors affecting optimal solutions. The procedure of the LOFO method is shown in Fig. 1. LOFO samples, in which the factor to be considered (X_i) was removed from the original dataset (X_1, X_2, ..., X_{i-1}, X_{i+1}, ..., X_n), were prepared. A number of duplicated samples with replacement (BS-LOFO samples) were generated using the BS resampling method. Details of the BS method have been reported elsewhere (Efron and Tibshirani, 1993). RSM-S was then applied to the BS-LOFO samples and the cluster of the BS-LOFO optimal solutions (X^*_{optim}, Y^*_{optim}) was then estimated using SOM. A set of BS optimal solutions can be regarded as a mixture of several clusters composed of a single global and other local optimal solutions. In evaluating the reliability of the original optimal solution, extracting a cluster of global optimal solutions from the BS solutions is inevitable. Details of the SOM clustering of optimal solutions have been fully described previously (Arai et al., 2007; Onuki et al., 2008). The difference between the BS-LOFO and BS-original optimal solutions (X_{optim}, Y_{optim}) were evaluated using statistical quantities. That is, the ratio of the mean square of the BS-original optimal response to that of the BS-LOFO optimal response was estimated.

Student's t was used when the mean square of the BS-original optimal solutions was equal to that of BS-LOFO optimal solutions; otherwise, Welch's t was used.

2.2. Sensitivity analysis based on the random number technique

To overcome the controversial points attributed to the LOFO method, another approach to evaluating the effects of factors on the optimal solution was proposed, one that uses a random number technique. The concept of the random number technique is shown in Fig. 2. All the factors except the factor to be considered were fixed at the optimal solution ($X_{1,optim}$, $X_{2,optim}$, ..., $X_{i-1,optim}$, $X_{i+1,optim}$, ..., $X_{n,optim}$), and the relevant factor ($X_{i,optim}$) was perturbed by generating normally distributed random number variables in proximity to the optimal solution within the range of the BS-original optimal solution $\pm SD$ value, and then the response variables were estimated by RSM-S. The ratio of the mean square of the estimated responses (SD^2_P) to that of the BS-optimal responses (SD^2_B) was defined as the

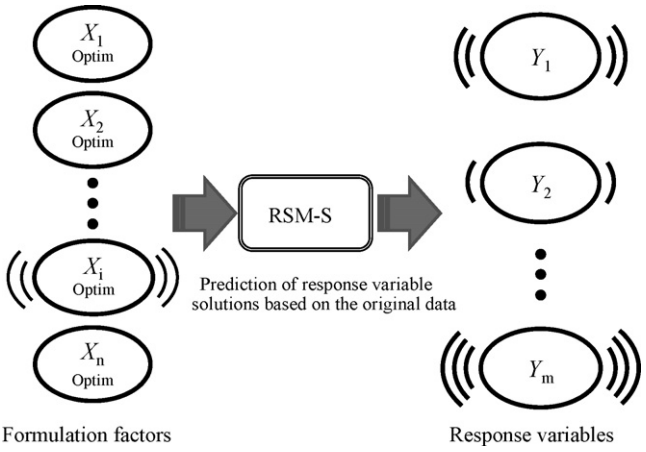

Fig. 2. Schematic representation of the sensitivity analysis of the formulation factors using a normally distributed random number technique. All the factors except the factor to be considered were fixed at the optimal solution ($X_{1,optim}$, $X_{2,optim}$, ..., $X_{i-1,optim}$, $X_{i+1,optim}$, ..., $X_{n,optim}$), and the relevant factor ($X_{i,optim}$) was perturbed by generating a normal distribution of random number variables in proximity to the optimal solution, and then the response variables were estimated by RSM-S. The ratio of the mean square of the estimated responses (SD^2_P) to the BS-optimal solutions (SD^2_B) was defined as the judging index, evaluating the effect of the relevant factors on the response variables in the optimal solution. The scheme shows that the optimal Y_m is the most sensitive to the perturbation of the optimal X_i.

"judging index", which evaluated the effect of the factors on the response variables in the optimal solution (Eq. (1)).

$$R = \frac{SD^2_P}{SD^2_B} \quad (1)$$

The effect of the relevant factor on the response variables is increased as the R-value increases. This method is a sensitive detector that allows us to understand the effects of factors on the area neighboring the optimal solutions.

2.3. Identification of the possible design space and control space

To set up a possible design space that can satisfy the various specifications requested for the response variables, a novel method using a random number technique was investigated. The concept of the method is illustrated in Fig. 3. To understand the concept clearly, model data for solid dispersions were used, referred to as case C,

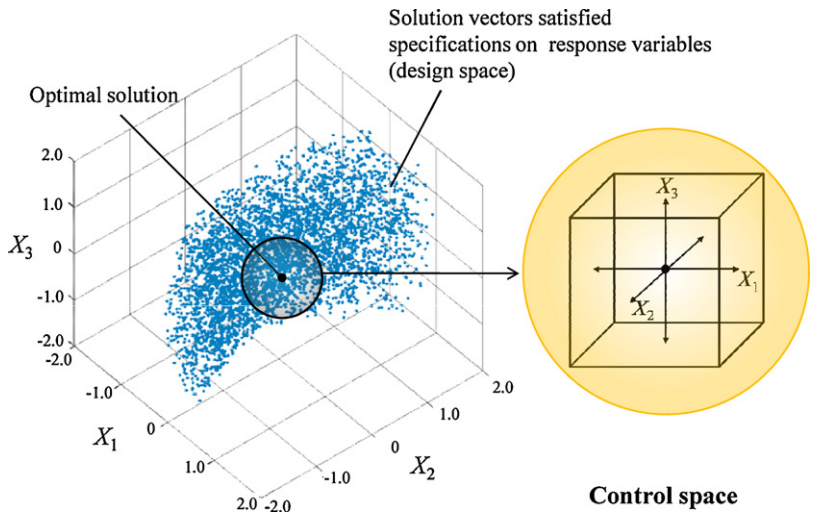

Fig. 3. Typical example of the setup of the possible design space and control space using a uniform random number technique. A large number of formulation vectors were generated in the neighborhood of the optimal solution using a uniform random number technique. The design space was defined as the set of formulation vectors that satisfy the overall specifications of the responses. A dataset of solid dispersions (case C in Table 1) was used to draw this graph. Three factors (X_1–X_3) were preliminarily normalized using the BS-optimal mean and σ values, and they are shown in the range of −2 to 2 in Fig. 3 (left). The control space can be defined as the set of formulation vectors within a super-spherical range. In addition, the upper and lower limits in the control space are defined as a super-cubic area inscribed in the super-spherical range in Fig. 3 (right).

and are described in Section 3. All the formulation factors (X_1, X_2, …, X_n) were perturbed using uniform random number variables, and a large number of formulations were generated as neighbors of the optimal solution, for instance, within the range of the BS-original optimal solution $\pm 2\sigma$, where σ is the calculated standard deviations of the BS-optimal solutions. Each factor was preliminarily normalized using the BS-optimal mean and σ values, and then a group consisting of solution vectors that satisfied the specifications for the response variables was defined as the design space in Fig. 3 (left). The solution vectors were restricted within a range from 95% to 105% of the optimal solution as proper specifications. A set of Euclidian distances (d) between the optimal solution ($\bar{X}_i$) and the formulation vectors ($X_{v,i}$), which violate the specifications for the response variables, was calculated as:

$$d = \sqrt{\sum_{i=1}^{n} \left(\frac{X_{v,\,i} - \bar{X}_i}{SD_i} \right)^2} \tag{2}$$

where SD_i is the standard deviation of the BS-original optimal solutions and n is the number of factors. The formulation vector located on the shortest Euclidian distance (d_{min}) from the optimal solution was explored. The vectors ($X_{cs,i}$) within a super-spherical range can be regarded as the "control space" (MacGregor and Bruwer, 2008) and are defined as:

$$\sqrt{\sum_{i=1}^{n} \left(\frac{X_{cs,\,i} - \bar{X}_i}{SD_i} \right)^2} < d_{min} \tag{3}$$

It is also desirable that the factors are orthogonally flexible within the super-spherical range described above. The normalized range (r) of the upper and lower limits is defined as the length of the side of the super-cubic area inscribed in the super-spherical range:

$$-\frac{1}{\sqrt{n}} d_{min} \le r \le +\frac{1}{\sqrt{n}} d_{min} \tag{4}$$

3. Materials and methods

3.1. Model data

Three cases of formulation optimization were chosen as the model data from previously published articles, as summarized in Table 1 (Takayama et al., 1985; Wu et al., 2001; Fan et al., 2004). Ketoprofen hydrogels, containing 1-*O*-3-*n*-butylcyclohexanol (OEBC) and diisopropyl adipate (DIA) as chemical enhancers and isopropanol (IPA) as a solvent, were used as case A (Wu et al., 2001). The amounts of OEBC, DIA, and IPA were selected as the causal factors. All other components in the hydrogels were fixed at constant values. The penetration rate (R_p) of ketoprofen across the skin was determined from blood concentration–time profiles after the administration of the hydrogels to rat abdominal skin. A total irritation score (TIS) at the application site on the skin was estimated according to pathological observations with an optical microscope. The R_p and TIS values for the model formulations were used as the pharmaceutical responses.

Table 1
Data sets for the simultaneous optimizations estimated by RSM-S.

Formulation factors	Response variables
(A) Ketoprofen hydrogels (48 formulations)	
X_1: 1-*O*-Ethyl-3-n-butylcyclohexanol (OEBC, %)	Y_1: Penetration rate (R_p, μg/h)
X_2: Diisopropyl adipate (DIA, %)	Y_2: Total irritation score (TIS)
X_3: Isopropanol (IPA, %)	
(B) Paclitaxel emulsions (45 formulations)	
X_1: Soybean oil (mg/mL)	Y_1: Entrapment efficiency (%)
X_2: PEG-DSPE (mg/mL)	Y_2: Particle size (nm)
X_3: Polysorbate 80 (mg/mL)	
(C) Indomethacin solid dispersions (11 formulations)	
X_1: Polyvinyl polypyrrolidone (PVPP, g)	Y_1: 50% Dissolution time (min)
X_2: Methyl cellulose (MC, mg)	Y_2: Stability (%)
X_3: Ethanol (mL)	

Table 2
The simultaneous original optimal solution and the BS-optimal solution of the formulations.

	X_1 (%)	X_2 (%)	X_3 (%)	Y_1 ((g/h)	Y_2
(A) Ketoprofen hydrogels					
Original optimum[a]	1.26	4.87	31.7	341.6	4.17
BS-optimum[b]	1.23 ± 0.12	4.71 ± 0.26	31.5 ± 1.7	338.3 ± 47.8	4.14 ± 1.04
	X_1 (mg/mL)	X_2 (mg/mL)	X_3 (mg/mL)	Y_1 (%)	Y_2 (nm)
(B) Paclitaxel emulsions					
Original optimum[a]	5.75	2.32	44.6	97.3	269.3
BS-optimum[b]	5.84 ± 0.16	2.28 ± 0.06	45.1 ± 1.0	97.8 ± 0.8	270.3 ± 1.5
	X_1 (g)	X_2 (mg)	X_3 (mL)	Y_1 (min)	Y_2 (%)
(C) Indomethacin solid dispersions					
Original optimum[a]	2.44	216.1	165.0	1.48	92.5
BS-optimum[b]	2.69 ± 0.22	200.1 ± 37.4	162.5 ± 5.7	1.39 ± 0.13	91.7 ± 0.7

[a] Original optimal solution was estimated from original dataset.
[b] BS-optimal solution was estimated from BS-dataset and represented as a mean±SD for 1000 of BS-resampling data.

As case B (Fan et al., 2004), a polyethylene glycol (PEG)ylated emulsion including paclitaxel was used, with soybean oil as the internal phase and polysorbate 80, PEG–distearoylphosphatidylethanolamine (PEG–DSPE), and cholesterol as the emulsifiers or coemulsifiers. The amounts of soybean oil, PEG–DSPE, and polysorbate 80 were selected as the causal factors. The entrapment efficiency of the paclitaxel in the emulsions and the particle sizes of the paclitaxel emulsions were used as the pharmaceutical responses.

As case C (Takayama et al., 1985), solid dispersions of indomethacin (IMC) were used, containing crosspovidone (polyvinylpolypyrrolidone, PVPP) as the carrier of the solid dispersions and methylcellulose (MC) as the stabilizing agent for the dissolution of IMC. The amounts of PVPP, MC, and ethanol, the solvent used in the preparation of the solid dispersions by the solvent evaporation method, were selected as the causal factors. The chemical stability of IMC in the solid dispersions was expressed as the residual amount of IMC after the samples were stored for 30 days at 60 °C under 75% relative humidity. The 50% dissolution time of the IMC and the chemical stability of the IMC in the model formulations were used as the pharmaceutical responses.

3.2. Computer programs

Optimization based on RSM-S and BS resampling was performed using dataNESIA® version 3.2 (Yamatake Corp., Fujisawa, Japan). Viscovery SOMine® version 4.0 (Eudaptics Software GmbH, Vienna, Austria), based on the algorithm of Kohonen's self-organizing map, was used to identify the global optima from the diverse optimal solutions estimated with the BS resampling method. A random number technique was applied with Microsoft Excel® for Windows 2007, and the macroprograms were written by the authors with Visual Basic version 6.5 (Microsoft Corp., Tokyo, Japan). Chemish® version 4.60 (ChemInfoNavi, Iwakuni, Japan) and JMP® version 7 (SAS Institute Japan Ltd., Tokyo, Japan) were used to visualize the design space and control space.

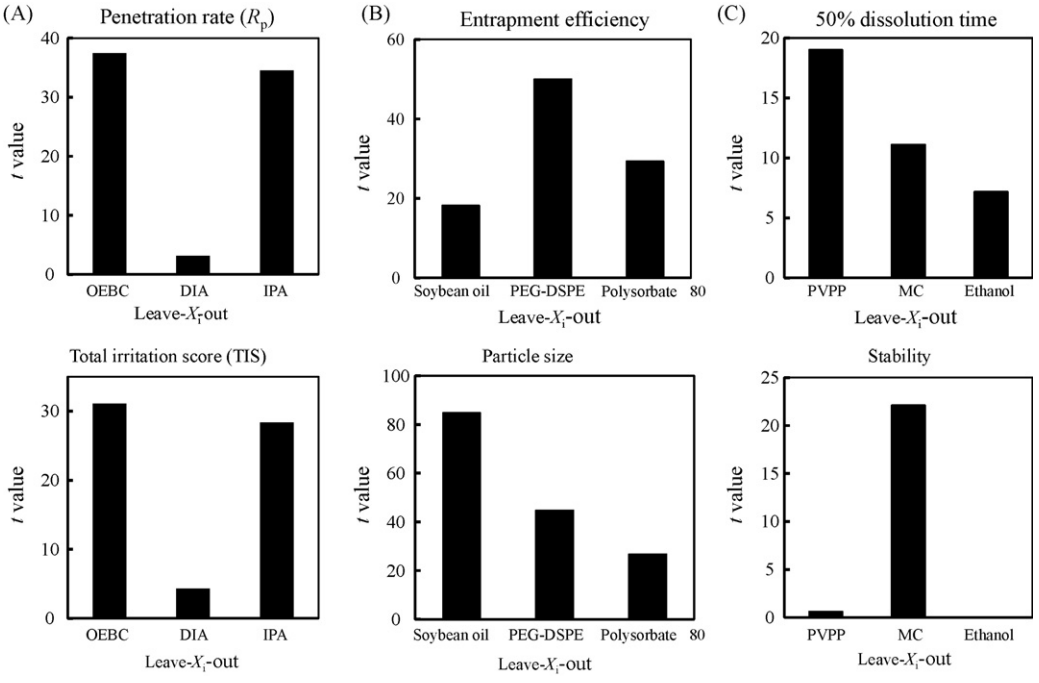

Fig. 4. Sensitivity analysis of the causal factors of the optimal responses based on the LOFO method using *t* values in (A) ketoprofen hydrogels, (B) paclitaxel emulsions, and (C) indomethacin solid dispersions.

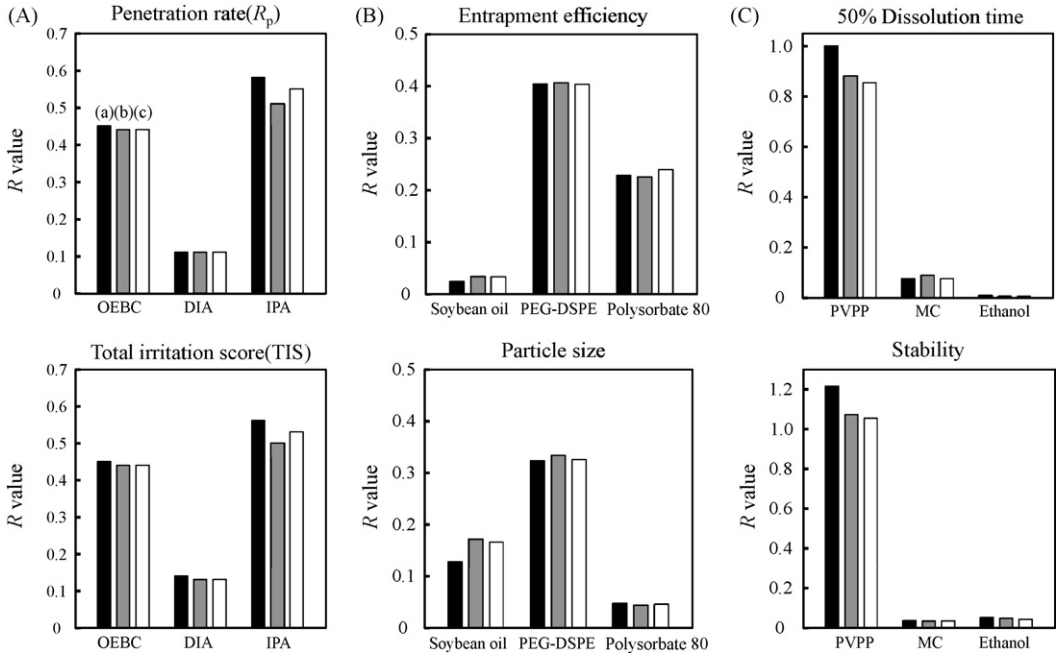

Fig. 5. Sensitivity analysis of the causal factors of the optimal responses based on the random number technique using *R* values in (A) ketoprofen hydrogels, (B) paclitaxel emulsions, and (C) indomethacin solid dispersions. The *R* values are shown as a function of the generated number of (a) 100 sets, (b) 300 sets, and (c) 500 sets of the relevant factor in the range of the BS-original optimal solution ± *SD* value.

4. Results and discussion

4.1. Original optimal solutions and BS-optimal solutions

The individual datasets summarized in Table 1 were simultaneously optimized using RSM-S and the original optimal solutions were estimated. The BS resampling method was applied to each case and the BS-optimal means and their standard deviations were calculated. The results are shown in Table 2. Further details about estimating the original optimal and BS-optimal solutions have been fully described in previous papers (Arai et al., 2007; Onuki et al., 2008). In cases A and B, the original optimal values were within the range of the lower and upper limits of the BS-optimal solutions. Conversely, in case C, the original optimal values, such as X_1 and X_2, were somewhat different from the BS-optimal solutions. In particular, the original optimal value X_1 was lower than the lower limit of the BS-optimal range in case C. This suggests that the number of model formulations in cases A and B were sufficient to estimate high-precision optimal solutions, but this was not true for case C because of the limited number of model formulations.

4.2. Sensitivity analysis based on the LOFO method

The effect of each factor on the optimal solution was analyzed based on the LOFO method, i.e., the BS-original optimal solutions were compared with the BS-LOFO optimal solutions. Welch's *t* value was used as the judging index because the mean square values for the BS-original and BS-LOFO optimal solutions were statistically unequal in all cases. The results are shown in Fig. 4. In case A (Fig. 4A), when the factor "the amount of OEBC" or "the amount of IPA" was omitted, markedly high *t* values were observed in both the optimal responses R_p and TIS. This suggests that these factors are important in promoting the activity of the skin delivery of ketoprofen from the hydrogels and the induction of skin damage. It also suggests that the effect of DIA is rather weak compared with those of OEBC and IPA. In case B (Fig. 4B), all the factors showed some degree of significance for the optimal responses in the paclitaxel emulsions. The factor "the amount of PEG–DSPE" strongly affected the entrapment efficiency, whereas the factor "the amount of soybean oil" most affected the sizes of the particles. In case C (Fig. 4C), the amount of PVPP was the most important factor for the dissolution of the indomethacin in the solid dispersions, but the amount of MC predominantly affected the chemical stability of the indomethacin.

4.3. Sensitivity analysis based on the random number technique

Although the sensitivity analysis based on the LOFO method provided insight into the factors affecting the optimal formulations, there were some discrepancies between the LOFO results and the experimental facts (Takayama et al., 1985; Wu et al., 2001). In case A, omitting the factor "OEBC" from the original dataset (BS-LOFO samples) resulted in a higher estimate of the optimal R_p value than that calculated for the BS-original value (data not shown). It is difficult to provide an appropriate reason for the LOFO result because OEBC is a promising chemical enhancer in the delivery of drugs via the skin. It was also experimentally evident that the chemical stability of indomethacin in the solid dispersions in case C was greatly affected by the amount of PVPP rather than by the amount of MC (Takayama et al., 1985). Nevertheless, the LOFO method showed that the amount of MC was the most important factor affecting the chemical stability of indomethacin. Although further investigation should be required, these findings suggest that the LOFO method is occasionally an inaccurate approach when the number of causal factors is relatively small, such as in cases A, B, and C. The LOFO method may well be appropriate for sensitivity analysis when the number of causal factors is sufficiently large.

To overcome some controversial points attributed to the LOFO method, we developed a novel sensitivity analysis based on the random number technique. The basic concept of this approach has been discussed in Section 3. Fig. 5 shows the *R* values for cases A, B, and C as a function of the number of generated values for the factor to be considered. In all cases, the *R* values were stable despite the number of values generated for the relevant factor. In case A (Fig. 5A), the magnitude of the *R* values was close to that of the *t* values in Fig. 4A. However, case B showed some differences between the *R* and *t* values (Fig. 5B). In case C (Fig. 5C), the amount

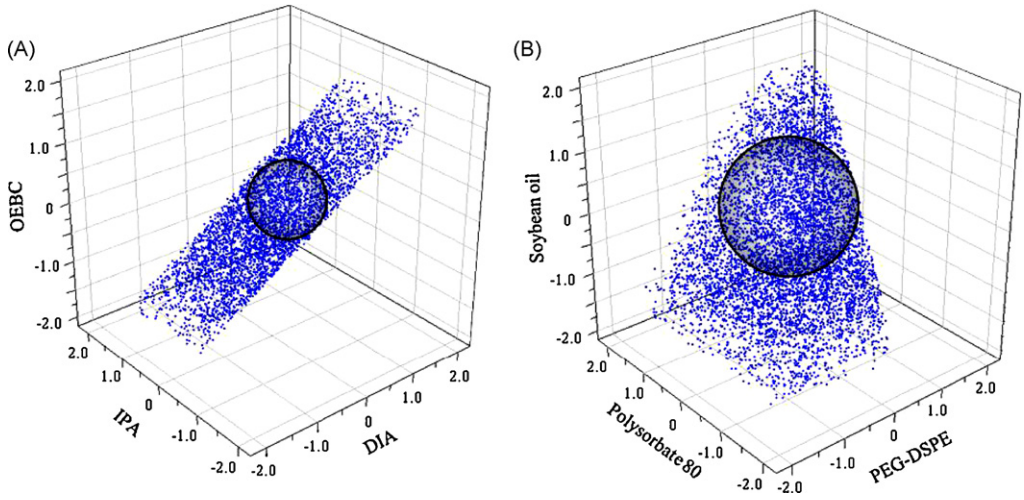

Fig. 6. The design space represented by the solution vectors that can satisfy the specifications of the responses and the control space represented as a super-spherical range in the design space in (A) ketoprofen hydrogels and (B) paclitaxel emulsions. Each axis was represented as the normalized level using the BS-optimal mean and σ values.

Table 3
The orthogonally flexible ranges of the formulation factors in the possible control space.

	X_1 (%)	X_2 (%)	X_3 (%)
(A) Ketoprofen hydrogels			
	1.20–1.26	4.65–4.77	31.1–31.9
	X_1 (mg/mL)	X_2 (mg/Ml)	X_3 (mg/mL)
(B) Paclitaxel emulsions			
	5.74–5.95	2.24–2.32	44.4–45.8
	X_1 (g)	X_2 (mg)	X_3 (mL)
(C) Indomethacin solid dispersions			
	2.65–2.74	192.5–206.9	161.4–163.6

of PVPP strongly affected the 50% dissolution time, as well as the chemical stability of indomethacin in the solid dispersions. This suggests that the random number technique explains the experimental results well (Takayama et al., 1985; Wu et al., 2001; Fan et al., 2004) compared with the LOFO method. The *R* values in cases A, B, and C were also calculated as a function of the range of the generating relevant factor. In all cases, the magnitude of *R* values was similar, irrespective of a difference in the range of *SD* values from 0.5 to 1.5 (data not shown). These findings support the proposition that the random number technique reflects the response surface proximate to the optimal solution estimated by RSM-S and that it is a useful way to evaluate the causal factors affecting the responses in computer-based optimization studies.

4.4. Design space and control space

To set up a design space and control space (MacGregor and Bruwer, 2008) based on a scientific rationale that can satisfy the specifications of the pharmaceutical responses, a novel approach taking advantage of the random number technique was investigated. In all cases A, B, and C, the causal factors were generated within the range of the BS-original optimal solution $\pm 2\sigma$. More specifically, 10,000 formulations were generated in the neighboring spaces of the BS-original optimal solution. Fig. 6 illustrates schematically the design space in cases A and B, represented as the solution vectors that satisfy the specifications of the response, and the control space as a super-spherical area in the design space. The design space and control space of case C have been shown previously (Fig. 3). The lower and upper limits in the control spaces are summarized in Table 3. Within the range of the control space, individual factors can be independently changed because of the orthogonality among the factors.

In case A, a range from 90% to 110% of the optimal solution was set as the specifications for R_p and TIS. The formulation vectors that satisfy the specifications are shown in Fig. 6A. The amount of DIA occurred across a wide range of the design space, whereas the amounts of OEBC and IPA were strictly restricted. In the sensitivity analysis, the amount of DIA only slightly affected the optimal formulation, whereas OEBC and IPA had prominent effects on the optimal formulation (Fig. 5A). The sensitivities of each factor on the optimal formulation correlated well with the design space and the control space. In case B, more than 97% of the entrapment efficiency and less than 271.8 nm of the particle size were constrained to the responses, as specification examples. As shown in Fig. 6B, the design space was greatly influenced by the specification of particle size, i.e., the design space was predominantly regulated by the amounts of soybean oil and PEG–DSPE, as factors determining the particle size. As in case A, all the factors in the control space can be varied individually in case B (Table 3B). In case C, the design space strongly reflected the nonlinear relationship between the factors and the responses. Even though the amount of PVPP was fairly low, it satisfied the specifications on the responses when the amount of MC was far from the optimal solution. The effects of MC and ethanol on the design space were rather weak, although the amount of PVPP was a factor strictly defining the design space (Fig. 3 left and Table 3C).

5. Conclusions

The simultaneous optimization of a model dataset was performed using RSM-S. Reliability and reproducibility were estimated

with the BS resampling method and a sufficient number of model formulations were required to estimate high-precision optimal solutions. To analyze the sensitivities of the factors on the optimal formulations, the random number technique was a better approach than the LOFO method. To establish the design space and control space that can satisfy the specifications of the pharmaceutical responses, based on a scientific rationale, a novel approach taking advantage of the random number technique was investigated. As a result, the control space was successfully defined as a superspherical area in the design space of the formulation factors.

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