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Key functions in polymer carriers for intestinal absorption of insulin

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Abstract

This work aimed to clarify the relationship between polymer function and insulin absorption, and to evaluate the optimized preparative formulation predicted from this relationship.

Insulin-loaded polymer (ILP) carrier systems were prepared following a two-factor composite second-order spherical experimental design. To investigate the polymer function, we evaluated its insulin release, bioadhesiveness, and protective effect. Each ILP was administered intestinally, and glucose reduction was monitored as the pharmacological effect. Based on these data, an optimized formulation was predicted and how the polymer function affects insulin absorption was clarified by multivariate spline (MVS) interpolation.

A greater pharmacological effect was apparent in ILPs with a smaller particle size and loaded with more insulin. The pharmacological effect predicted by MVS after the administration of ILP made under optimized preparative conditions was almost identical to the observed effect. Moreover, MVS clarified the relationship between the polymer function and the pharmacological effect. These results supported that MVS can be an effective tool with which to approximate the relationship between the function of a dosage form and its absorption, and to explore the optimized preparative conditions.

Keywords: Insulin; Intestinal absorption; Multivariate spline interpolation; Optimization; Polymer

1. Introduction

For developing the pharmaceutical dosage form, numerous experimental sets must be tested for getting effective pharmacological effect and less toxicity since chemicals sometimes work as the suppression and implement of biological activity including some transporters and membrane characteristics. Recently, the computational analysis is demonstrated that from minimal experimental data sets, the dissolution profile of drugs and optimized formulation is calculated, and these predictions are reliable since the prediction is close to the observed data (Takayama et al., 2000).

In our groups, a polymeric carrier system for peptide composed of poly(methacrylic acid) and poly(ethylene glycol) (P(MAA-*g*-EG)) has been developed and feasibility of this carrier system could be demonstrated since at the desirable site, insulin was quickly released (Morishita et al., 2004), and the absorption of released insulin is facilitated by the multi-function of polymer like inhibiting the insulin degradation (Yamagata et al., 2006), and bioadhesiveness to intestinal wall (Morishita et al., 2004; Goto et al., 2006), resulting in hypoglycemia effect following oral administration of insulin loading P(MAA-*g*-EG) (ILP) to any types of diabetics rats (Morishita et al., 2006). However, we did not clarify how these effects are related to insulin absorption, or the optimal particle size and insulin loading for oral insulin delivery although in parts, the important factor in ILP might be elucidated.

At present, various strategies are used to predict and optimize drug formulations. The response surface method (RSM) is now widely used to explore the optimized formulation because

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seeking the best formulation from thousands of experimental data is both complicated and difficult without a mathematical method. Although various algorithms have been reported to generate response surfaces, their prediction of pharmaceutical responses is not always adequate because their estimates are poor and the optimization procedures are complex. Recently, we developed a novel optimization technique that incorporates multivariate spline (MVS) interpolation (Takayama et al., 2004; Onuki et al., 2004, 2005). MVS is basically a boundary element method, in which a Green function is used to interpolate the minimum curvature of multidimensional data points (Sandwell, 1987). As usual, observational data include experimental error. This technique can naturally interpolate between observational data points, including the experimental error (Wahba, 1990). The generation of response surfaces using MVS has provided much detailed information. MVS is expected to be a candidate prediction tool in this context. Furthermore, the utilization of this program should make clear the key factor in developing the dosage form from the results obtained.

Here, we evaluated the effectiveness of P(MAA-*g*-EG) as a drug delivery carrier, especially for macromolecules, using MVS. We focused on how P(MAA-*g*-EG) polymers affect insulin absorption, and which of the parameters tested is the key factor in facilitating insulin absorption. Furthermore, from the factor relationships, we explored the optimal preparative conditions for ILP that will provide the best pharmacological effect following the administration of the ILP.

2. Materials and methods

2.1. Materials

Methacrylic acid (MAA) was purchased from Sigma–Aldrich Co. (St. Louis, MO). Dimethoxy phenyl acetophenone (DMPA) was purchased from Sigma–Aldrich Co. In this study, the inhibitor contained in MAA was removed by passage through a column packed with DE-HIBIT 200 (Polyscience, Inc., Warrington, PA). Methoxy terminated poly(ethylene glycol) monomethacrylate 1000 (PEGMA) and tetraethylene glycol dimethacrylate (TEGDMA) were purchased from Polyscience, Inc. Human recombinant insulin was purchased from Wako Chemical Co. Ltd. (Osaka, Japan). All other chemicals were at least reagent grade.

2.2. Experimental design

In this study, we assumed, based on the results of a previous study, that insulin absorption could be facilitated by insulin encapsulated in a polymer, and constructed the correlation scheme shown in Fig. 1. The input was the amount of insulin loaded and the size of the polymer particles, which are controllable parameters. The pharmacological effect, which was represented by the glucose reduction after the administration of insulin, was the output. The amount of insulin released from the polymer, the insulin degradation rate, and the bioadhesiveness of the ILP to the intestinal wall were chosen as the latent factors because these parameters are already known to influence insulin absorption (Table 1).

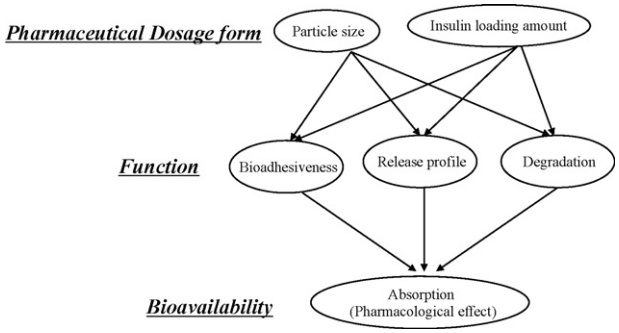

Fig. 1. Scheme of the correlation between ILP characteristics, polymer function, and insulin absorption.

A simultaneous optimization study, based on the RSM, was performed according to a previously reported procedure. A two-factor composite second-order spherical experimental design was used to select model formulations.

2.3. Animals

This research was performed at Hoshi University and complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals. Normal male Sprague-Dawley rats weighing approximately 180 g were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). While animals were housed in rooms controlled between $23 \pm 1\,^{\circ}C$ and $55 \pm 5\%$ relative humidity, they had free access to water and food during acclimatization. They were fasted for 24 h prior to experiments.

2.4. Synthesis of P(MAA-g-EG) microparticles

Microparticles of P(MAA-*g*-EG) were prepared by the free-radical photopolymerization of MAA and PEGMA (Morishita et al., 2002) The monomers were mixed in the appropriate molar ratios to yield a 1:1 ratio of MAA:EG units in the gel. The solutions were diluted to 44.4% (w/w) of the total monomers with a 1:1 (v/v) solution of ethanol and water. TEGDMA was

Table 1
Experimental design for optimization

No.	X1	X2	Size (μm)	Insulin loading amount (%, w/w)
1	−1	−1	51.5	2.5
2	1	−1	262.9	2.5
3	−1	1	51.5	10
4	1	1	262.9	10
5	−1.412	0	25.2	6
6	1.412	0	338.8	6
7	0	−1.412	140.5	0.50
8	0	1.412	140.5	12
9	0	0	140.5	6
10	0	0	140.5	6

added as the crosslinking agent, with 0.075 mol of TEGDMA per mole of MAA.

DMPA equivalent to 1% of the weight of the monomers was added as initiator. The reaction mixture was pipetted between glass plates spaced 0.8 mm apart, to create a monomer film. Polymerization was initiated by exposing the monomer film to ultraviolet (UV) light at 365 nm (Aicure, ANUP5204, Matsushita Electric Works, Tokyo, Japan), and was allowed to react for 30 min. The resultant hydrogels were removed from the glass plates and rinsed for one week in deionized water (changed daily) to remove the unreacted monomers and the sol fraction. After the unreacted monomers were removed, the hydrogels were dried *in vacuo*. The dried hydrogels were then crushed in a coffee mill to produce the powdered polymer. Finally, the resultant fine polymers were classified within a wide range of polymer sizes using sieves with various mesh sizes. The particle sizes of the obtained polymers were calculated based on scanning electron microscope (JSM-5600, JEOL) images of the polymers, and particle size was presented as the average value of 200 particles randomly selected from the images.

2.5. Preparation of ILP

To 140 mg P(MAA-*g*-EG), 20 ml of insulin solution in a concentration range of 0.1–1.0 mg/ml, dissolved in phosphate-buffered saline (PBS) at pH 7.4, was added and stirred for 2 h. To collapse the hydrogels, 10 ml of 0.1 M HCl was added, and the insulin-loaded polymers were collected for filtering through a 0.45 μm membrane filter. The resultant polymers were dried at room temperature for 2 days *in vacuo*. Powdered polymers of various particle sizes were thus obtained.

Percent incorporation of insulin was calculated as follows,

$$\%\ \text{incorporation of insulin}(\%, \text{w/w}) = \frac{\text{insulin incorporated amount}}{\text{weight of ILP}} \times 100$$

2.6. Study of insulin release from ILPs

A 10 mg sample of each ILP was placed in a beaker, and 20 ml of PBS was added. The solution was stirred and aliquots were taken at predetermined times through a membrane filter. Finally, the insulin concentration was calculated based on high-performance liquid chromatography (HPLC) data. The released insulin was then calculated with the following the equation:

$$\%\ \text{insulin released from ILPs} = \frac{\text{amount of insulin released}}{\%\ \text{incorporation of insulin} \times \text{weight of ILPs}} \times 100$$

2.7. Preparation of intestinal fluid for the degradation study

The rats were fasted for 24 h and anesthetized by i.p. injection of 50 mg/kg sodium pentobarbital (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). The rats were secured to the operating table in a supine position and a midline abdominal incision was made.

To obtain the intestinal fluid, a sonde needle was inserted into the upper portion of the small intestine. The intestine was cannulated on the lower side (length = 20 cm) to remove the intestinal fluid. The small intestine contents were washed out with 20 ml of PBS. The intestinal fluid has a high lipid content, which may interfere with the HPLC analysis of insulin. Therefore, this efflux was treated with two volumes of methylene chloride to remove the lipids (Yamagata et al., 2006; Asada et al., 1994). This extraction process was repeated five times.

To compare the results of different sets of insulin degradation studies, the concentration of the intestinal enzyme fluid was adjusted to yield 50% degradation of the initial insulin level in 30 min at 37 °C.

2.8. Study of insulin degradation

Firstly, the amount of polymers used in this study was determined based on how much amount of ILPs with different size and incorporated insulin amount should be administered to rats. The calculated amount of P(MAA-*g*-EG) polymers of variously sized particles were incubated for 15min with intestinal fluid diluted with PBS. The incubated solution was centrifuged to remove the polymers, and 1.9 ml of the supernatant was collected. Finally, 100 μl of insulin solution at a concentration of 4 mg/ml was added to the resultant solution, and reincubated for 30 min at 37 °C. As control, 2.0 ml of the intestinal enzyme fluid diluted with PBS (pH 7.3) and pH-adjusted PBS in a range of 4.5–7.0 was incubated for 15 min, and second incubation was conducted for 30 min and at 37 °C after 100 μl of insulin solution was added to 1.9 ml of incubated intestinal enzyme fluid.

At the predetermined time, 50 μl samples were taken and prepared for HPLC analysis by the addition of an aliquot of ice-cold stop solution consisting of acetonitrile and 0.1% TFA in a ratio of 30:70 (v/v) to terminate insulin degradation. A 10 μl aliquot of this sample was injected directly into the HPLC system.

2.9. Bioadhesiveness study

In this study, a fluorescently labeled polymer was used because it offers an easy and highly sensitive method for quantifying the polymer. 9-Anthracenylmethyl methacrylate (PolyFluor 407, Polyscience, Inc.) was selected as the fluorescent marker (Goto et al., 2006). Marker corresponding to 0.1 mol% of the total monomer was added to the monomer solution, and polymerization occurred in the way described above for the polymer preparation. This resulted in labeled polymer in which the florescent marker was directly polymerized into the backbone of the MAA chain.

Following anesthetization by i.p. injection of sodium pentobarbital (50 mg/kg; Dainippon Pharmaceutical Co., Ltd.), rats were restrained in a supine position on a thermostatically controlled board at 37 °C. A front middle incision was made in the abdomen and the ileal segment was isolated. A 7 cm length of isolated segment was washed with 20 ml of PBS at 37 °C, and then the abdomen was clamped. The appropriate amount of fluorescent polymer was then administered to the rats 1 h after washing isolated segment. The non-adherent polymers were

collected by washing the treated segments with PBS 30 min later. The collected fluorescent polymer was quantified with fluorescence spectrometry (emission, 365 nm; excitation, 425 nm). Finally, bioadhesiveness was calculated with the following equation.

$$\%\ \text{adhesion} = \frac{\text{amount administered} - \text{amount collected}}{\text{amount administered}} \times 100$$

2.10. In situ insulin absorption study

Following anesthetization by i.p. injection of sodium pentobarbital (50 mg/kg; Dainippon Pharmaceutical Co., Ltd.), rats were restrained in a supine position on a thermostatically controlled board at 37 °C. During the experiments, additional i.p. injections of sodium pentobarbital (12.5 mg/kg) were necessary at every 1 h following administration to maintain the anesthesia.

In this study, the *in situ* loop method was used. A front middle incision was made in the abdomen and the ileal segment was isolated. A 10 cm length of isolated segment was washed with 20 ml of PBS at 37 °C, and then the abdomen was clamped. Glucose levels were allowed to stabilize for 1 h after surgery. The ILPs were suspended in 0.3 ml of PBS, and were infused into the 7 cm ileal segment. A syringe was washed with 0.2 ml of PBS, then loaded with ILP, and the ILP was injected into the loop. This procedure was repeated twice. A blood sample was withdrawn from the jugular vein before the administration of ILP and at intervals after its administration. After the blood samples were taken, glucose levels were measured using NovoAssist Plus (Novo Nordisk Pharmaceuticals, Tokyo, Japan).

2.11. HPLC method to determine insulin concentrations

The assay for insulin concentrations in the incorporation, release, and degradation studies was as previously reported (Morishita et al., 2002). Briefly, the mobile phase consisted of acetonitrile and water containing 0.1% NaCl and 1% TFA in a ratio of 30:70 (v/v). A 10 μl sample was injected into an HPLC system equipped with a UV detector. An insulin peak, with a retention time of around 7 min at flow rate of 1.0 ml/min, was detected at 220 nm.

2.12. Data analysis

The data measured for the model formulations were analyzed with a computer program in which the MVS had been incorporated (Takayama et al., 2004; Onuki et al., in press). The computer program dataNESIA (Yamatake Corp., Tokyo, Japan) was used. MVS has recently been recognized as a superior method for the high-precision modeling of multidimensional data points. In dataNESIA, observational data, including experimental error, can be naturally interpolated by means of a thin-plate spline, which is represented as the sum of the interpolation with the Green function and a linear polynomial equation. The details of dataNESIA have been described in full elsewhere (Takayama et al., 2004). The multiobjective simultaneous optimization was performed based on a standardized Euclidian distance function method, as described previously (Takayama et al., 2004).

To evaluate the significant levels of each causal factor in the prediction of responses in MVS, a contribution index is calculated based on Monte Carlo approach. Wu et al. have already demonstrated that the comparison of contribution index value is the reliable for understanding the degrees of contribution of each factor in the prediction of responses (Wu et al., 2001).

3. Results

3.1. Influence of polymer particle size on insulin incorporation

Fig. 2 shows the response surface based on the insulin incorporation study. Clearly, the percentage incorporated amount of insulin into the polymers is dependent on the insulin concentration, irrespective of the particle sizes used in this study since the correlation coefficient, *r*, is almost 1 in a leave-one-out cross validation. From this result, the targeted percentage of insulin incorporated amount to polymer (w/w) is controllable by changing the concentration of insulin solution in a concentration range of 0.1–1.0 mg/ml.

3.2. Differences in insulin release between formulations

Fig. 3 shows the response surface (A) and the contribution index (D) for insulin release from the ILPs. Insulin release increased as the size of the ILP particles decreased (Fig. 3(A)) and insulin release seems to be mainly dependent on the particle size of the ILP since the contribution index in particle size is approximately 90% (Fig. 3(D)). From these findings, the particle sizes of the ILPs are a key factor in controlling insulin release.

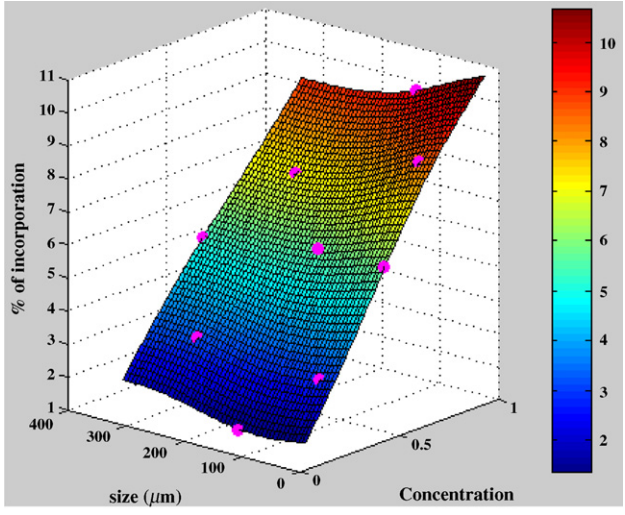

Fig. 2. Response surface for insulin incorporation into P(MAA-*g*-EG).

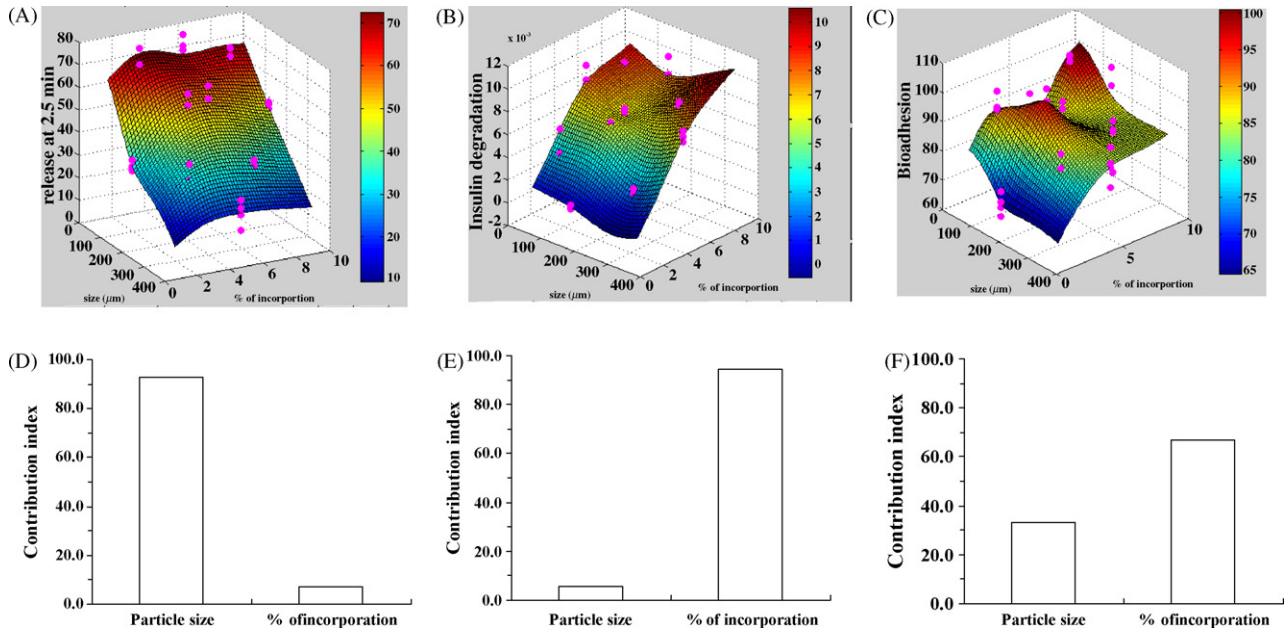

Fig. 3. Response surface (A–C) and contribution index (D–F) for the amount of insulin released from ILP at 2.5 min (A and D); effect on insulin degradation of ILP (B and E); and bioadhesive properties of the polymer (C and F). Leave-one-out cross-validation value Insulin release (%), $r = 0.92471$ (in leave-one-out cross validation) Degradation (mg/ml/min), $r = 0.94801$ (in leave-one-out cross validation) Bioadhesiveness (%), $r = 0.71379$ (in leave-one-out cross validation).

3.3. Inhibition of insulin degradation in the presence of polymers

Fig. 3(B) shows the response surface and the contribution index based on the inhibition study of insulin degradation by the polymers. Previously, we reported that the carboxylic groups on PMAA backbone chain are contributed to the capture of calcium ion from the surrounding medium. The calcium ion was knows as the activator of enzymatic activity related to the insulin degradation in the intestine (Yamagata et al., 2006; Madsen and Peppas, 1999). The insulin degradation is one of major barriers for the insulin permeation through the intestinal mucosa (Aoki et al., 2005), and the inhibitory effect of insulin degradation would play a crucial role for the enhancing effect of insulin absorption by the polymers.

As showing the effect of polymers on the insulin degradation, it showed the dose dependent manner of polymer since polymers with greater amount inhibited the insulin degradation strongly and the contribution index of particle size is extremely low (less than 10%) (Fig. 3(B and E)).

3.4. Bioadhesiveness to the intestinal tract of polymers with particles of various sizes

Polymers with a hydrophilic tether, such as PEG, can penetrate the mucus layer, resulting in bioadhesion (Serra et al., 2006; Sahlin and Peppas, 1997). It has already been reported that the bioadhesiveness of a polymer with PEG is dependent on the molecular weight of PEG (Serra et al., 2006). The particle size of the polymer also contributes to the strength of its bioadhesion to the intestinal tract. Thus, the effect of the particle size of the polymer on its bioadhesiveness in ileal segments was evaluated. As shown in Fig. 3(F), the size of the polymer particles and polymer dose seem to play key roles in polymer adhesion to ileal segments although the contribution index for bioadhesiveness of polymers is superior in percentage of incorporation to particle size. The relationship between particle size and bioadhesiveness has been clarified by Morishita et al. (2004), who showed that polymers with larger particles had less bioadhesive strength in each intestinal segment. Regarding with the influence of the administration dose and bioadhesiveness of polymer against the intestinal wall, a larger dose might saturate the interaction with the intestinal wall.

3.5. Effects of ILP particle size and degree of insulin incorporation on glucose reduction

The response surface for the pharmacological effect following the administration of ILPs to ileal segments is shown in Fig. 4. In this study, the index of the pharmacological effect was the area above the curve (AAG) calculated from glucose reduction profiles. Clearly, more insulin loaded into a polymer with a small particle size was predicted by the response surface to have the greatest pharmacological effect (Fig. 4(A)), and the contribution of percentage of incorporation to AAG is higher than particle size (Fig. 4(B)).

3.6. Insulin absorption study following ILP administration under optimized preparative conditions

Based on these experimental results, the optimized solution was estimated to be a polymer with a particle size of 25.2 μm, with 9.7% insulin loading. When the optimized formulation was prepared, 140 mg of polymer (25.2 μm particles) was stirred in 10 ml of insulin solution at a concentration of 1.0 mg/ml, yielding an ILP with 10.4% insulin incorporation. This dosage form

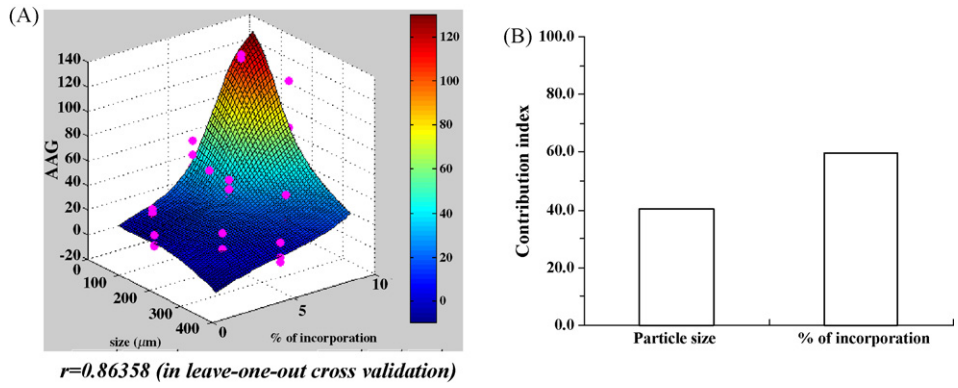

Fig. 4. Response surface of (A) and contribution index (B) to pharmacological effect.

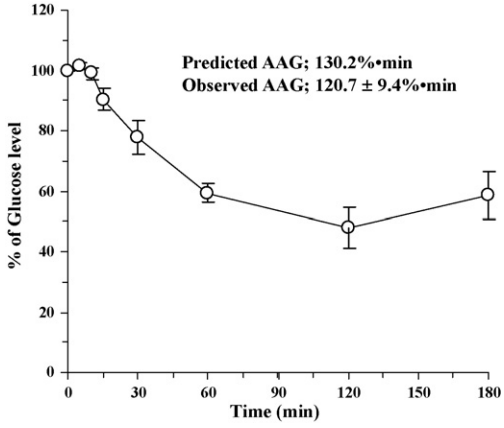

Fig. 5. Glucose reduction after the optimized formulation of the insulin-loaded polymer was administered. Optimized formulation: particle size, 25.2 μm; incorporation, 9.7%. Obtained formulation: particle size, 25.2 μm; incorporation, 10.4%. Data was represented as the mean ± S.E. (n = 4–5).

was administered to the intestine using an *in situ* method. The AAG value calculated from the glucose reduction profiles was almost identical to the predicted value, and this value was also the highest among all the formulations tested in this study (Fig. 5). The optimized preparative conditions calculated from the results of a series of experiments based on the spherical experimental design showed the greatest pharmacological effect, and the predicted effect was consistent with that observed (predicted AAG, 130.2%/h; observed, 120.7 ± 9.4%/h).

4. Discussion

To date, researchers have devoted themselves to developing pharmaceutical dosage forms that maximize the effective absorption of the active substance and minimize the side effects. The development of dosage forms that include peptide and protein drugs is one of the most challenging projects. Most peptide and protein drugs are easily denatured in harsh environments, as in the presence of degradative enzymes in the intestinal tract. Their permeation through the intestinal mucosa is also lower than that of other drugs. A number of researchers have used various strategies to counter these disadvantages, including chemical absorption enhancers (Onuki et al., 2000; Uchiyama et al., 1999), enteric coatings (Hosny et al., 1998; Agarwal et al., 2001), and so on. Unfortunately, these studies focused only on individual effects. In the development of pharmaceutical dosage forms, drug absorption will depend on the total balance of factors. For example, drug absorption from a dosage form with bad release kinetics will be reduced even if that dosage form has a good permeation capacity.

We have already reported that ILPs have great potential as an oral insulin delivery system because the ILP protects the insulin in an intact form from enzymatic attack in the stomach and intestinal fluid (Yamagata et al., 2006). This results in accelerated insulin absorption from the intestinal fluid. Moreover, the bioadhesiveness of the polymer seems to play a crucial role in effective insulin absorption (Morishita et al., 2004; Goto et al., 2006). However, these attributes were evaluated individually, and until now we had not clarified how these parameters complement one another to facilitate insulin absorption. Therefore, we explored the optimal preparative conditions and clarified the contributions of the polymeric characteristics to insulin absorption, using MVS.

From our previous data, it is likely that the relationship shown in Fig. 1 has been realized. Polymers with different levels of incorporation and different particle sizes affect the following three factors: insulin release, inhibition of insulin degradation, and bioadhesiveness. These factors might then directly affect insulin absorption. Therefore, we investigated how particle size and insulin incorporation are related to these three parameters. Particle size greatly affected insulin release (Fig. 3(D)), but the contribution of insulin incorporation to insulin degradation is much greater than the effect of polymer particle size (Fig. 3(E)). This reversed effect might be due to different mechanisms. Insulin release originates from polymeric swelling, and polymers with smaller particles necessarily have larger contact areas with water. As a result, the swelling rate of small-particle polymers will be relatively higher, causing a faster release of insulin. However, insulin degradation is due to an interaction between trafficking components and enzyme activity, and this is independent of the swelling rate. Detailed profiles of insulin degradation are not given in this report. However, the manner of insulin degradation is obviously dependent on the amount of insulin incorporated. In this study, the amount of dispersed polymer was determined based on the doses given to rats, and this amount was

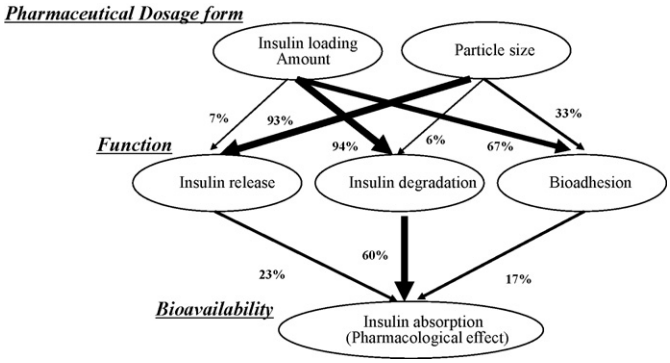

Fig. 6. Contribution indices for causal factors on intermediate variables, and these reliables on the output signal.

lower for polymers with higher insulin incorporation. Insulin degradation after treatment with small amounts of polymer was greater than that after treatment with larger amounts of polymer. Therefore, the inhibitory effect on insulin degradation exerted by the polymer is related to the amount of polymer administered. However, insulin degradation was suppressed by 50% even after the administration of the least inhibitory polymer relative to control, which was not treated with polymer (data not shown). These findings indicate that the polymer has a strong inhibitory effect on insulin degradation, even when low doses are administered.

The bioadhesion properties of the dosage form are also important in the enhancement of drug absorption. The greater the strength of the polymer adhesion to the intestinal lumen, the greater is the concentration gradient formed, resulting in increased drug permeation through the mucosal membrane. From our data, the contributions of particle size and level of incorporation are almost equal (Fig. 3(F)). The PEG tether is an important factor in bioadhesion and previous reports have demonstrated that the nontangled chain of PEG confers elevated adhesion properties in the intestine, due to the ease with which the hydrophilic chain penetrates into the mucus matrix (Sahlin and Peppas, 1997; Peppas, 1998). These data suggest that the available area for the penetration of the PEG chain and the dose of polymer have a close relationship because smaller doses of polymer render the available area for bioadhesion larger. Most polymers can interact with the intestinal wall when small amounts of polymer are applied to the intestine.

Thus, particle size and the amount of insulin incorporated influence various functions that contribute to drug absorption. A one-factor-at-a-time experiment could not clarify which factors most affect drug absorption because the estimation of these contributions was so complicated. But, the computational analysis by MVS can show which factors have the great impact on the drug absorption as the contribution index since for the insulin absorption following ILPs administration, contribution indices against pharmacological effect are calculated as, 60% insulin degradation, 23% insulin release, and 17% bioadhesion (Fig. 6).

The dosage form is determined based on numerous pharmaceutical experiments. To date, there are no effective tools with which to estimate the best formulation to maximize effective drug absorption, or to identify the key functions in developing the pharmaceutical dosage form. However, we have shown here that computational methods, especially MVS, are the most effective tools in determining the best preparative conditions during preformulation research, and in predicting the factors that contribute most to the pharmacological effect. Basically, MVS was used to determine the optimized conditions from the experimental data sets (Takayama et al., 2004; Onuki et al., 2005). However, this report demonstrates that MVS is a useful tool in extracting the key factor in drug absorption, and in exploring the optimal preparative conditions. From an experimental data set constructed from a well-designed experiment, MVS identified the optimal preparative conditions to produce the greatest pharmacological effect. At the same time, MVS clarified the contributions of the polymer functions to insulin absorption.

5. Conclusion

In this study, the optimal preparative conditions for a polymeric carrier of an oral peptide delivery system were estimated. The conditions predicted to have the greatest pharmacological effect were consistent with the observed conditions following the intestinal administration of the optimized formulation. Furthermore, MVS demonstrated how the functions of P(MAA-*g*-EG) as an insulin-delivery carrier impact on insulin absorption enhancement and/or its pharmacological effects.

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