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Research Article

Formulation Optimization of an Indomethacin-Containing Photocrosslinked Polyacrylic Acid Hydrogel as an Anti-inflammatory Patch

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Abstract. Photocrosslinked polyacrylic acid hydrogel, made from polyacrylic acid (PAA) modified with 2-hydroxyethyl methacrylate (HEMA), is a promising candidate adhesive for dermatological patches. In this study, we investigated the further availability of hydrogel as an adhesive for dermatological patches using a hydrogel containing indomethacin (IDM) as a model anti-inflammatory patch. From an orthogonal experimental study, we clarified the relationships between formulation factors and characteristics of model formulation. Formulations with a lower degree of swelling were prepared by increasing the degree of HEMA modification and the addition of Tween 80. Apparent permeation rate was increased by addition of L-menthol and Tween 80. A tendency for higher HEMA modification to be accompanied by the prolongation of the lag time of IDM was observed. To obtain an applicable anti-inflammatory patch, we conducted a formulation optimization study using a novel optimization method, a response-surface method incorporating multivariate spline interpolation (RSM-S). Consequently, a highly functional anti-inflammatory patch in terms of its adhesive properties and bioavailability was successfully obtained. Since a wide range of functions can be fully controlled by manipulating the formulation factors, photocrosslinked polyacrylic acid hydrogel is an attractive candidate adhesive for dermatological patches.

KEY WORDS: dermatological patch adhesive; formulation optimization; indomethacin; multivariate spline interpolation; photocrosslinked hydrogel.

INTRODUCTION

Hydrogels are formed by three-dimensional polymer networks in the aqueous phase. They can retain a large amount of water as well as maintaining their mechanical strength. Today, numerous hydrogels are used in pharmaceuticals, especially as adhesives for dermatological patches, such as cataplasm, which is used for local inflammation and as a wound dressing.

To act as adhesive for dermatological patches, a wide range of functions are required. Appropriate adhesiveness and gel strength are necessary for sound attachment to the skin. In addition, we can expect an efficient cooling effect of the hydrogel because of its large amount of incorporated water. Thus, water capacity is a crucial characteristic of dermatological patch adhesive. Furthermore, when the hydrogels are regarded as drug carriers, drug release from the hydrogel is very important.

In general, most conventional hydrogels used as adhesives for dermatological patches are composed of polyacrylic acid (PAA) and its salts, and chemical crosslinking is used to increase their mechanical strength. This chemical crosslinking is achieved by the formation of ionic interactions between the carboxyl groups of the polymer and polyvalent cations such as calcium, copper, and aluminum. Although chemical crosslinking methods are well accepted for this purpose, plenty of room for improvement exists. For instance, this crosslinking is affected by changes in the surrounding aqueous phase, such as changes in pH, temperature, or polymer concentration, because the ionic bond is weak and unstable by nature. Thus, it is difficult to adjust all of the characteristics to appropriate levels using conventional methods.

In order to obtain a hydrogel acceptable as an adhesive for dermatological patches, application of a photopolymerization method to gel formation is rational solution. Photopolymerization is initiated by irradiation with light such as ultraviolet light (UV) and visible light. It enables rapid conversion of a monomer or macromer solution to a gel or solid under physiological conditions. This method has been gaining much attention in medical fields. A number of functional hydrogels prepared with this method have been applied to drug delivery (1–3) and scaffolding of tissue (4–6).

In a previous study (7), we developed a photocrosslinked PAA hydrogel, which was made from PAA modified with 2-hydroxyethyl methacrylate (HEMA) (Fig. 1), and evaluated its usefulness as an adhesive for dermatological patches. From a series of experiments, we clarified the relationships between the formulation factors and physical properties of the adhesive, and succeeded in obtaining a highly functional

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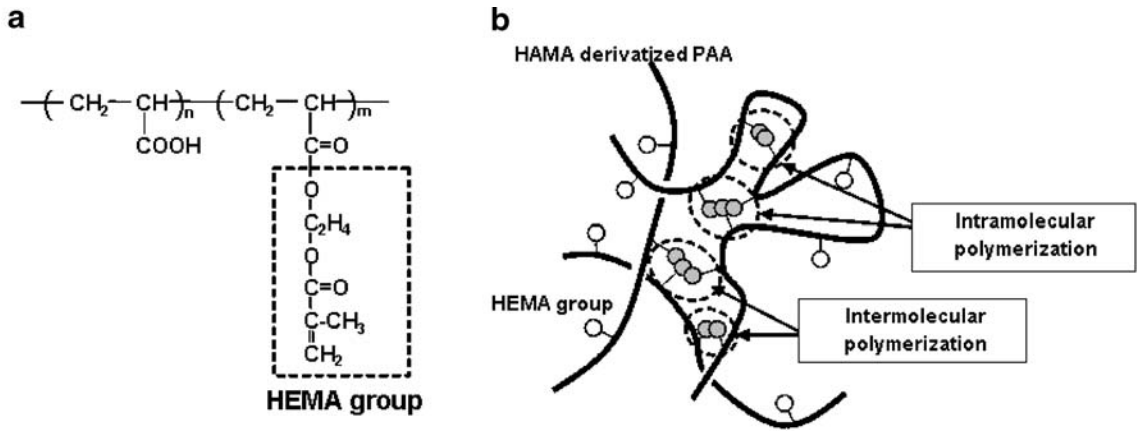

Fig. 1. Photocrosslinkable PAA–HEMA and photocrosslinked PAA–HEMA hydrogel: **a** chemical structure of PAA modified with HEMA, **b** photogelation mechanism by the formation of crosslinked PAA networks by intermolecular and intramolecular polymerization of HEMA groups in PAA molecules

hydrogel in terms of adhesiveness, gel strength, and water capacity. We also investigated the pharmacological activity of the hydrogel using carrageenan-induced edema in rats and reported its effective cooling and anti-inflammatory effects. From these studies, our hydrogel was considered promising as a candidate adhesive for dermatological patches.

In this study, we employed a hydrogel containing indomethacin (IDM) as a model anti-inflammatory patch and evaluated the further use of the hydrogel as a dermatological patch adhesive. Following an L8 orthogonal experiment, model formulations with different conditions were prepared, and various characteristics were examined. Selected characteristics of the formulation included gel fraction, degree of swelling, apparent permeation rate of IDM through the skin, and lag time. The relationships between formulation factors and observed characteristics were clarified using an analysis of variance (ANOVA). Furthermore, to obtain an applicable anti-inflammatory patch, we conducted a formulation optimization study using a novel response-surface method incorporating multivariate spline interpolation (RSM-S) optimization method.

MATERIALS AND METHODS

Materials

PAA (molecular weight 2.5×10^5), 2,2-dimethoxy-2-phenylacetophenone (DMPA) and polyoxyethylene sorbitan monooleate (Tween 80) were purchased from Wako Pure Chemical Industries (Osaka, Japan). HEMA, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), and IDM were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Menthol was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All other reagents were of chemical grade.

Synthesis of Photocrosslinkable Polymers

Photocrosslinkable PAA–HEMA was synthesized according to a method described previously (7,8). In brief, after the elimination of the inhibitor by filtration, 3.61 g of HEMA was added to 2.00 g of PAA dissolved in purified water, and the mixture was stirred. A designated amount of EDAC dissolved in purified water was added to the mixture, which was then stirred for 4 h at room temperature to complete the reaction. The reaction mixture was dialyzed five times in the dark against purified water using a seamless cellulose tube (size 36; Union Carbide, Houston, TX, USA). The pH of the solutions was adjusted to pH 4.5 by the addition of 5 mol/l NaOH as required. The solution was lyophilized using a freeze dryer (FD-1; Tokyo Rikakikai, Tokyo, Japan) under reduced pressure. A white cotton-like substance was obtained.

Preparation of IDM-Containing Photocrosslinked PAA–HEMA Hydrogels

Model formulations used in this study are listed in Tables I and II. In brief, the designated amounts of photocrosslinkable

Table I. Formulations for IDM-Containing Photocrosslinked PAA–HEMA Hydrogels Based on L8 Experiment Design for Three Factors

Formulation number	Modification with HEMA (mol%)	Tween 80 concentration (%)	L-Menthol concentration (%)
Rp.1	5	0.0	0.0
Rp.2	5	0.0	2.0
Rp.3	5	2.0	0.0
Rp.4	5	2.0	2.0
Rp.5	15	0.0	0.0
Rp.6	15	0.0	2.0
Rp.7	15	2.0	0.0
Rp.8	15	2.0	2.0

Table II. Formulations for IDM-Containing Photocrosslinked PAA-HEMA Hydrogels Based on a Composite Spherical Experiment Design for Three Causal Factors

Formulation number	Modification with HEMA (mol%)	Tween 80 concentration (%)	L-Menthol concentration (%)
Rp.1	7.11	0.42	0.42
Rp.2	7.11	0.42	1.58
Rp.3	7.11	1.58	0.42
Rp.4	7.11	1.58	1.58
Rp.5	12.89	0.42	0.42
Rp.6	12.89	0.42	1.58
Rp.7	12.89	1.58	0.42
Rp.8	12.89	1.58	1.58
Rp.9	5.00	1.00	1.00
Rp.10	15.00	1.00	1.00
Rp.11	10.00	0.00	1.00
Rp.12	10.00	2.00	1.00
Rp.13	10.00	1.00	0.00
Rp.14	10.00	1.00	2.00
Rp.15	10.00	1.00	1.00
Rp.16	10.00	1.00	1.00

PAA–HEMA polymer were dissolved in phosphate-buffered saline (PBS, pH 7.4) and IDM, Tween 80, and L-menthol were added to the solution. Subsequently, DMPA dissolved in ethanol was added to the mixture solution as an initiator. The amount of photocrosslinkable PAA–HEMA was fixed at 15.0%, IDM at 2.0%, and DMPA at 0.15%. UV irradiation was applied using a UV curing system (Aicure ANUP5204; Matsushita Electric Works, Tokyo, Japan). The wavelength of illumination ranged from 200 to 400 nm, and the irradiation time was 5 min. The light intensity at 365 nm was 28 mW cm^{-2}, as measured with a photometer (UIT-150; Ushio, Tokyo, Japan).

Stability of IDM Photopolymerization

IDM was dissolved in 10 ml of methanol (5 mg/ml), and then DMPA was added to the solution at concentrations of 0, 1, 2, and 3 mg/ml, respectively. UV irradiation was performed (28 mW cm^{-2} at 365 nm), and 100 µl of solution was withdrawn at designated intervals. The concentration of IDM in the samples was determined using a Hitachi LaChrom Elita HPLC System (Hitachi High-Technologies, Tokyo, Japan). Other chromatographic conditions were as follows: column, YMC-pack A-302 S-5 A 150×4.6 mm i.d. (Yamamura Chemical Laboratories, Tokyo, Japan); UV detection, 254 nm; mobile phase, 0.1% phosphoric acid-methanol (25:75, *v*/*v*); flow rate, 1.00 ml/min; internal standard, *p*-hydroxybenzoic acid *n*-hexyl ester.

To examine the influence of gel formation on stability of IDM, hydrogel containing IDM was prepared. The concentrations of IDM and polymer in hydrogel were fixed at 0.5% and 15%, respectively. UV irradiation was performed for the designated time. The prepared hydrogel was immersed in 15 ml of a water and methanol mixture (1:3, *v*/*v*) for 48 h at room temperature to extract IDM from hydrogel. The concentration of IDM in the supernatant was determined with HPLC.

Gel Fraction and Degree of Swelling

The gel fraction and degree of swelling were measured according to previous reports (7,8). Photocrosslinked PAA hydrogels of 0.5 g (weight of the solid content, W_{solid}) on a polystyrene disk were equilibrated in 60 ml of purified water for 24 h at room temperature and then weighed after the excess water was carefully removed (W_{water}). After the elimination of water by freeze-drying, the remaining solid was weighed (W_{gel}).

The gel fraction (%) was calculated as:

$$\text{Gel fraction} = W_{gel}/W_{solid} \times 100. \quad (1)$$

The degree of swelling was calculated as:

$$\text{Degree of swelling} = W_{water}/W_{gel}. \quad (2)$$

In Vivo Study

The animal experiments in this study complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals at Hoshi University. Male HWY/Slc rats weighing 180–200 g were purchased from Sankyo Labo Service (Tokyo, Japan). The animals were housed under conditions of controlled temperature (23±1°C) and relative humidity (55±5%) and were allowed free access to water and food during acclimatization. Rats were anesthetized with an intraperitoneal injection of 50 mg/kg sodium pentobarbital (Dainippon Pharmaceuticals, Osaka, Japan), and restrained in a supine position on a thermostatically controlled board at 37°C. The model formulations (2.0 g) with a backing layer of polyethylene terephthalate film were applied to the abdominal skin of rats. Blood samples (0.4 mL) were taken via the jugular vein at 0, 2, 4, 6, 8, and 10 h after the application.

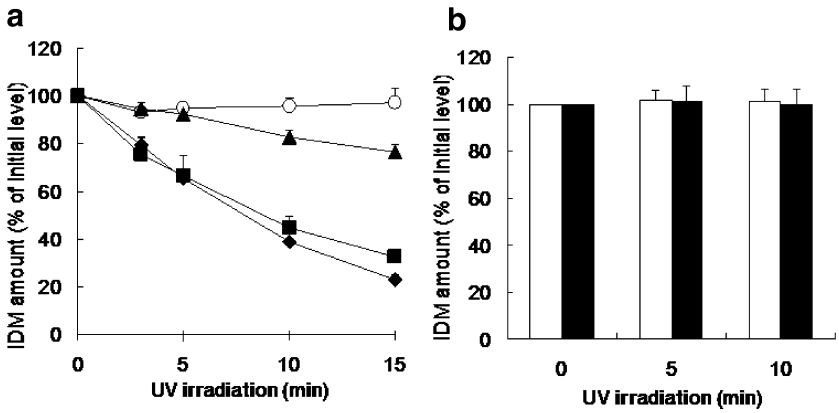

Fig. 2. Stability of IDM in photopolymerization. **a** Changes in IDM concentration in methanol after the UV irradiation. DMPA concentration was fixed at 0 (*open circle*), 1 (*filled triangle*), 2 (*filled square*) and 3 mg/ml (*filled diamond*). **b** Changes in IDM amounts after photopolymerization. Reaction solutions were 15% PAA–HEMA solution containing 1.5 (*open square*) or 3.0 mg/mL (*filled square*) of DMPA. UV irradiation was conducted for the designated time. Each value represents the mean±SD for three determinations

Each blood sample was centrifuged (13,400×*g*, 2 min) and the plasma separated from blood cells. A methanolic solution of internal standard (100 μl) and chloroform (2 ml) were added to the plasma sample (200 μl), and then the sample was mixed using vortex mixer. Subsequently, the organic solvent was evaporated under a gentle stream of nitrogen at room temperature. Finally, the sample was redissolved in 200 μl of mobile phase and the concentration of IDM was determined with HPLC.

The apparent permeation rate (PR) of IDM was estimated from a two-compartment model based on the assumption that the rate of permeation of indomethacin from the gel is constant after a lag time according to the following equation (9):

$$C = \frac{\mathrm{PR}}{\mathrm{V_c}k_{10}}\left\{1 + \frac{\beta - k_{10}}{\alpha - \beta}\mathrm{e}^{-\alpha(t-t_\mathrm{L})} + \frac{k_{10} - \alpha}{\alpha - \beta}\mathrm{e}^{-\beta(t-t_\mathrm{L})}\right\}, \quad (3)$$

where C is the plasma concentration, PR is the rate of permeation, t is the time, t_L is the lag time, V_c is the distribution volume of the central compartment, k_{10} is the elimination rate constant from the central compartment, and α and β are the hybrid first-order rate constant. The mean values of V_c, k_{10}, α and β, obtained from the intravenous administration of IDM. In brief, IDM solution (1 mg/ml) was prepared by dissolving IDM in a tiny amount of ethanol with heat and diluting with 1/15 M, PBS. The solution (2.5 ml/kg) was administered by bolus injection via the right jugular vein of rats. Blood samples were taken via the left jugular vein at 5, 10, 15, 20, 30, 60, 90, and 120 min. The blood samples were then handled the same way as already described.

Evaluation of Skin Irritation

Irritation of rat skin evoked by model formulations was judged microscopically after the end of percutaneous absorption experiments. The site of application of each hydrogel formulation on the skin was excised from rats. The separated skins were fixed in 20% neutral buffered formalin solution. Tissues were stained with hematoxylin and eosin (HE). All sections were examined by light microscopy as previously described (9,10).

Data Analysis

The relationship between the causal factors and characteristics were analyzed using the ANOVA component of Statistica (Statsoft, Tulsa, OK, USA); dataNESIA (Yamatake Corp., Tokyo, Japan) was used for RSM-S and BS resampling. Viscovery (Eudaptics Software, Vienna, Austria) was used for SOM clustering.

Table III. Experimental Values of Characteristics of IDM-Containing Photocrosslinked PAA-HEMA Hydrogels Prepared According to L8 Orthogonal Experimental Design for Three Causal Factors

Formulation number	Gel fraction (%)	Degree of swelling	Apparent permeation rate (μg/h)	Lag time (h)
Rp.1	77.1±4.1	407.0±8.8	1.92±1.11	1.22±0.64
Rp.2	80.5±2.2	378.1±10.1	1.81±0.87	1.74±0.33
Rp.3	83.3±4.6	363.1±8.0	1.28±0.26	1.01±0.94
Rp.4	84.2±2.7	351.1±4.7	2.64±0.37	1.19±0.35
Rp.5	77.5±1.1	314.8±14.4	1.16±0.81	1.60±0.65
Rp.6	77.5±1.7	311.6±14.4	1.97±0.82	1.33±0.30
Rp.7	78.4±6.0	287.4±17.7	1.62±0.51	1.62±0.16
Rp.8	81.3±3.4	290.7±19.0	3.90±1.32	1.58±0.22

Each value represents the mean±SD for three determinations.

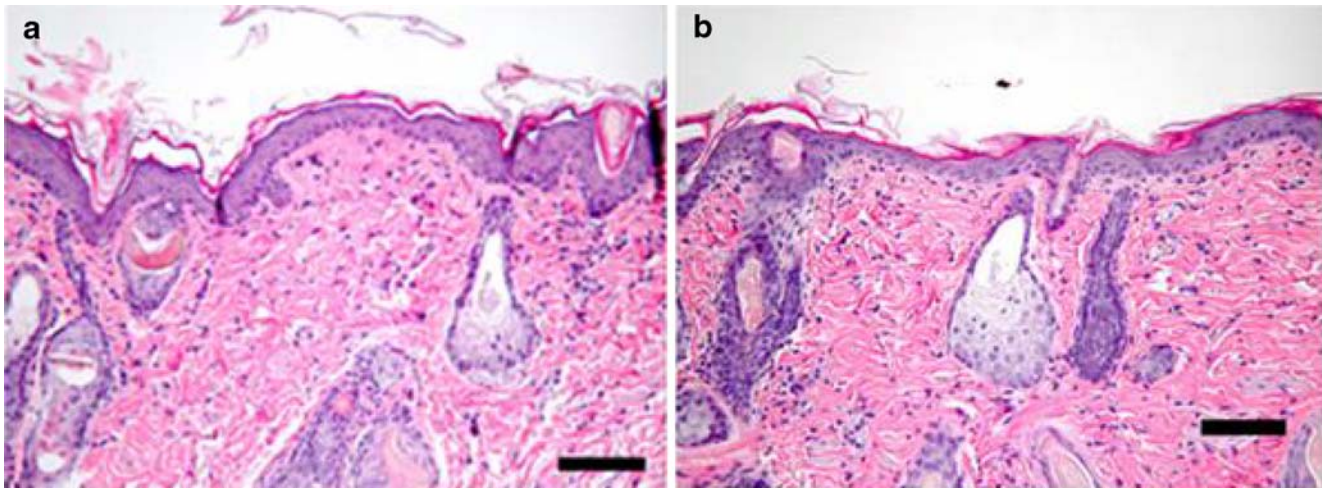

Fig. 3. Histological observations of a vertical section of rat skin at 10 h. HE stain; ×100: **a** control, **b** Rp.8

RESULTS AND DISCUSSION

Stability of IDM in Photopolymerization

In general, dermatological patch structures were classified into a matrix type and a reservoir type (11). Between them, the matrix-type formulation was suitable for this study because of its easy preparation. However, in the course of preparation of the formulations, the reaction solution undergoes exposure to UV light and free radical generation. The drug incorporated in the formulation might therefore be degraded and altered.

We firstly evaluated the stability of IDM to the photopolymerization. Figure 2a shows the changes of IDM concentration in methanol after the UV irradiation. DMPA was added to the IDM solution as an initiator at 0, 1, 2, and 3 mg/ml. In DMPA free solution, the IDM concentration did not change throughout the experimental period. On the other hand, DMPA had great impact on the IDM concentration. The amount significantly decreased with increase in the DMPA concentration. This indicated that IDM was sensitive not to UV irradiation but to free radical exposure.

Subsequently, we evaluated the stability of IDM during gel formation. As shown in Fig. 2b, the concentration of IDM

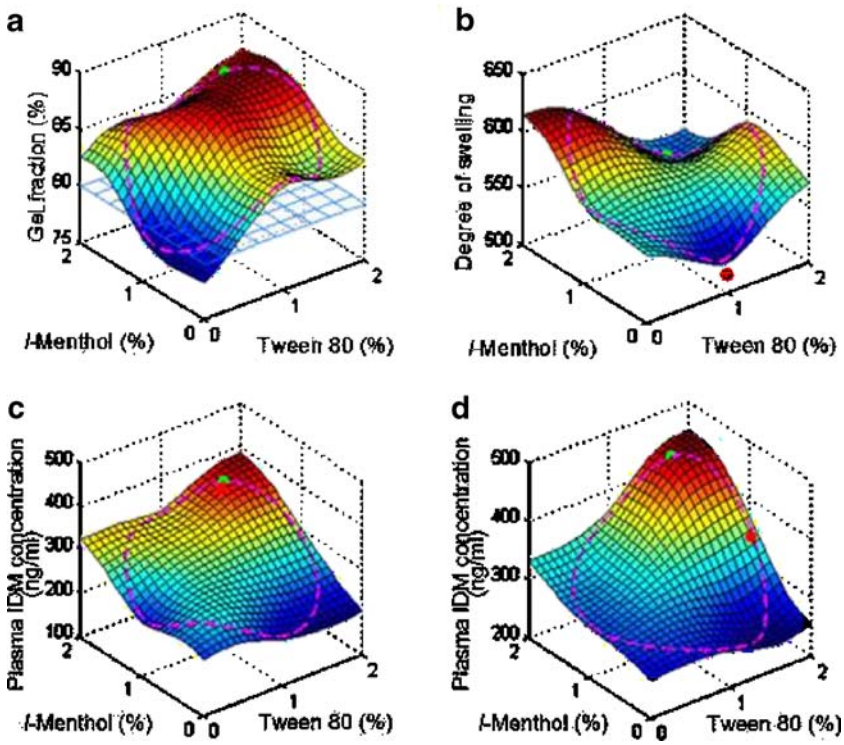

Fig. 4. Response surface of characteristics prediction using the MVS as a function of Tween 80 concentration and L-menthol concentration (modification with HEMA, 11.3%). **a** Gel fraction, **b** degree of swelling, **c** plasma IDM concentration at 6 h, **d** plasma IDM concentration at 10 h

Table IV. Original Optimal Formulation of IDM-Containing Photocrosslinked PAA-HEMA Hydrogel Estimated by RSM-S

Factors	Original optimal formulation[a]	Bootstrap optimal formulation[b]
Modification with HEMA (mol%)	11.34	11.1±2.2
Tween 80 concentration (%)	1.60	1.5±0.1
L-Menthol concentration (%)	1.75	1.6±0.2

[a] Simultaneous optimal formulation was estimated from original data set with RSM-S.
[b] Bootstrap optimal formulations were estimated with bootstrap re-sampling and SOM clustering.

did not change regardless of the DMPA concentration. Probably, the polymer plays a role in preventing radicals from attacking IDM. IDM is hydrophobic substance by nature. Most IDM exists in the aqueous solution by interacting with polymer. Under the circumstance, IDM can escape from the attack of radicals. IDM can be therefore incorporated into formulation without any damage. Thus, we conducted the following experiments using the matrix-type formulations dispersing IDM homogeneously in hydrogel.

Effect of Preparation Conditions on Formulation Properties

Formulations are determined by several formulation factors. It is thought that these factors consequently affect the characteristics of the final product. If we can clarify the relationships between formulation factors and product characteristics, this offers profound insight into formulation design. We investigated the relationships according to the following experimental design. Modifications with HEMA, Tween 80 concentration, and L-menthol concentration were selected as a causal formulation factors, and eight kinds of model formulations were prepared according to an L8 orthogonal experimental design (Table I). Gel fraction and degree of swelling were measured as physical properties of the formulations. They are widely used to understand the properties of the hydrogels (12–17). The gel fraction represents the degree of conversion to hydrogel. As described in the methods section, after the hydrogel was swollen, excess amounts of water were eliminated. At the same time, polymers that were not involved in gel formation were removed with the water. Therefore, the value of the gel fraction decreases with a decrease in gel formation. The degree of swelling is affected by numerous factors, such as crosslink density (7), entanglement of the polymer chains (8), and the hydrophilicity of the polymer (8). In general, because a solid hydrogel swells less in water, we can estimate the mechanical strength of the hydrogel from the degree of swelling. Moreover, we recently reported that higher gel strength is accompanied by greater adhesiveness in such a water-rich hydrogel (7). Thus, we can evaluate the potential of a hydrogel as an adhesive for dermatological patches by these characteristics. As well as examining physical properties, we conducted an *in vivo* absorption study using hairless rats and evaluated the bioavailability of IDM incorporated in the formulations. The apparent permeation rate and the lag time calculated from a plasma IDM concentration–time curve were selected as characteristics.

The observed values of each characteristic are shown in Table III. All characteristics were markedly changed, and causal factors seemed to affect individual characteristics significantly. To clarify the effect of causal factors on individual characteristics, an ANOVA of observed values was conducted. The gel fraction was significantly affected by the addition of Tween 80 and the value became higher with the addition to the reaction solution. As for degree of swelling, significant contributions of HEMA modification and addition of Tween 80 to the degree of swelling were observed, and formulations producing less swelling were prepared by increases in HEMA modification and the amount of the nonionic surfactant Tween 80. When Tween 80 was added to the reaction solution, the solubility of IDM in the reaction solution improved, and reaction solution becomes more transparent. It enhanced the photopolymerization, resulting in a higher gel fraction and lower swelling of the resulting gel. Increase in the HEMA modification increased the number of points of crosslinking. Thus, using such conditions, hydrogel having high crosslinking density were prepared, and the degree of swelling was decreased.

Formulation factors also affected bioavailability of IDM incorporated in the formulation. Apparent permeation rate was increased by addition of L-menthol and Tween 80. Especially, the effect of L-menthol was pronounced. The enhancing effect of L-menthol on the skin permeation is well known (18–21), and the marked increase in bioavailability of IDM was caused by the enhancing effect. The improvement of IDM solubility was no doubt associated with the enhancement of IDM permeation rate by Tween 80. Although there was not any significant contribution, a tendency for higher

Table V. Evaluation of the Reliability of Predicted Characteristics for Optimal Formulation of IDM-containing Photocrosslinked PAA-HEMA Hydrogel

Characteristics	Original optimal formulation[a]	Bootstrap optimal formulations[b]	Observed values[c]
Gel fraction (%)	86.7	86.4±1.1	87.3±5.5
Degree of swelling	545	546.3±14.8	558.0±28.8
Plasma IDM concentration at 6 h (ng/ml)	368	367.5±22.0	370.4±79.1
Plasma IDM concentration at 10 h (ng/ml)	444	427.8±55.8	404.9±115.4

[a] Simultaneous optimal formulation was estimated from original data set with RSM-S.
[b] Bootstrap optimal formulations were estimated with bootstrap re-sampling and SOM clustering.
[c] Each value represents the mean ± S.D. for five determinations.

HEMA modification to be accompanied by a prolongation of the lag time of IDM was observed. This indicates the possibility a change in the polymer composition affects the drug release properties of the hydrogel.

We examined skin irritation after the application of gel formulations for 10 h (Fig. 3). No damage was observed in any samples, suggesting that the hydrogel can be safely used as an adhesive for dermatological patches.

Formulation Optimization

From the orthogonal experimental study, we found out that formulation factors significantly affected various characteristics. We assumed that various characteristics could be fully controlled by manipulating the formulation factors. To obtain an acceptable anti-inflammatory patch having sufficient adhesive properties and high bioavailability of IDM, we optimized the formulation using RSM-S. RSM-S is a nonlinear response surface method developed in our laboratory. Multivariate spline interpolation (MVS) is integrated into RSM-S as a method of generating the response surface. The basic concept of MVS involves a boundary element method (22). Green functions are used for the minimum curvature interpolation of multidimensional data points. As usual, observational data include experimental error. To avoid problem over estimation, the multi-dimensional data surface including experimental error is estimated as a sum of interpolation with a Green function in a liner polynominal equation (thin-plate approximation) (23). The smoothing parameter, which is the ratio of the Green function interpolation and thin-plate approximation, is automatically estimated using a generalized cross-validation technique. Further details of RSM-S have been described fully in a previous paper (24). Since it can estimate nonlinear relationships between factors and characteristics with high accuracy, we can obtain a stable optimal formulation. Various findings have suggested that RSM-S is a promising tool for formulation optimization (7,24–27). In addition, we have recently offered a novel method for evaluating the reliability of nonlinear optimal formulation. Ensuring the reliability of the optimal solution estimated by a nonlinear method such as RSM-S has been remained a challenge. We integrated bootstrap (BS) resampling and self-organizing map (SOM) clustering into RSM-S, and suggested a methodology to solve this issue. Details of these concepts and the optimization procedure have been previously described (27).

In the optimization study, gel fraction, degree of swelling, and plasma concentration of IDM after 6 and 10 h application of formulation were selected as criteria. Figure 4 shows the response surfaces generated by RSM-S. Since the shapes of response surfaces are complicated, nonlinear relationships between the causal factors and gel characteristics were clearly observed. Based on these response surfaces, a simultaneous optimal formulation was estimated. More than 80% of gel fraction, the minimum degree of swelling and the maximum values of plasma concentration of IDM at 6 and 10 h were defined as individual ideal characteristics in seeking an optimal formulation. The optimal formulations from the original data set and BS samples are shown in Table IV. To evaluate the accuracy of these optimal formulations, we prepared a formulation according to the original optimal formulation and measured characteristics. The observed values were coincident with predicted values (Table V). Thus, we concluded that the estimated optimal formulations could be considered reliable.

CONCLUSIONS

Using an orthogonal experimental study, we clarified the relationships between formulation factors and characteristics of a model formulation. Furthermore, by means of the novel optimization technique, RSM-S, a high functional anti-inflammatory patch in terms of its adhesive properties and bioavailability of incorporated IDM in the formulation was successfully obtained. A wide range of functions of our hydrogel can be fully controlled by manipulating formulation factors at our disposal. Thus, our hydrogel is a promising candidate adhesive for dermatological patches.

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