Formulation Study on Retinoic Acid Gel Composed of Iota-Carrageenan, Polyethylene Oxide and Emulgen® 408

Keishi Kawata, Takehisa Hanawa, Naoko Endo, Masahiko Suzuki, and Toshio Oguchi*

Department of Pharmacy, University of Yamanashi Hospital; 1110 Shimokato, Chuo, Yamanashi 409–3898, Japan.

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In the present study, all-trans retinoic acid (RA) gels formulated with various compositions of polyethylene oxide (Emulgen®) and iota-carrageenan (ι-CG) were prepared and their physicochemical properties were evaluated. The compression energy, which is the work required to compress the product through a fixed distance, increased with increasing amount of ι-CG or Emulgen®. The adhesion energy and displacement decreased with increasing amount of ι-CG or Emulgen® due to the progression of gel formation. From the results of the sensory tests, the properties of RA gels such as adhesiveness, gel strength and spreadability seemed to be adjustable depending on the condition of skin by varying the components of RA gels. Through photostability study, the expiration date and storage conditions of RA gels were determined as “4°C for 28 d with no exposure to light.”

Key words all-trans retinoic acid; gel; texture; photostability

Retinoids are well known as a group of natural and synthetic derivatives of vitamin A, and many studies have been directed towards the characterization and application of vitamin A as a therapeutic agent for various diseases. In particular, all-trans retinoic acid (RA), which is one of the derivatives of vitamin A, has been widely used as an anti-inflammatory agent in inflammatory arthritis, an anticancer agent in different types of cancers such as acute promyelocytic leukemia, and an ophthalmic agent for corneal wounds.

In the dermatological field, topical RA has been used in proliferative and inflammatory diseases such as acne vulgaris, multiple plane warts following laser skin resurfacing, hyperpigmentation, and acquired dermal melanocytosis. Various drugs for external use such as ointments, cream or lotion containing tretinoin have been prepared and evaluated. The advantages of using such drugs in treatment include the fact that there is no need for surgical procedures by clinicians, and that patients are able to apply the treatment themselves.

However, because RA is water-insoluble, emulsification is necessary to dissolve RA uniformly. Yoshimura et al. prepared an aqueous RA gel by dispersing RA in Emulgen® 408 which is nonionic emulsifier, and in Carbopol® 940. In previous studies, we investigated the use of a mixture of polyethylene oxide and iota-carrageenan (ι-CG) as the base ingredient of mouthwash. Polyethylene oxide was obtained by ring-opening polymerization of ethylene oxide, which has a high mucoadhesion property and spinnability, whereas iota-carrageenan has been used as a texture modifier and gelling agent in the food and/or cosmetic industry. Since both compounds are tasteless and odorless, it was thought that these compounds are suitable as additive agents. The results demonstrated that the use of allopurinol mouthwash containing both polyethylene oxide and ι-CG decreased the chance of stomatitis caused by cancer chemotherapy and radiotherapy.

In the present study, we prepared various compositions of RA aqueous gels (RA gels) consisting of polyethylene oxide, ι-CG and Emulgen® 408, and evaluated their physicochemical properties, such as stability, discoloration, adhesiveness, and spreadability. The feasibility of the mixture consisting of polyethylene oxide, ι-CG and Emulgen® 408 as the base ingredient for a topical treatment was also examined.

Experimental

Materials All-trans retinoic acid (RA) was purchased from Wako Pure Chemical Industries (Osaka, Japan). ι-CG, Alkox® E-30 (Alkox®) and polyoxyethylene oleyl ether (Emulgen 408, Emulgen®) were generously supplied by Ina Food Co. (Nagano, Japan), Higuchi Inc. (Tokyo, Japan) and Kao Co. (Tokyo, Japan), respectively.

Preparation of RA Gels To clarify the effect of ι-CG or Emulgen® addition on the physicochemical properties, the various formulas of RA gels were prepared as shown in Table 1. RA gels were prepared by the emulsification method. A fixed amount of hydrogel prepared with various ratio of Alkox® (1.48–1.98%) and Emulgen® (0–1.88%) was withdrawn into a syringe A as shown in Fig. 1. A solution consisting of a fixed amount of cetanol (1.0%) and various amount of Emulgen® (2.0–10.0%) was poured into syringe B. After connecting syringes A and B with a syringe connector (ϕ 1.0 mm), the pistons were pushed reciprocally more than 20 times and the mutual solutions were mixed and emulsified. Phase separation was not observed for each sample during this study.

Measurement of the Viscosity of RA Gels The viscosity of the RA gels were measured using a Toki RE-80U Viscometer (Tokyo, Japan) equipped with a cone and plate fixture (14 mm diameter, 3° angle).

Measurement of Color Difference Approximately 1.5 mL of sample was poured into the glass mold (25 mm diameter, 3 mm thickness). The surface of the sample was covered with Dura Seal™ (Diversified Biotech, MA, U.S.A.), and stored

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*Present address: Faculty of Pharmaceutical Sciences, Tokyo University of Science; 2641 Yamazaki, Noda, Chiba 278–8510, Japan.

To whom correspondence should be addressed. e-mail: toshioo@yamanashi.ac.jp © 2012 The Pharmaceutical Society of Japan
under various conditions. The discoloration of the sample was evaluated by color difference. The color difference ΔE*ab was defined and calculated as the change in Hunter’s parameters of the sample using a Minolta calorimeter CR-300 (Osaka, Japan).

Measurement of RA Concentration  RA concentration in the sample was determined by high-performance liquid chromatography (HPLC). The HPLC conditions were: column, Shodex® Asahipak ODP-50 4E (4.6mm i.d.×250mm, Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, 50:50 methanol:acetonitrile; elution rate, 1.5mL/min; internal standard, vitamin K1; detector, SPD-10AVP; column oven, CTO-10AVP; and calculator, C-R8A (220nm, Shimadzu Co., Kyoto, Japan).

In Vitro Assessment of the Adhesiveness of RA Aqueous Gels  The apparatus and procedures are schematically illustrated in Fig. 2. The measurements of adhesive force were performed with using a creep meter (Yamaden model 33005S, Tokyo, Japan) at 25°C and 60% relative humidity (RH). A fixed volume (40mL) of the RA gel was measured in a beaker (45mm diameter, 25mm depth). In these assessments, we designated a Teflon® plunger (20mm diameter) that was lowered onto the surface of the RA gel. After loading downwards at a velocity of 1mm/s until 30% load-strain was obtained, the plunger was pulled up at a rate of 1mm/s. The adhesive force and the displacement were measured when the plunger was completely separated from the surface of the RA gel. In this study, the force loaded at 30% load-strain was assumed to be the adhesion force, and the distance from the gel surface at separation was estimated as an index of spinnability.

Sensory Evaluation of RA Gels  The sensory evaluation was designed to clarify whether the various formulas had any impact on individual sensory properties. In this study, sensory evaluations regarding RA gels were conducted with the help of eight pharmacists from the hospital of University of Yamanashi (females, mean age of 25.5±2.27 years). The pharmacists were questioned on the samples’ spreadability, adhesiveness, gel strength, and spinnability using a scale of minus to plus five, with the names of the samples covered. White petroleum was used as the standard, and if the texture was the same as the standard, 0 was chosen from the calibrated scores. Higher scores indicated better spreadability, lighter adhesiveness, lower gel strength and higher spinnability.

Stability of RA in RA Gels  About 1.5mL of sample (Rp. 3 in Table 1) was poured into the glass mold (25mm diameter, 3mm thickness). The surface of the sample was covered with quartz plate and stored at 4°C, 20°C, and 40°C for 0–21d under various light intensity conditions (Table 2).

Table 1. Component of RA Gels

<table>
<thead>
<tr>
<th>Component (%)</th>
<th>Rp. 1</th>
<th>Rp. 2</th>
<th>Rp. 3</th>
<th>Rp. 4</th>
<th>Rp. 5</th>
<th>Rp. 6</th>
<th>Rp. 7</th>
<th>Rp. 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkox</td>
<td>1.88</td>
<td>1.88</td>
<td>1.88</td>
<td>1.88</td>
<td>1.88</td>
<td>1.93</td>
<td>1.78</td>
<td>1.48</td>
</tr>
<tr>
<td>t-CG</td>
<td>0</td>
<td>0.38</td>
<td>0.75</td>
<td>0.94</td>
<td>0.88</td>
<td>0.77</td>
<td>0.71</td>
<td>0.59</td>
</tr>
<tr>
<td>RA</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Emulgen 408</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Cetanol</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dist. water</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 3 depicts the flow curves for the RA gels prepared with various compositions of t-CG and Emulgen®. Overall, the viscosity of the RA gel decreased with increasing shear rate, and typical shear thinning (but non-thixotropic) behavior was observed.

The fluidity index was calculated using the following relationship:

\[ \eta_{app} = m\gamma^n \]

where \( \eta \) = apparent viscosity (mPas), \( m \) = coefficient of viscosity, \( \gamma \) = shear rate (s\(^{-1}\)), and \( n \) = fluidity index. The values of the fluidity index \( n \) characterize the fluidity; the exponent takes the value of \( n=1 \) for Newtonian flow, and a value in the range of \( 0<n<1 \) for non-Newtonian flow. The values of the fluidity index are listed in Table 3. All samples were observed as non-Newtonian flows.

Adhesive Properties of RA Gels  To investigate the effects of the addition of t-CG or Emulgen® on adhesiveness, the adhesive energy and spinnability of the samples were
evaluated in vitro by load–strain analysis on a creep meter. Load–strain curve for each RA gel is shown in Fig. 4. In this study, the gel strength of the samples was defined as the highest load point at a strain of 30%, corresponding to part “a” as shown in Fig. 2. In these curves, the lifting process of the plunger starts at a load–strain of 30%. At the curve below the x-axis, the value of the load represents the tension received by the plunger, and the peak area of the curve indicates the adhesion energy between the surface of the plunger and that of the samples; the larger the area of the load–strain curve, the higher the adhesion energy of the sample solution. At the same time, distance from the solution surface at negative load, corresponding to part “b” as shown in Fig. 2, was considered to be an index of spinnability; the higher the load–strain, the larger the spinnability of the sample solution.

In this study, to evaluate the relationship between the results of physicochemical properties and those of sensory tests such as adhesiveness, spinnability and spreadability, these results are depicted on the same figures (Figs. 5–7) and the scores obtained from the sensory tests are listed in Table 4.

As for adhesiveness measured from the sensory tests, it showed a “sticky” tendency with increasing adhesion energy (Fig. 5). Figure 6 shows both the displacement and scores for spinnability obtained from the sensory tests. The displacement decreased by 0.5% of the concentrations of the added l-CG and 2.5% of that of added Emulgen®. These tendencies were also observed in the scores of sensory tests; e.g., the scores regarding spinnability indicated a “sticky” tendency as concentrations of the added l-CG and/or Emulgen® increased. Shin et al. demonstrated that spinnability gives an indication of the inner structure of polymers; i.e., polymers with a linear structure show spinnability, whereas polymers with a 3-dimensional structure do not show spinnability. The decrease in spinnability, as observed in this study, seemed to be due to the promotion of gelation by the increased concentrations of l-CG or Emulgen®.

Figure 7 depicts both the compressibility and the scores regarding spreadability obtained from sensory tests. The compressibility value increased as a function of the concentrations of l-CG and/or Emulgen®. In addition, a good correlation was observed between the spreadability obtained from the sensory and the compressibility tests. Jones et al. demonstrated that the compressibility is correlated to the spreadability of the gel on skin surface. Furthermore, Garg et al. demonstrated that the spreadability is an important characteristic of topical formulations and is responsible for correct dosage transfer to the target site, ease of application on the substrate and consumer preference. Based on these considerations, the texture of RA gels, including adhesiveness, gel strength and spreadability, seems to be adjustable depending on the condition of skin by varying its components.

Stability of RA in RA Gels RA is well known to be easily oxidized, thermally unstable and susceptible to photodegradation. In this study, in order to investigate discoloration, color differences before and after storing under various conditions were observed. It has been reported that color difference measurements were attempted to evaluate the kinetic and forecast the discoloration of medicines. The color of the RA gel was pale yellow immediately after preparation. Figure 8 shows the color differences of RA gels stored at various lighting intensities and temperatures. At 0% light-transmittance, the color difference became more apparent with increasing temperature. A change in color of RA from pale yellow to white was observed during storage.
Fig. 4. Compression and Decompression Curves of RA Gels
(a) Addition effect of \( \iota -CG \): —, Rp. 1; – – – – –, Rp. 2; – – – –, Rp. 3; – – –, Rp. 4; – – –, Rp. 5. (b) Addition effect of Emulgen®: – – –, Rp. 6; – – – – –, Rp. 3; – – – –, Rp. 7; – – –, Rp. 8.

Table 4. Mean Values Obtained from Sensory Tests of Various RA Gels

<table>
<thead>
<tr>
<th>Rp. No.</th>
<th>Spreadability</th>
<th>Adhesiveness</th>
<th>Gel strength</th>
<th>Spinnability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.00±1.00(^b)</td>
<td>–1.25±22.31(^b)</td>
<td>4.33±1.32(^b)</td>
<td>–0.78±2.73(^b)</td>
</tr>
<tr>
<td>2</td>
<td>3.22±0.97(^b)</td>
<td>1.33±1.80(^b)</td>
<td>3.01±1.12(^b)</td>
<td>2.00±1.41(^b)</td>
</tr>
<tr>
<td>3</td>
<td>3.67±0.71(^b)</td>
<td>2.11±1.69(^b)</td>
<td>3.01±0.71(^b)</td>
<td>1.56±1.59(^b)</td>
</tr>
<tr>
<td>4</td>
<td>3.00±1.18(^b)</td>
<td>1.57±1.51</td>
<td>2.56±1.51</td>
<td>1.50±1.32</td>
</tr>
<tr>
<td>5</td>
<td>2.56±1.42(^b)</td>
<td>1.43±1.81</td>
<td>2.11±1.76</td>
<td>1.33±2.74</td>
</tr>
<tr>
<td>6</td>
<td>2.63±1.39(^b)</td>
<td>1.88±1.13</td>
<td>3.00±0.76</td>
<td>1.56±1.51</td>
</tr>
<tr>
<td>7</td>
<td>2.88±0.84(^b)</td>
<td>2.00±1.41</td>
<td>2.89±0.78</td>
<td>1.44±2.79</td>
</tr>
<tr>
<td>8</td>
<td>2.38±1.69(^b)</td>
<td>1.50±1.85</td>
<td>2.33±1.00</td>
<td>0.67±2.29</td>
</tr>
</tbody>
</table>

\( a) p<0.05, \ b) 0.01<p<0.05, \ c) p<0.01 \) (Mann–Whitney’s U-test).

Fig. 5. Adhesion Energies and Scores for “Adhesiveness” Obtained from Sensory Tests
(a) Addition effect of \( \iota -CG \): ○, adhesion energies (J/m\(^3\)); ■, scores for “adhesiveness” obtained from sensory tests. (b) Addition effect of Emulgen®: ○, adhesion energies (J/m\(^3\)); ■, scores for “adhesiveness” obtained from sensory tests.

Fig. 6. Displacements and Scores for “Spinnability” Obtained from Sensory Tests
(a) Addition effect of \( \iota -CG \): ○, displacements (mm); ■, scores for “spinnability” obtained from sensory tests. (b) Addition effect of Emulgen®: ○, displacements (mm); ■, scores for “spinnability” obtained from sensory tests.
conditions. As shown in Fig. 9, RA degrades by apparent first-order kinetics.

To clarify the light intensity and/or temperature dependency in degradation behavior, the relationship between the apparent first-order rate constants and light intensity or the reciprocal of absolute temperature were plotted (Fig. 10). When the RA gels were stored at a constant temperature (25°C), the degradation rate increased with increasing light intensity (Fig. 10a). Similarly, the degradation rate of RA increased with increasing temperature (Fig. 10b). Where RA gels were stored under light shielding condition, the rate constants were lower than those of light irradiated condition. In this study, the temperature and light intensity showed a strong effect on the apparent first-order rate constant of RA. From the results of the photostability study, the expiration date and storage conditions of RA gels were determined as “4°C for 28 d with no exposure to light.”

**Conclusion**

In conclusion, this study has characterized various RA gel compositions containing s-CG and Emulgen® in terms of rheological, textural and photostability properties. Such information can provide a more rational basis for the selection of formulations depending on the condition of skin by varying
the components of RA gels.

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References