Manuscript for submission as a regular article in J. Drug Del. Sci. Tech.

Purification of isopropyl fatty acids markedly changed the skin permeation of a model hydrophilic chemical

Dai Qi, Hiroaki Todo, Kenji Sugibayashi*

Faculty of Pharmaceutical Sciences, Josai University, 1-1- Keyakidai, Sakado, Saitama, 350-0295, Japan.

* Correspondence: sugib@josai.ac.jp

Abstract

We determined the difference in the skin-penetration enhancing effects of conventional (C-) and super-refined (SR-) isopropyl fatty acids (IPFAs) including isopropyl myristate (IPM), isopropyl palmitate (IPP), and isopropyl oleate (IPO). *In vitro* permeation experiments were performed with excised hairless rat skin using a model mal-absorbed chemical, calcein. As a result, SR-IPM pretreatment markedly increased the skin permeation of calcein from aqueous solution more than C-IPM pretreatment, in spite of a small difference in IPM purity (98.78 vs 99.96%). Similar phenomena were recorded even when using SR-IPM emulsion compared with C-IPM emulsion without pretreatment. In contrast, C-IPP pretreatment showed higher skin permeation than SR-IPP. In addition, no difference was observed in skin permeation between C-IPO and SR-IPO. Skin impedance was also determined as an index of skin barrier function. SR-IPM changed the skin barrier function more than C-IPM, which supported the penetration-enhancing order of C-IPM and SR-IPM shown above. It was unexpected to find such a big difference in the skin-penetration enhancing effect of C-IPM vs SR-IPM and C-IPP vs SR-IPP. The present results suggested that SR-IPM could be a promising ingredient in pharmaceutical and cosmetic products for topical use.

Keywords: super-refined isopropyl fatty acid, super-refined isopropyl myristate, chemical enhancer, skin permeation

Searching for potent skin-penetration enhancers is a key issue in the development of new topical and transdermal drug delivery systems. Through many previous exploratory studies, a variety of delivery systems containing chemical enhancer(s) have been developed. However, it has become more difficult to find new potent enhancers over the past decade or two, which suggests that the present methodology and ideas are not sufficient to find good skin-penetration enhancers. Several researchers have already applied physical enhancement methods such as iontophoresis and microneedle puncture with chemical enhancers [1,2]. A combination of two or more chemical enhancers is also useful to further increase the skin permeation of drugs [3-5]. Because the purity of most enhancers in previous studies has not been 100.0%, these studies even using single enhancers may be thought of as examining the effect of a kind of enhancer mixture. We then paid attention to the purity level of the enhancers.

Isopropyl fatty acids (IPFAs) such as isopropyl myristate (IPM), isopropyl palmitate (IPP), and isopropyl oleate (IPO) are major ingredients (as skin penetration enhancers) in topical and transdermal delivery systems as well as cosmetic products. IPM is the most used among such fatty acid esters, and it was expected to especially enhance the penetration of active chemicals through the skin [4,6]. The previous experiments were done using crude IPFAs (such as IPM) as described above. Nowadays, super-refined IPFAs are available as a result of the rapid progress in purification technology for IPFAs. This technology using column chromatographic purification physically removes primary impurities to have super-refined IPFAs (SR-IPFAs). The impurities in the conventional IPFAs (C-IPFAs; such as IPM) may be related to the increased skin irritation and decreased stability as well as lowering the penetration enhancing ability of drugs through the skin.

In the present study, the effect of SR-IPFAs especially SR-IPM on the skin-penetration enhancing effect was investigated to reveal the usefulness of newly developed SR-IPFAs compared with C-IPFAs using a conventional *in vitro* skin permeation experiment and skin impedance determination. The purity of C-IPM used in this experiment was 98.78%, whereas that of SR-IPM was 99.96% (Table 2).

I. MATERIALS AND METHODS

1. Materials

C-IPM, SR-IPM, C-IPP, SR-IPP, C-IPO, SR-IPO, and the mixture of impurities remaining in the column after the purification process were gifts from Croda Japan (Tokyo, Japan). Other reagents and solvents were of analytical grade and used without further purification. Table 1 shows the chemical structures and molecular weights (M.W.) of the chemicals analyzed. Table 2 compares impurities in the C-IPM and SR-IPM used in this experiment.

Tables 1 and 2

2. Experimental animals

Male hairless rats (WBM/ILA-Ht, 7–9 weeks old, body weight: 200–250 g) were purchased from either Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experiment Animal Laboratories (Fukaya, Saitama, Japan). They were housed in temperature-controlled rooms (25 ± 2°C) with a 12 h light-dark cycle (7:00–19:00 h). The rats were allowed free access to food (Oriental Yeast Co. Tokyo, Japan) and tap water. All breeding procedures and the experiments on animals were

performed in accordance with the guideline of the Animal Experiment Committee of Josai University.

3. Preparation of formulations

Oil-in-water (O/W) emulsions containing calcein as a model mal-absorbed hydrophilic penetrant were prepared with 30% (v/v) C-IPFAs or SR-IPFAs and 0.1% (v/v) polysorbate (Tween) 80 as an emulsifier. The mixture of IPFA and Tween 80 was added slowly to calcein in pH 7.4 phosphate-buffered saline–EDTA solution (PBS-EDTA). The obtained solution was agitated thoroughly at 14,000 rpm for 5 min using a Polytron PT3100 (Kinematica AG, Littau-Lucerne, Switzerland) to obtain O/W emulsions.

4. *In vitro* skin permeation experiment

4.1. *In vitro* skin permeation of calcein from PBS-EDTA after pretreatment with SR-IPFA

Abdominal full-thickness skin from hairless rats was excised under anesthesia by *i.p.* injection of medetomidine (0.375 mg/kg), butorphanol (2.5 mg/kg), and midazolam (2 mg/kg), and excess subcutaneous fat and blood was carefully removed. Then, the hairless rats were sacrificed immediately by injection of pentobarbital sodium salt (40 mg/kg). The stratum corneum surface of the skin (application area; 0.95 cm^2) was pretreated with 3.0 mL of IPFAs for 1 h. Then, the pretreated skin was wiped and washed with PBS-EDTA to remove excess IPFAs from the skin surface. The skin piece was mounted in a side-by-side diffusion cell with an effective diffusion area of 0.95 cm^2 [7]. Then, 0.1 M calcein solution (3.0 mL) was placed on the stratum corneum side of the skin.

The receiver solution was 3.0 mL of PBS-EDTA, which was maintained at 32°C using a thermo-regulated water bath. A magnetic stirrer bar was added in the donor and receiver compartments, which moved at about 1,200 rpm throughout the experiment. The receiver solution samples (0.5 mL) were withdrawn every 1 h, and the same volume of PBS-EDTA was added to the receiver compartment to keep the volume constant.

4.2. *In vitro* skin permeation experiment with calcein from O/W emulsion without pretreatment

The O/W emulsion (3.0 mL) containing calcein with C-IPFA or SR-IPFA was placed on the stratum corneum side of the skin (without pretreatment) in the side-by-side diffusion cell, as above. The other methods were the same as above.

4.3. Determination of calcein

Each receiver sample was examined for calcein using a fluorospectrophotometer (RF 5300PC, Shimadzu, Kyoto, Japan) at an excitation wavelength of 488 nm and fluorescent emission wavelength of 515 nm.

5. Skin impedance determination

Pretreated abdominal full-thickness skin of hairless rats by C-IPM or SR-IPM or O/W emulsion containing each IPM was mounted in a side-by-side diffusion cell as above. The donor and receiver solutions were 3.0 mL of PBS, which was maintained at 32°C using a thermo-regulated water bath. Both solutions were stirred using a magnetic stirrer bar at about 1,200 rpm throughout the experiment. Skin impedance was determined using an impedance meter (10 Hz AC, Advance, Tokyo, Japan) at various intervals over 2 h.

6. Statistical analysis

Statistical analysis was performed using an unpaired Student's *t*-test. A *p* value less than 0.05 was considered significant.

II. RESULTS

1. Effect of C-IPFAs and SR-IPFAs on the *in vitro* skin permeation of calcein

Figure 1 shows the pretreatment effect of C-IPFAs and SR-IPFAs on the time course of the cumulative amount of calcein that permeated from PBS-EDTA through excised full-thickness hairless rat skin. Figures 1a, b and c show the results for IPM, IPP, and IPO pretreatment, respectively. PBS-EDTA was pretreated as a control. Permeation experiments were done using calcein in PBS-EDTA after one of these three pretreatments (pretreated by C-IPFAs or SR-IPFAs or PBS-EDTA). Pretreatment with C-IPM slightly enhanced the skin permeation of calcein compared with a control solvent, PBS-EDTA. In contrast, pretreatment with SR-IPM markedly enhanced the skin permeation of calcein compared with C-IPM. When evaluated statistically, the cumulative amount of skin permeation over 8 h, a significant difference was observed between these two groups. On the other hand, C-IPP showed a much higher enhancing effect than that with SR-IPP, and significant difference was observed between them. It was also of note in the case of IPO that there was little difference in the skin permeation of calcein between with C-IPO and SR-IPO.

Fig. 1

Figure 2 shows the effect of IPFA-containing O/W emulsion on the time course of the cumulative amount of calcein that permeated through excised full-thickness

hairless rat skin without any pretreatment. Tween 80 at a concentration of 0.1% was used to prepare the O/W emulsion, so the same concentration of Tween 80 as well as PBS-EDTA were used as the calcein donor solution. The C-IPM emulsion enhanced the skin permeation of calcein compared with PBS-EDTA, but the effect was not different from that of Tween 80 solution, suggesting that the effect for the C-IPM emulsion may be dependent on Tween 80 in the emulsion. On the other hand, the SR-IPM emulsion markedly increased the skin permeation of calcein. A significant difference was observed for the cumulative permeation over 8 h between the C-IPM and SR-IPM groups.

No enhancing effect was observed on the skin permeation of calcein with C-IPP and SR-IPP emulsions. In contrast, C-IPO and SR-IPO emulsions increased skin permeation, but the effect was much lower than with SR-IPM emulsion.

Fig. 2

Because both SR-IPM and its emulsion markedly increased the *in vitro* skin permeation of calcein compared with C-IPM, other IPFAs and their emulsions, the following experiments were done using only C-IPM and SR-IPM.

2. Effect of C-IPM and SR-IPM on skin impedance

Skin impedance was found to be directly related to the skin permeability of hydrophilic compounds [8]. To further evaluate the positive enhancing effect of SR-IPM rather than C-IPM, skin was pretreated with C-IPM or SR-IPM or their O/W emulsions for 60 min. Table 3 shows results for the impedance change (% against that before

pretreatment). The skin impedance changes after pretreatment with SR-IPM and SR-IPM emulsion were significantly greater than those for C-IPM and its emulsion, respectively.

Table 3

III. DISCUSSION AND CONCLUSION

It was of particular interest in the present study that SR-IPM pretreatment markedly increased the skin permeation of calcein more than C-IPM pretreatment (Fig. 1a), in spite of only a small difference in IPM purity (98.78 vs 99.96%). A similar phenomenon was observed even when using SR-IPM emulsion than C-IPM emulsion (Fig. 2a). In contrast, C-IPP pretreatment showed higher skin permeation than SR-IPP (Fig. 1b). In addition, no difference was observed on the skin permeation by the C- and SR-IPO (Fig. 2b). It will be important to evaluate why C-IPP showed higher skin permeation than SR-IPP. In the present study, however, we just focused on the higher skin-penetration enhancing effect of SR-IPM, because our main focus was to find a good enhancer system or to establish a good searching method for chemical enhancers.

The present results shown in Figures 1a and 2a suggested that impurities in C-IPM decreased skin permeation. Then, a pretreatment experiment on skin was done by the addition of impurities (at a concentration of 5%) obtained in the column chromatographic purification process from C-IPM into the SR-IPM specimen. As a result, skin permeation was decreased to a similar level as for C-IPM pretreatment (Fig. 3). Further experiments will be needed with changing impurity concentrations. Higher fatty acids, such as dodecanoic acid and tridecanoic acid, and fatty acid esters with similar total carbon numbers, such as butyl myristate and IPP, were found in the impurities in C-IPM

(Table 2). Because IPP, one of the impurities in C-IPM, had little enhancement effect on the skin permeation of calcein, as shown in Figures 2b and 3b, the higher effect of SR-IPM than C-IPM may not be surprising.

The result of skin impedance determination (Table 3) showed that SR-IPM changed the skin barrier function more than C-IPM. This result supported the penetration-enhancing order of C-IPM and SR-IPM in Figures 1a and 2a. Several mechanisms such as lipid extraction, phase separation, inverted micelle formation, or bilayer fluidization [9] may be related to the skin impedance change with SR-IPM. In addition, these are also related to the penetration-enhancing effect of SR-IPM. The effect of pure IPM and several impurities must be evaluated separately.

We believe that few researchers would expect such a big difference in the skin-penetration enhancing effects with C-IPM vs SR-IPM and C-IPP vs SR-IPP. Further attention will be needed on the use of crude chemical enhancers, and we have to compare the enhancing effect between crude and pure chemicals when extraordinarily pure chemicals are available.

The present results suggested that newly developed SR-IPM could be a promising ingredient in pharmaceutical and cosmetic products for topical use. Further studies will need to be conducted to evaluate the usefulness of SR-IPFAs in pharmaceutical and cosmetic applications.

ACKNOWLEDGEMENT

We thank Croda Japan for providing C-IPFAs and SR-IPFAs.

REFERENCES

1. Liu K.C., Green C.R., Alany R.G., Rupenthal I.D. - Synergistic effect of chemical penetration enhancer and iontophoresis on transappendageal transport of oligodeoxynucleotides. - Int. J. Pharm., **441**, 687-692, 2013.

2. Yang Y., Kalluri H., Banga A.K. - Effects of chemical and physical enhancement techniques on transdermal delivery of cyanocobalamin (vitamin B12) *in vitro*. - Pharmaceutics, **3**, 474-484, 2011.

3. Shi J., Cong W.J., Wang Y.M., Liu Q.F., Luo G.A. - Synergistic effect and mechanism of cineole and terpineol on *in-vitro* transdermal delivery of huperzine A from microemulsions. - Iran J. Pharm. Res., **12**, 271-280, 2013.

4. Kobayashi Y., Nakamura H., Sugibayashi K., Morimoto Y. - Estimation of action site of L-lactic acid-ethanol-isopropyl myristate mixed system on the skin permeation of ketotifen. - *Int. J. Pharm.*, **156**, 153-162, 1997.
5. Kobayashi D., Matsuzawa T., Sugibayashi K., Morimoto Y., Kimura M., Analysis of combined effect of l-menthol and ethanol as skin permeation enhancers based on 2-layer skin model. - Pharm. Res., **11**: 96-103, 1994.

6. Sato K., Sugibayashi K., Morimoto Y., Effect and mode of action of aliphatic esters on the *in vitro* skin permeation of nicorandil. - Int. J. Pharm., **43**, 31-40, 1988.

7. Okumura M., Sugibayashi K., Ogawa K., Morimoto Y., Skin permeability of water soluble drugs, Chem. Pharm. Bull., **37**, 1404-1406, 1989.

8. White E.A., Orazem M.E., Bunge A.L. - Characterization of damaged skin by impedance spectroscopy: chemical damage by dimethyl sulfoxide. - Pharm. Res., **30**, 2607-2613, 2013.

9. Barry B.W. - Breaching the skin's barrier to drugs. - Nature Biotech. **22**, 165-167, 2004.

Figure captions

Figure 1. Effect of C-IPFA and SR-IPFA pretreatment on the time course of the cumulative amount of calcein permeating through full-thickness hairless rat skin from its aqueous solution.

Figures 1a, b and c show for IPM, IPP, and IPO, respectively. Symbols: C-IPFAs (○); SR-IPFAs (●); PBS-EDTA (▲). Significant difference was observed between C-IPM and SR-IPM and between C-IPP and SR-IPP. Each point represents the mean ± S.D. (n = 3 - 12).

Figure 2. Effect of C-IPFA and SR-IPFA emulsions on the time course of the cumulative amount of calcein permeating through full-thickness hairless rat skin without pretreatment.

Figures 2a, b and c show for IPM, IPP, and IPO, respectively. Symbols: C-IPFAs (○); SR-IPFAs (●); 0.1% Tween 80 (△); PBS-EDTA (▲). Significant difference was observed between C-IPM and SR-IPM emulsions. Each point represents the mean ± S.D. (n = 3 - 19).

Figure 3. Effect of impurities on the time course of the cumulative amount of calcein permeating through full-thickness hairless rat skin.

Symbols: C-IPM (○); SR-IPM(●); impurities spiked SR-IPM (■); PBS-EDTA (▲). Each point represents the mean ± S.D. (n = 3 - 12).

Fig. 1

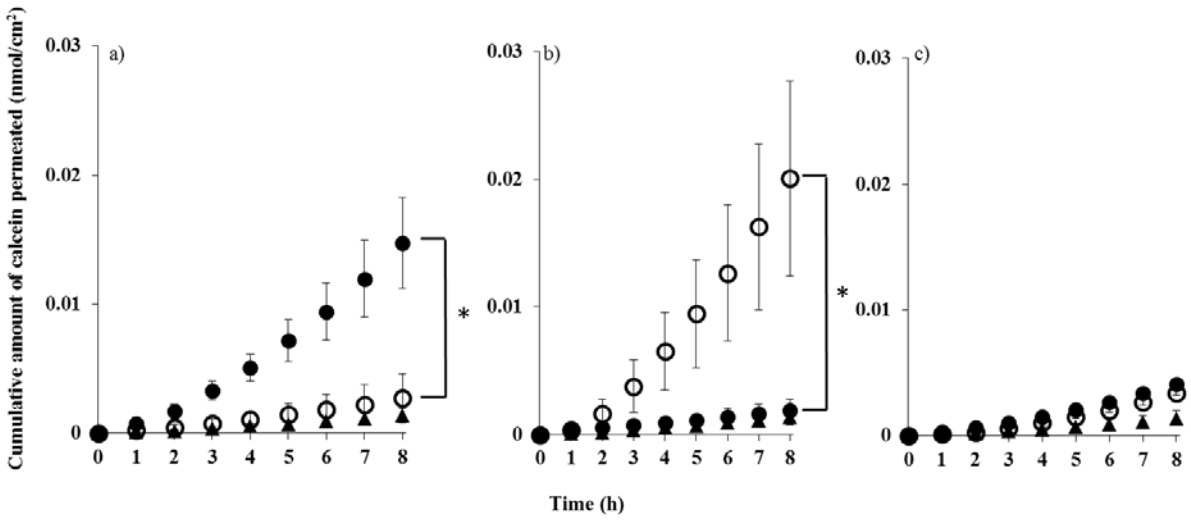

Cumulative amount of calcein permeated (nmol/cm²)
a)
b)
c)
0.03
0.02
0.01
0
0 1 2 3 4 5 6 7 8
*
*
Time (h)

Fig. 2

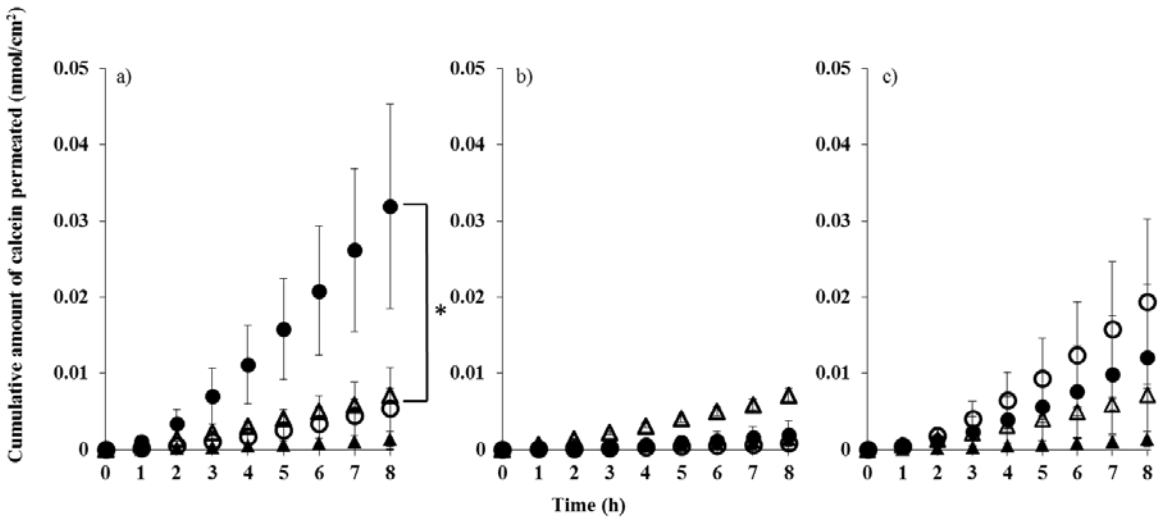

Cumulative amount of calcein permeated (nmol/cm²)
a)
b)
c)
0.05
0.04
0.03
0.02
0.01
0
0 1 2 3 4 5 6 7 8
*
Time (h)

Fig. 3

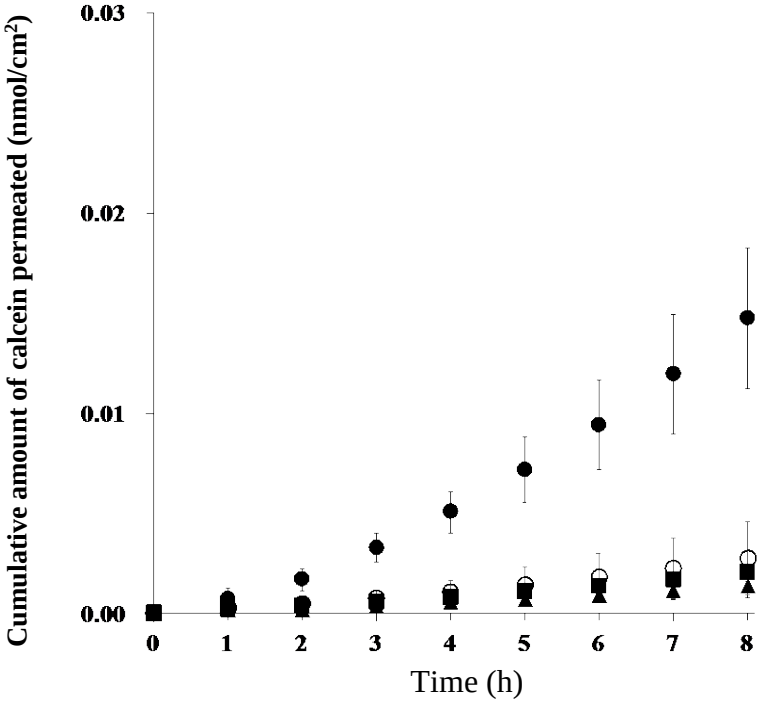

Cumulative amount of calcein permeated (nmol/cm²)
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6
7
8
Time (h)

Table 1 Structure and molecular weight of IPFAs used in the present study

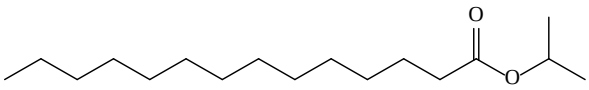
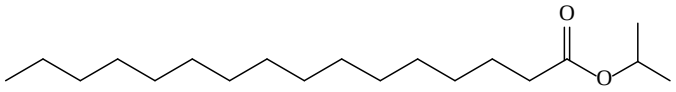
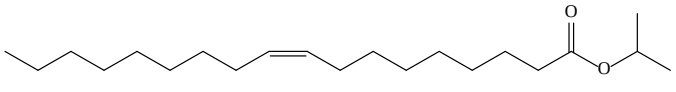
IPFAs	Abbreviation	Molecular formula *M.W.*
Isopropyl myristate	C-IPM	 270.5
	SR-IPM	
Isopropyl palmitate	C-IPP	 298.5
	SR-IPP	
Isopropyl oleate	C-IPO	 324.5
	SR-IPO	

Table 2 Impurities in C-IPM and SR-IPM

	C-IPM	SR-IPM
Benzenesulfonic acid	0	0
Dodecanoic acid	0.24	0
Tridecanoic acid	0.21	0.01
Tetradecanoic acid	0	0
Isopropyl 13-methyltetradecanoate	0	0
Butyl myristate	0.03	0
Isopropyl palmitate	0.74	0
Unidentified compounds	0	0.03
Total (impurity)	1.22%	0.04%

These values are for the C-IPM and SR-IPM used in this experiment.

Table 3 Skin impedance change after 60-min application of C-IPM or SR-IPM or one of their emulsions

	Impedance ratio (%)
C-IPM	45.8 ± 8.3
SR-IPM	25.6 ± 4.4*
C-IPM emulsion	83.5 ± 5.5
SR-IPM emulsion	63.6 ± 8.2*

Impedance before application was set to 100%. Each value represents the mean ± S.D. (n = 5). *Significant difference was observed between C-IPM and SR-IPM and between C-IPM and SR-IPM emulsions.