# Survey of Formaldehyde (FA) Concentration in Cosmetics Containing FA-donor Preservatives

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**We measured the amount of formaldehyde (FA) in cosmetics containing FA-donor preservatives [imidazolidinyl urea (IU), dimethyloldimethyl hydantoin (DM), diazolidinyl urea (DU), quaternium-15 (QU), and bronopol (BP)] and explored the factors affecting FA release. FA was detected in all the 89 cosmetic products tested. FA concentrations of cosmetics declared to contain DM and DU were significantly higher than those of cosmetics declared to contain IU and BP. Detected FA concentration of samples produced in the U.S.A. was significantly higher than that of samples produced in European countries. A weak proportional relationship was observed between the pH value and the released FA concentration of the cosmetic products containing DM and DU. There were no significant differences in the FA concentrations of various categories of cosmetics (lotion, gel, conditioner, shampoo, body wash, and others). Cosmetics containing a blend of amines, amides, or hydrolyzed proteins together with FA-donor preservatives had a lower FA concentration than the others.**

**Key words** —— formaldehyde-donor preservative, free-formaldehyde reducer, cosmetic, hydrogen ion concentration

## INTRODUCTION

A wide variety of antimicrobial preservatives are used in cosmetics. Formaldehyde (FA)-donor compounds are one of the most frequently used preservatives in cosmetics, except for parabens.[1] Some reports show that FA-donor preservatives have an antibacterial and antifungal effect because of their chemical compositions and FA released upon their decomposition.[2]

In previous studies, several cosmetic ingredients were reported to have potency to dermal sensitization.[3–6] A retrospective European survey of allergic contact reaction reports that 165 of 475 patients showed positive reactions to preservatives.[3] In that study, more than half of the cases of positive reaction to preservatives were caused by FA itself or by FA-donor preservatives. In another report on the patch tests of North American Contact Dermatitis Group, quaternium-15 (QU), an FA-donor preservative, was the most common cosmetic allergen in both males and females.[4] In addition to QU, FA and four more FA-donor preservatives, namely, diazolidinyl urea (DU), dimethyloldimethyl hydantoin (DM), imidazolidinyl urea (IU), and bronopol (BP), were listed as the top 20 allergens with a cosmetic source. It remains controversial whether FA is the major cause of the allergic reaction caused by FA-releasing compounds.[6–8] However, previous reports showed that the threshold of allergic reaction to FA is 30 mg/kg[9] or 250 mg/kg[10] in FA-sensitive individuals; hence, the involvement of FA levels in allergic activity is not negligible.

In Japan, IU and DM are permitted to blend at the maximum concentration of 0.3% only for the rinse-off cosmetics that are not to be used for cleansing the mucosa. In contrast, formalin, a 37% concentrated solution of FA, is included in the list of prohibited ingredients.[11] There is a discrepancy between permission for blending two FA-releasing preservatives and prohibition for the inclusion of formalin. In other countries, the regulation related to the use of FA-donor preservatives is more relaxed than in Japan. In European Union (EU) and Association of Southeast Asian Nations (ASEAN) countries, nine FA-donor preservatives are permitted as ingredients for cosmetics, and the maximum allowed concentrations of IU and DM are 0.6%, twice as much as in Japan.[12, 13]

Recently, with the development of information

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technologies, such as the internet, consumers in Japan can easily obtain products from foreign countries. In the last decade, the amount of cosmetics imported to Japan also increased by 100%.[14] Hence, it is expected that the increase in the opportunity to obtain cosmetics that contain FA-donor preservatives leads to an increase in the risk of dermal sensitization.

Thus far, there have been few studies on the survey of FA concentration of cosmetics containing FA-releasers.[10, 15, 16] Because of the threat posed by an FA-releaser as a dermal sensitizer, it is important to know the relationship between sensitizing potential and free-FA concentration. In this study, FA concentration measurement and statistical analysis of purchased cosmetic samples are described. The present study result is helpful in evaluating the risk of contact dermatitis from FA-donor preservatives in cosmetic samples and is expected to provide useful information for developing strategies to avoid health hazard from FA-releasing preservatives.

## MATERIALS AND METHODS

**Cosmetic Samples** —— Most of the cosmetics ($n$ = 68) were obtained via personal import ordered over the internet. The others ($n$ = 21) were purchased from the cosmetic sections of various shops in Osaka prefecture. All the cosmetic samples were obtained from December 2008 to January 2009. Purchased samples were stored at ambient temperature until FA measurement. The FA concentrations were measured within 2 months at the latest from arrival of the samples. Sample numbers classified with sample type and containing FA-donor are shown in Table 1.

**Chemicals and Reagents** —— 2,4-Dinitrophenylhydrazone, acetonitrile, ethylacetate, and formalin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Determination of FA Concentration** —— The concentrations of the cosmetic samples were determined by a method described previously with little modification.[17] Briefly, 5 ml of 2,4-dinitrophenylhydrazone diluted in 2 mol/l $H_3PO_4$ [0.02% (w/v)] and 2.5 ml of distilled water was added to 0.05 mg of cosmetic sample, and left standing at ambient temperature for 20 min after vortex. The mixture was shaken with 2.5 ml of ethyl acetate for 10 min, and the ethyl acetate layer diluted with acetonitrile to $\times 5$ was subjected to HPLC. HPLC was carried out using a Shimadzu LC-10A series instrument equipped with a UV detector. The determination wavelength was set at 355 nm. An octadeaylsilanized silica gel (ODS) column of L-column ODS (5 μm, 4.6 mm I.D. × 150 mm, Chemicals Evaluation and Research Institute (C.E.R.I.), Saitama, Japan) was used. The mobile phase was acetonitrile : water (1 : 1) delivered at a flow rate of 1.2 ml/min. The column temperature was held at 40°C, and the volume of sample injected was 10 μl.

**Statistical Analysis** —— Statistical analysis was performed by using the software STATCEL2 (OMS Co., Ltd., Tokyo, Japan). One-way analysis of variance (ANOVA) was used for comparing the difference between blended donor-preservative, sample type and the country of origin. If the ANOVA was significant, Tukey-Kramer's test was performed for multiple comparisons. A $p$-value $< 0.05$ was considered to indicate significant difference. The relationship between the pH value of the sample cosmetics and the detected FA concentrations was investigated using Pearson's correlation coefficient test. To compare the FA concentrations of samples blended with or without amino group compounds, Welch's $t$-tests (2-tailed) were carried out.

**Table 1.** Sample Numbers Classified with Sample Type and Containing FA-donors

| | Lotion* | Gel | Body wash | Conditioner | Shampoo | Other** | Total |
|---|---|---|---|---|---|---|---|
| IU | 9 | 0 | 3 | 0 | 2 | 2 | 16 |
| DM | 7 | 1 | 12 | 0 | 2 | 2 | 24 |
| DU | 9 | 8 | 1 | 9 | 11 | 0 | 38 |
| QU | 5 | 0 | 1 | 0 | 2 | 1 | 9 |
| BP | 1 | 0 | 0 | 1 | 0 | 3 | 5 |
| Total | 31 | 9 | 17 | 10 | 17 | 8 | 92 |

* In three lotion samples, two of the FA-donors are blended. Two lotions contain IU and QU, and one lotion contains DU and DM.They are counted up for both of the group. Actual sample numbers of lotions are 28 and the total sample numbers are 89. ** Hair liquid, fragrance, cleansing clothes and eye make up remover, *etc.*

## RESULTS AND DISCUSSION

### Detected Free-FA Concentration

FA was detected in all samples analyzed. The free FA concentrations ranged from 2.70 mg/kg to 876 mg/kg. The highest FA concentration was detected from a DM containing lotion, which was made in the U.S.A. and measured pH value was 6.17. The detected levels were more than 30 mg/kg in 83 samples and more than 250 mg/kg in 44 samples out of the 89 cosmetic samples. These concentrations are the previously reported thresholds for dermal sensitization in FA-sensitive individuals.[8,9] It is suspected that most of the cosmetics that claimed to contain FA-donor preservatives are possible dermal sensitizers for FA-sensitive individuals.

### Difference of FA Concentration with Blended FA-donor Preservatives

FA levels detected in cosmetics containing FA-donor preservatives are shown in Fig. 1. These data are classified into five groups according to the blended FA-donor compounds. FA concentrations of samples containing IU, DM, DU, QU, and BP are ranged from 39.7 to 265, from 25.6 to 876, from 18.1 to 704, from 57.4 to 538, and from 2.70 to 57.4 mg/kg, respectively. Classified on the basis of the contained FA-donor preservatives, a statistically significant difference can be seen in the result of the one-way ANOVA ($p < 0.001$). Tukey-Kramer's test was performed for the multiple comparisons on blended donor preservatives. The results are shown in Fig. 1. The mean concentration ± standard error of FA in samples containing IU, DM, DU, QU, and BP are 132 ± 19.8, 316 ± 42.6, 325 ± 30.0, 284 ± 58.3, and 26.8 ± 9.93 mg/kg, respectively. Detected FA concentrations are significantly different between IU and DM ($p < 0.05$), IU and DU, DM and BP, and DU and BP ($p < 0.01$). The mean FA concentration of QU containing samples is about the same level of DU or DM-containing samples, but the statistical analysis does not reveal a significant difference from that of IU or BP blended samples. A reason for this is that the number of QU-containing samples is less than that of DU and DM-containing ones. These results show that the FA concentration of cosmetic samples depends on the type of FA-donor preservative in them.

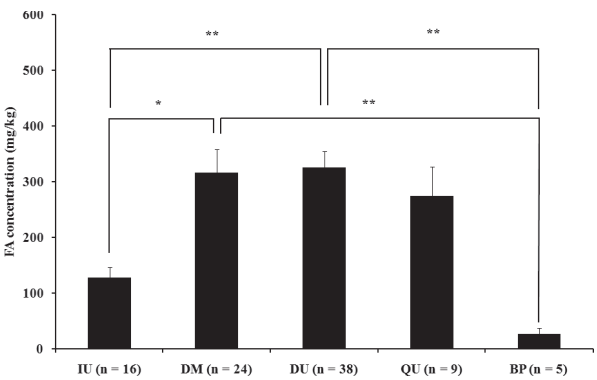


**Fig. 1.** Comparison of Detected FA Concentrations in Cosmetics Containing FA-donor Preservatives

The columns and the bars are the mean FA concentrations of each group and S. E. of the mean, respectively. Measured sample numbers of each group are shown below. The statistical difference was determined with Tukey-Kramer's test ($^{*}p < 0.05$, $^{**}p < 0.01$).

The release of FA and decomposition property of BP in buffers and homemade cosmetics were previously reported.[17] In that report, 20 mg/l FA was detected in a model lotion that contained BP (1 g/l) after incubation at 25°C for 30 days. The average FA concentration of BP blended cosmetics was 26.8 ± 9.93 mg/kg; this matched well with the result. The IU and DU concentrations (1 g/l) in the model lotion revealed the FA concentration to be approximately 100 mg/l and 250 mg/l after incubation at 25°C for 30 days (data not shown). The result of the analysis of homemade cosmetics was close to the average of the commercial cosmetics purchased. It is useful to investigate the decomposition properties of FA-donor preservatives in the model cosmetics for assuming the chemical property of FA-donor compounds in cosmetics.

### Difference of FA Concentration with Sample Type

To test the FA concentration dependency on the sample type (lotion, gel, conditioner, shampoo, body wash, and others), a one-way ANOVA was carried out. However, the result was not significant ($p = 0.61$). FA concentrations of lotions, gels, body washes, conditioners, shampoos, and others are ranged from 18.8 to 876, from 101 to 425, from 25.6 to 480, from 12.1 to 391, from 68.1 to 704, and from 2.70 to 538 mg/kg, respectively. The detected FA levels of each group are shown in Fig. 2. The mean concentration ± standard error of FA detected from lotions, gels, body washes, conditioners, shampoos, and others are 299 ± 47.0, 233 ± 45.8, 247 ± 36.2, 251 ± 36.2, 299 ± 43.7, and 117 ± 60.7 mg/kg, respectively. Free-FA levels of cosmetics, preserved with FA-donors, are believed to be independent of their sample types and are determined mainly with other factors such as blended

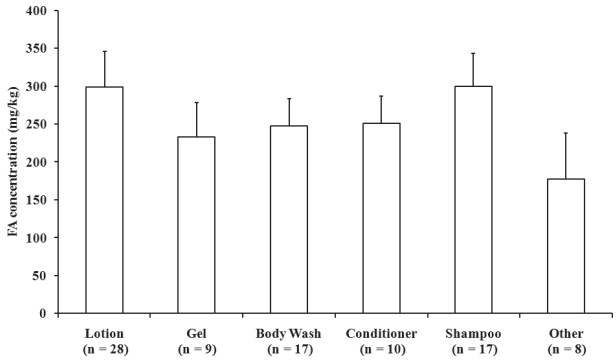


**Fig. 2.** Comparison of Detected FA Concentrations between Different Sample Types

The columns and the bars are the mean FA concentrations of each sample type and S. E. of the mean, respectively. Measured sample numbers of each type are shown below.

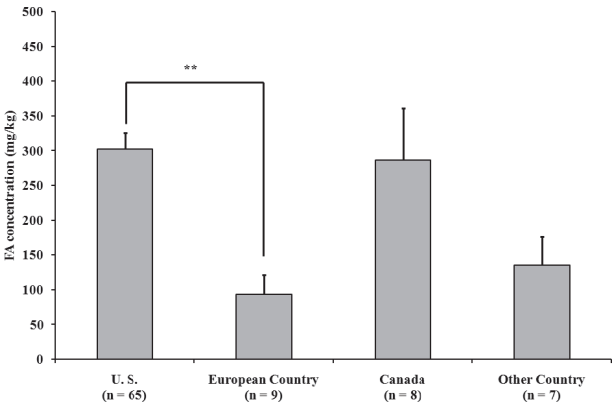


**Fig. 3.** Differences of Detected FA Concentrations in Cosmetics between Produced Countries

The columns and the bars are the mean FA concentrations of each group and S. E. of the mean, respectively. Measured sample numbers of each group are shown below. The statistical difference was determined with Tukey-Kramer's test ($^{**}p < 0.01$).

FA-donor preservatives, or blended concentration of FA-donors, *etc.*

## Difference of FA Concentration with Country of Manufacture

Comparison of FA concentration classified with the country of origin (U.S.A., European countries, Canada, and other countries) was shown in Fig. 3. FA concentrations of samples produced in U.S.A., European countries, Canada, and other countries are ranged from 12.1 to 876, from 2.7 to 261, from 18.8 to 654, and from 25.6 to 321 mg/kg, respectively. Classified on the basis of the country of manufacture, a statistically significant difference can be seen in the result of the one-way ANOVA ($p < 0.01$). Tukey-Kramer's test was performed for the multiple comparisons. The results are shown in Fig. 3. The mean concentration ± standard error of FA in samples produced in U.S.A., European countries, Canada, and other countries are 302 ± 23.2, 93.5 ± 27.8, 286 ± 75.1, and 135 ± 41.2 mg/kg, respectively. Detected FA concentrations are significantly different between the samples made in European countries and made in the U.S.A. ($p < 0.05$). In the EU countries, maximum concentrations of FA-donors are shown in the positive list of preservatives.[12] While in the U.S.A., there are no limitations for the concentration of FA-donor preservatives.[18] The difference of FA concentrations seems to be partially ascribed to the difference in legislation of FA-donor preservatives.

## FA Concentration of the Cosmetic Sample and its pH Value

The relationship between the pH value of the cosmetic and the detected FA concentration was examined by using Pearson's correlation coefficient test (Fig. 4). A statistically significant ($p < 0.05$) weak positive correlation was observed in samples containing DU and DM ($r = 0.396$ and $r = 0.426$, respectively). It is expected that the cosmetics blended with DU and DM show low free-FA levels when they are formulated at low pH values. Although the relationship between pH and free-FA concentration was reported previously on the basis of the pH dependence of decomposition in buffer solutions,[17] there have been no studies that have reported the relationship of free-FA concentration in the actually purchased cosmetics and their pH values. Further investigation on the activities of an FA-donor as a preservative is necessary, but this knowledge may be useful in developing strategies to reduce the occurrence of FA-derived contact dermatitis with cosmetics containing FA-donors.

On the other hand, no significant correlations are found between pH values and the detected FA levels in the cosmetics containing IU, QU, and BP. Insufficient sample number or the influence of other components may contribute to this result. Further investigation with considerably more samples or with another approach is necessary to conclude the relationship between pH values and the FA liberation from the FA-releaser in cosmetics.

The pH value of lotions, gels, body washes, conditioners, shampoos, and others are ranged from 4.1 to 9.0, from 5.2 to 7.2, from 4.6 to 7.6, from 4.7 to 7.7, from 3.6 to 5.8, and from 3.6 to 7.1, respectively. The median pH value in the measurement of

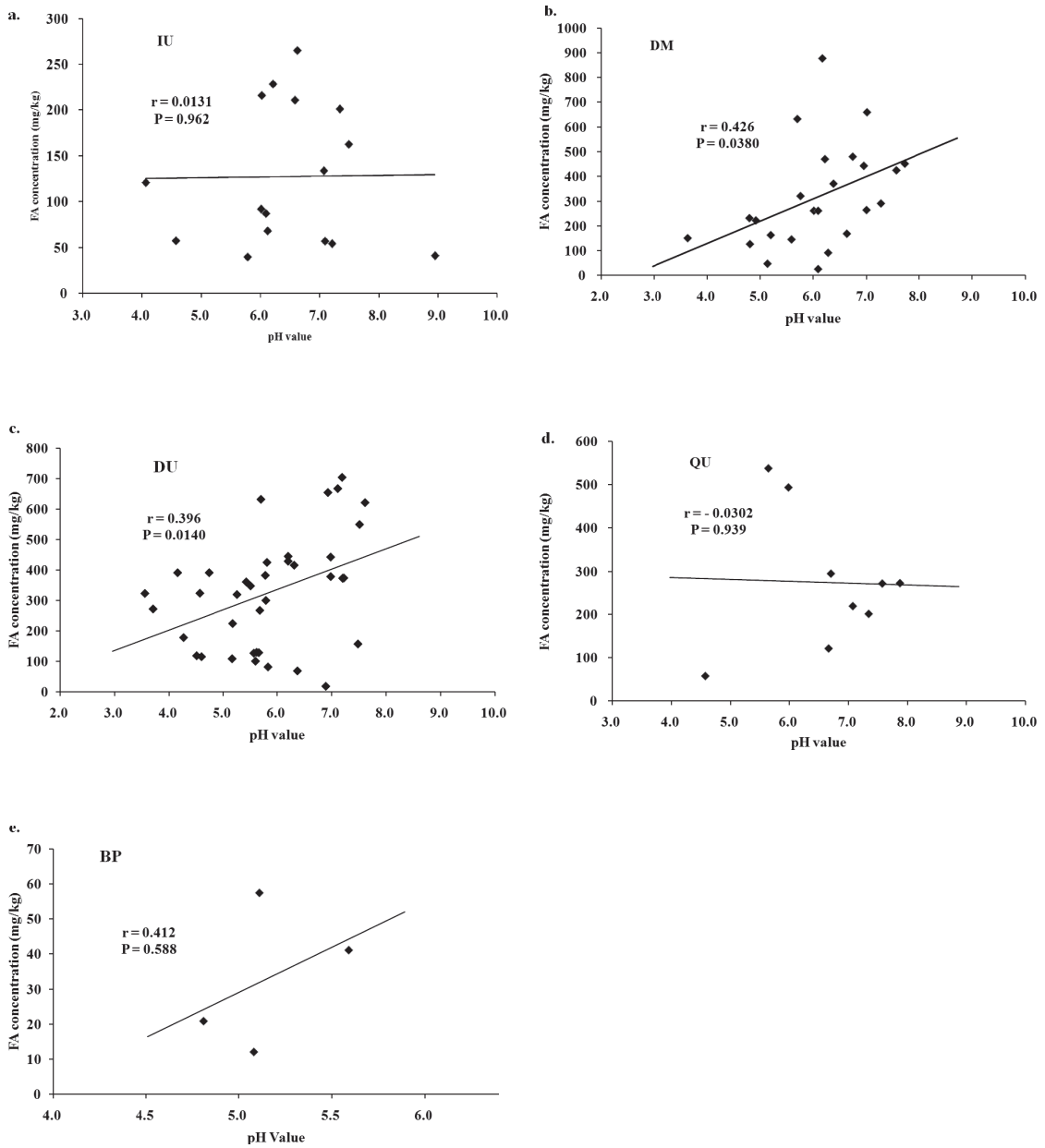


**Fig. 4.** Relationship between pH and FA Concentration of Cosmetic Samples Containing (a) IU, (b) DM, (c) DU, (d) QU, and (e) BP
The relationship between pH value and detected FA concentration was investigated using Pearson's correlation coefficient test ($p < 0.05$).

lotions, gels, body washes, conditioners, shampoos, and others are 6.8, 5.7, 6.4, 6.2, 4.5, and 5.6, respectively.

## Effect of Compounds with Amino Groups on FA Concentration

To study the effect of compounds reacting with FA on the free-FA concentration in cosmetics, a *t*-test (2-tailed) was performed if the samples were blended with any amines, amino acids, hydrolyzed proteins or not. In cosmetics that contained any amines, amides, or hydrolyzed proteins together with FA-donor preservatives, FA concentrations were significantly lower than in the others ($p < 0.01$, Fig. 5). Amines, amino acids, and hydrolyzed proteins may be regarded as "free-FA reducers" and lower the occurrence of FA-derived contact dermatitis with cosmetics containing FA-donor preservatives. It was previously reported that protein reduced the concentration of free FA in shampoos preserved with FA-donor preservatives.[19] Reaction of FA with amine, amino acids, and proteins was also studied in some researches.[20–22] In one of the reports on the reaction of FA with proteins, it was

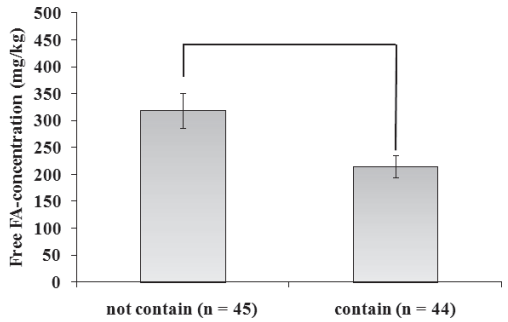


**Fig. 5.** Effect of Co-blended Amino Group Compounds on Free-FA Concentrations

The statistical difference was determined using Welch's $t$ test ($^{**}p < 0.01$).

shown that the reactions possibly occurred not with the primary amide and amino groups of proteins but with secondary amides.[21] Further research is necessary to know whether amines, amino acids, and hydrolyzed proteins can be used in day-to-day life.

Our analysis shows that most of the cosmetics blended with an FA-donor preservative act as a dermal sensitizer to FA-sensitive people. However, it is also expected that the low pH formulation or the blend with amines and amides may lead to a decrease in the free-FA concentration in cosmetics. Because of FA-donors' decomposition property, the dermal sensitivity of the decomposed product must be taken into consideration when we evaluate the FA-donor derived contact sensitivity in cosmetic samples. Several patch test studies were performed to elucidate the contact sensitivities of FA itself and of FA-releasers.[4, 23, 24] In this study, we measured the FA concentration of actually purchased cosmetics that contain FA-donor preservatives. In the future, studies on the decomposition properties of FA-donor compounds and contact sensitivities of decomposed FA-donors will be required to clarify dermal sensitivities caused by the FA-donors in cosmetics.

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