

The Release of Formaldehyde upon Decomposition of 2-Bromo-2-nitropropan-1,3-diol (Bronopol)

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We investigated the release of formaldehyde (FA) upon the decomposition of 2-bromo-2-nitropropan-1,3-diol (Bronopol: BP), an FA-releasing substance widely used as an antimicrobial preservative in cosmetics and toiletries in many Western countries. The decomposition process producing FA mainly depends on temperature and pH. Notably, the pH of the BP-diluted buffer solution was the factor most influencing the release of FA. The release was markedly in higher alkaline buffer compared with acidic buffer. When a BP solution [0.1% (w/v)] prepared with a weakly alkaline buffer (pH 8.0) was stored at 25°C, the concentration of FA reached 30 ppm after 24 hr. On the other hand, under acidic conditions (pH 2.0), little FA was produced over 50 days. Homemade cosmetics (lotions and jells) were made [with 0.1% (w/v) BP] and stored at a constant temperature of 25°C for 30 days. The concentration of FA increased with time, reaching 20 ppm after 50 days. We investigated seasonal variation in the release of FA upon decomposition of BP in Osaka. BP test solution [0.1% (w/v)] was prepared with 0.005 M sodium phosphate buffer (pH 6.0) and stored at ambient temperature for 30 days. The experiment was conducted three times in different seasons (mid winter, early spring, and early summer). It was found that the release of FA with decomposition of BP differed among the seasons.

Key words — 2-bromo-2-nitropropan-1,3-diol, formaldehyde, release, hydrogen ion concentration, temperature

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INTRODUCTION

Antibacterial preservatives are widely used in cosmetics to prevent spoilage due to microbial contamination. Several of these preservatives are known as formaldehyde (FA)-releasing compounds^{1–4} that release FA through decomposition or degradation. 2-Bromo-2-nitropropan-1,3-diol (bronopol: BP), one such substance, is active against a broad spectrum of bacteria, including Gram-negative species that are resistant to many antibacterial agents.^{5–7} In Japan, BP cannot be used in cosmetics; however, in the U.S.A. and many Western countries, this application is approved by law.⁸

In recent years, with the rapid development of information systems such as the World Wide Web, it has become easy to purchase cosmetics from overseas. In addition, Japanese traveling abroad have begun buying large amounts of cosmetics in other countries.⁹ BP, an FA-releasing substance, may be in these cosmetics. Special consideration is necessary about the safety of cosmetics, because they are used nearly constantly, and directly in contact with the skin. FA, the simplest aldehyde compound, is a causative agent of sick building syndrome,^{10,11} and a major public health concern. Many cases of systemic (*e.g.* anaphylaxis), and more often, localized (*e.g.* contact dermatitis) allergic reactions are attributed to FA.^{12–14} In addition, The International Agency for Research on Cancer (IARC, 2004) has classified FA in group 1 (carcinogenic to humans),¹⁵ based on limited evidence in humans, although the mechanism by which it induces tumors is not fully understood.

There have been some studies on the stability of BP in various conditions,^{16,17} but very little has been reported about the release of FA upon its decomposition. In the present study, we examined the effects of pH and temperature on the release of FA upon decomposition of BP.

MATERIALS AND METHODS

Preparation of Buffer Solutions — A 0.005 M sodium dihydrogen phosphate solution was prepared, and the pH adjusted to 2.0, 4.0, 6.0, and 8.0 with orthophosphoric acid or 0.005 M disodium hydrogen phosphate solution.

Preparation of Bronopol Test Solutions — BP (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan)

was diluted with the test buffer solution described above [0.1–0.01% (w/v)], and separated (25 ml) in a 50 ml polypropylene conical tube (Becton Dickinson, Franklin Lakes, NJ, U.S.A.) for the experiment. The effective concentration of BP in pharmaceutical preparations ranges from 0.01 to 0.1% (w/v).¹⁸⁾ All of the experiments were performed with shielding from light.

Determination of FA — The concentration of FA was determined according to the method of Fung and Groslean using formalin as the standard.^{19,20)} Briefly, 10 ml of 2,4-dinitrophenylhydrazine diluted in 2N H₃PO₄ [0.02% (w/v)] was added to 5 ml of test solution, and left standing at ambient temperature for 20 min. The mixture was extracted with 5 ml of ethyl acetate for 20 min, and the ethyl acetate layer diluted with acetonitrile to ×5 was subjected to HPLC. The chromatographic peak of the complex was confirmed by comparing its retention time and UV spectra. The concentrations were obtained by interpolation on standard curves. The formalin was standardized by the method of the Japanese Pharmacopoeia.²¹⁾ HPLC was carried out using a Shimadzu Prominence instrument equipped with a photodiode array detector. The determination wavelength was set at 355 nm. An Octadecyl silanized silicagel (ODS) column of L-column (5 μm, 4.6 mm I.D. × 150 mm, C.E.R.I., Saitama, Japan) was used. The mobile phase was acetonitrile: water (1 : 1) delivered at a flow rate of 1.0 ml/min. The column temperature was held at 30°C, and the volume of sample injected was 10 μl.

Determination of BP — The concentration of BP was determined according to the method of Wang with some modifications.¹⁶⁾ Briefly, each test solution was directly analyzed by HPLC with a photodiode array detector. BP standard solution was prepared with methanol. The chromatographic peak was confirmed by comparing its retention time and UV spectra. The concentration was obtained by interpolation on standard curves. The conditions for HPLC were as follows. The column used was a SUPELCOSIL LC-18 (5 μm, 4.6 × 250 mm), and was operated at 30°C. The mobile phase consisted of methanol: water: orthophosphoric acid (20 : 980 : 1) and the flow rate was 1.0 ml/min. The determination wavelength was 210 nm. The volume of sample injected was 10 μm. The HPLC system was as described above.

Preparations of Homemade Cosmetics (Lotion and Jelly) — The lotion was prepared as follows: 12.5 g of urea, 1.25 ml of glycerol, and 5 drops

of orange essential oil were dissolved in 450 ml of distilled water.²²⁾ The gel was prepared as follows: 2 g of xanthangum, 20 ml of glycerol, and 2 drops of 0.1% (w/v) hyaluronic acid were dissolved in 500 ml of distilled water.²²⁾ These homemade cosmetic formulations contained either 0.1 or 0.05% (w/v) BP.

Statistical Analysis — All data were subject to a one-way analysis of variance. A *p*-value < 0.05 was considered to indicate significant difference.

RESULTS

Effect of Temperature on Release of FA upon the Decomposition of BP

BP test solution [0.1% (w/v)] was prepared using a 0.005 M sodium phosphate buffer (pH 6.0), and incubated for 90 min in water baths of 25°C, 40°C, and 60°C. The release of FA upon the decomposition of BP was dependent on temperature (Fig. 1). The concentration of FA in the test buffer solution incubated at 60°C for 90 min reached about 50 ppm. A significant difference among the respective groups was recognized (*p* < 0.001).

Effect of Test Solution pH on Release of FA and Decomposition of BP

The pH of marketable cosmetics ranges from 2.0 to 8.0.²³⁾ BP test solutions [0.1 or 0.01% (w/v)] were prepared with several pH values (2.0, 4.0,

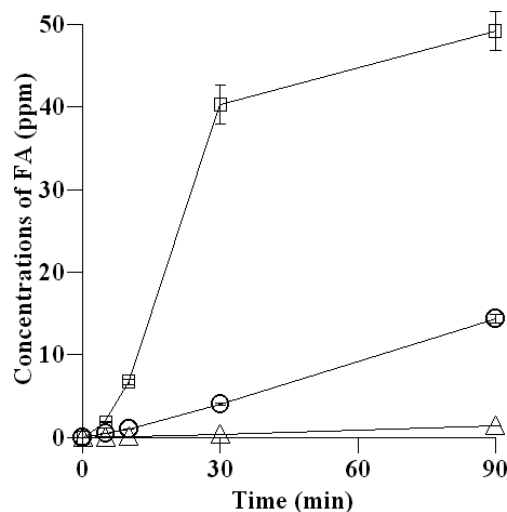


Fig. 1. Effect of Temperature on Release of FA upon Decomposition of BP

BP test solutions [0.1% (w/v)] were prepared with 0.005 M sodium phosphate buffer (pH 6.0). Each value represents the mean ± S.D. (*n* = 3). □; Incubated in a water bath at 60°C, ○; 40°C, △; 25°C.

6.0, and 8.0) of diluted 0.005 M sodium phosphate buffer, and stored in a constant temperature box at 25°C for 50 days. The release of FA upon the decomposition of BP was dependent on pH (Fig. 2). A marked FA-releasing effect was evident in alkaline buffer compared with the acidic buffer. When BP solution prepared with weakly alkaline buffer [pH 8.0, 0.1% (w/v)] was stored for 24 hr, the concentration of FA reached 30 ppm. In contrast, under acidic conditions [pH 2.0, with 0.1% (w/v) BP], little FA was produced over 50 days. A significant difference among the respective pH solutions was recognized ($p < 0.001$). The decomposition of BP in these solutions is shown in Fig. 3. The decomposition roughly corresponded to the release of FA.

Release of FA upon Decomposition of BP (Seasonal Variation)

We investigated seasonal variation in the

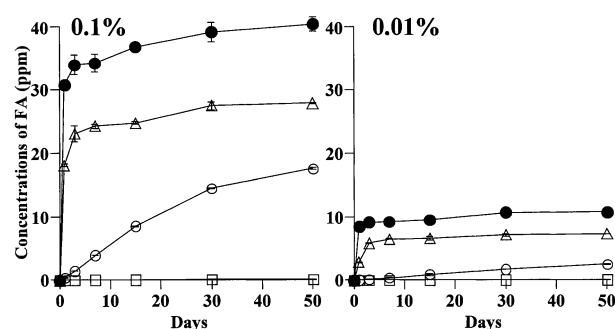


Fig. 2. Effect of pH on Release of FA upon Decomposition of BP

BP test solutions [0.1 or 0.01% (w/v)] were prepared with 0.005 M sodium phosphate buffer. Test solutions were stored in a constant-temperature box at 25°C. Each value represents the mean \pm S.D. ($n = 3$). ●, pH was adjusted to 8.0, Δ ; 6.0, \circ ; 4.0, \square ; 2.0.

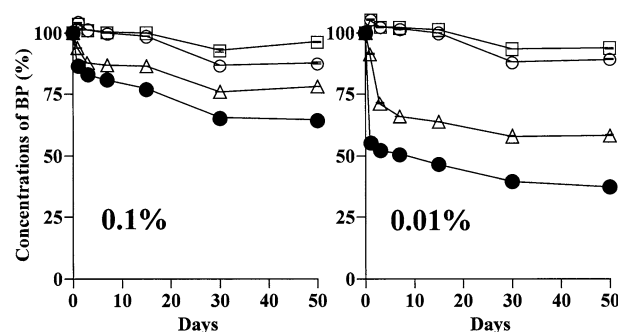


Fig. 3. Effect of pH on Stability of BP

BP test solutions [0.1 or 0.01% (w/v)] were prepared with a 0.005 M sodium phosphate buffer. Test solutions were stored in a constant-temperature box at 25°C. Each value represents mean \pm S.D. ($n = 3$). ●, pH was adjusted to 8.0, Δ ; 6.0, \circ ; 4.0, \square ; 2.0.

amount of FA released upon decomposition of BP in Osaka (Fig. 4). BP test solution [0.1% (w/v)] was prepared with a 0.005 M sodium phosphate buffer (pH 6.0), and stored at ambient temperature for 30 days. The experiment was conducted three times in different seasons (mid-winter, early spring, and early summer). It was found that release of FA upon decomposition of BP markedly differed among the seasons. A significant difference was recognized ($p < 0.001$).

Release of FA upon Decomposition of BP in Homemade Cosmetics (Lotion and Jell)

Homemade cosmetics (lotion and jell) were made [with 0.1 or 0.05% (w/v) BP], and stored at 25°C for 50 days. The concentration of FA in these cosmetics increased with time, and was 15 ppm in

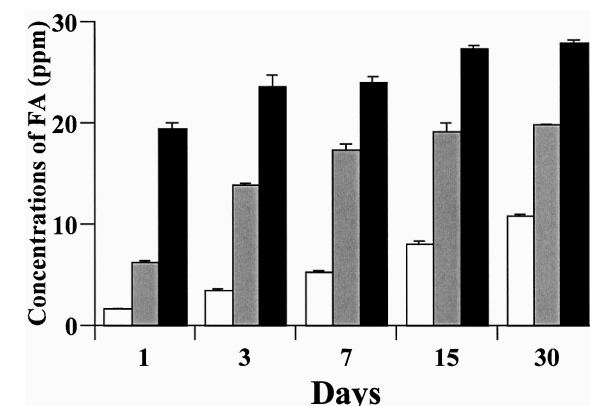


Fig. 4. Release of FA upon Decomposition of BP (Seasonal Variation)

BP test solutions [0.1% (w/v)] were prepared with 0.005 M sodium phosphate buffer (pH 6.0). Test solutions were stored at ambient temperature. Each value represents mean \pm S.D. ($n = 3$). \square ; Dec. 19, 2006–Jan. 18, 2007, \blacksquare ; Mar. 12, 2007–Apr. 11, 2007, \blacksquare ; May 14, 2007–Jun. 13, 2007.

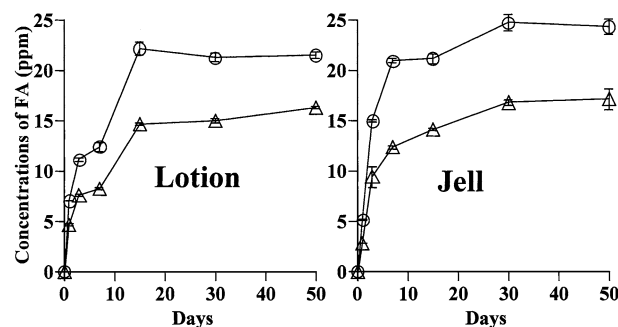


Fig. 5. Release of FA upon the Decomposition of BP in Homemade Cosmetics (Lotion and Jell)

Cosmetics were stored in a constant-temperature box at 25°C. Each value represents mean \pm S.D. ($n = 3$). \circ ; containing 0.1% (w/v) BP, Δ ; 0.05% (w/v).

all test samples after 50 days (Fig. 5).

The pH was either 6.37 (lotion) or 5.57 (jell). The dependency on pH as described above was not recognized in the homemade cosmetics.

DISCUSSION

There have been several studies on the stability or degradation of FA-releasing substances,^{16, 17)} but very little has been reported about the release of FA upon their decomposition. This paper is the first regarding the release of FA upon decomposition of BP.

There have been several observations of FA harming humans.^{24, 25)} Because FA is metabolized rapidly, harmful effects are observed primarily in those tissues or organs with which it first comes into contact. In a number of clinical studies, generally mild to moderate eye, nose, and throat irritation was experienced by volunteers exposed for short periods to levels of FA ranging from 0.25 to 3.0 ppm.^{26, 27)} Mucociliary clearance in the anterior portion of the nasal cavity was reduced following exposure to 0.25 ppm FA.²⁸⁾ In addition, an increased incidence of micronucleated buccal or nasal mucosal cells has been reported in individuals occupationally exposed to FA.²⁹⁾ Our results suggest that, when cosmetics containing BP are used, an amount of FA likely to be harmful may be released.

Bryce *et al.*¹⁶⁾ reported that the formation of bromonitroethanol from BP could take place via an irreversible retroaldol reaction with the liberation of an equimolar amount of FA. When BP solutions prepared with weakly alkaline (pH 8.0) or acidic (pH 6.0) buffer were stored at 25°C for 50 days, a sharp increase in the concentration of FA was observed from the 1st to 3rd day, and a gradual increase from the 3rd to 50th day. There was a corresponding decrease in the concentration of BP at these times. We surmised that a dynamic change in pH occurred from the 1st to 3rd day, and calculated the pH values of these solutions; however, no such change of pH was observed. Further studies are needed to clarify the details.

The decomposition process producing FA was dependent on temperature and pH. Notably, pH of BP-diluted solution was the factor most affecting the release of FA. Markedly higher release was evident in alkaline buffer compared with acidic buffer. Even when the alkaline buffer solution [pH 8.0, 0.1% (w/v)] was stored at 25°C, the concentration

of FA reached 30 ppm after 24 hr. On the other hand, under acidic conditions (pH 2.0), little FA was produced over 50 days.

Marked seasonal variation was found in the amount of FA released upon the decomposition of BP. When the BP test solution [0.1% (w/v)] was stored in mid-winter conditions in Osaka for 7 days, the concentration of FA was about 5 ppm. In contrast, in early summer, FA levels reached 25 ppm. We speculate that the difference in air temperature is the cause. These findings suggest that more FA was released in the mid-summer conditions.

Marked production of FA was observed for a brief period in the BP test solution prepared with 0.005 M sodium phosphate buffer (pH 6.0). In contrast, in the homemade cosmetics containing BP (pH of about 6.0), no marked FA production for a brief period was observed. Moreover, when BP solutions with various pH values prepared using diluted phosphate buffer were compared, the decomposition process producing FA was found completely dependent on pH. However, a similar dependency on pH was not observed in the homemade cosmetics containing BP. These results suggest that matrix compounds other than BP in the homemade cosmetics affected the release of FA upon decomposition of BP.

In the present study, all of the experiments were performed with shielding from light. Further investigations about the effect of light on release of FA are needed.

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