Liberation of Halide Ions from Xanthene Colors (Food Red Nos. 3, 104 and 105) by Photo-Irradiation

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Ion-chromatographic analyses were performed to quantitate the halide ions liberated by photo-irradiation from halogen-substituted xanthene colors (Food Red Nos. 3, 104 and 105). To elucidate the wavelength dependency of this phenomenon, aqueous solutions of the xanthene colors were photo-irradiated through various optical filters to provide selected transmission bands. The results suggest that the liberation of bromide and iodide from the xanthene moiety and chloride from the benzene moiety follows irradiation with visible light and UV light, respectively. It is known that solutions of these xanthene colors produce singlet oxygens *via* phtoactivation of the dye molecule and transfer of the energy to dissolved oxygen. Therefore, the liberation was also studied in the presence of the substances which alter the life-time of singlet oxygen. The results suggest that singlet oxygen has some effect on the liberation of halide ions from these food colors.

Keywords xanthene color; tar dye; halide ion; singlet oxygen; photochemical reaction; ion chromatography

In Japan, 12 synthetic colors (tar dyes in Japan) are presently permitted as food colors. Among them, Food Red No. 3 (R3, C.I. 45430, erythrosine, FD & C Red No. 3), No. 104 (R104, C.I. 45410, phloxine) and No. 105 (R105, C.I. 45440, rose bengal) are classified as xanthene colors with halogen substituents as shown by their chemical structures given in Fig. 1. In the course of our studies to determine inorganic halides[1)] by ion chromatography (IC) in commercial xanthene colors, we observed an increase in iodide levels in aqueous solutions of R3 and R105,[1b)] and an increase in the bromide level in solutions of R104[1c)] during strage under ordinary laboratory illumination. It was postulated that the increase in inorganic iodide and bromide in the aqueous solutions is due to the photochemical liberation of halogen atoms from the xanthene colors.

It is well-known that xanthene colors with halogen substituents are responsible for various photochemical reactions. In aqueous solution they act as a photochemical source of singlet oxygen, which is formed from the reaction of the color molecule, in the excited triplet state, with dissolved oxygen.[2)] It has been reported that xanthene colors may inhibit several enzymes *in vitro* through photochemical reactions.[3)] As reported by our own laboratory, the enzyme-inhibitory effects of the xanthene colors with halogen substituents are the most potent of the 31 synthetic colors permitted for use in foods and cosmetics.[4)]

In the field of food chemistry, the fading of halogenated xanthene colors in daylight has been reported by several authors. Kurayuki *et al.*[5)] studied the fading of aqueous solutions of R3 and some other halogenated xanthene colors under daylight and reported some organic and inorganic decomposition products. Umezawa *et al.*[6)] reported the time-dependent degradation of R3 and R104 in aqueous solution by ultraviolet light and daylight. However, the liberation of halide ions from xanthene colors by photo-irradiation has not been studied using modern chromatographic techniques. Therefore, we decided to investigate the liberation with IC, to learn more about the mechanism of the carbon–halogen bond scission.

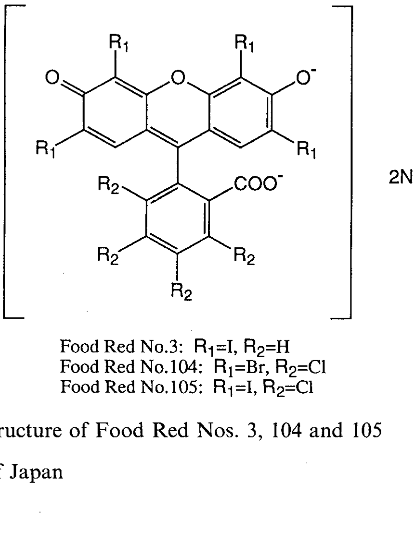
Materials and Methods

Reagents R3, R104 and R105 used in this study were standard products distributed by the NIHS. Deuterium oxide (D_2O, 99.75%) was purchased from Merck. Sodium azide (NaN_3), phthalic acid and tris(hydroxymethyl)aminomethane were purchased from Wako Pure Chemical Industries. A standard solution of sodium chloride (0.1 N), reagent grade sodium iodide (99.9%) and sodium bromide (99.9%), were purchased from Wako Pure Chemical Industries. Highly purified water obtained by a Milli-Q system (Millipore) was used for all analyses.

IC Conditions IC was conducted on a Shim-pack IC-A1 column with a guard column of Shim-pack IC-GA1 using a Shimadzu HIC-6A IC system equipped with a Shimadzu CDD-6A conductivity detector. The columns were maintained at 40 °C. The IC solvent was an aqueous solution (pH 4.0) containing phthalic acid (2.5 mM) and tris(hydroxymethyl)aminomethane (2.4 mM) at a flow rate of 1.5 ml/min. The peak areas of the detected halide ions were calculated using a Shimadzu C-R4AD Chromatopac integrator.

Preparation of Sample Solutions Each xanthene color (about 10 mg) was dissolved in highly purified water, D_2O or aqueous NaN_3 solution (0.1% or 0.0033%) to give a concentration of 1 mg/ml in a Pyrex or quartz test-tube (20 ml). In several experiments oxygen or nitrogen gas was bubbled into the sample solution at the rate of 500 ml/min for 30 s. Each test-tube was then stoppered during irradiation. Just before and after irradiation, 20 μl of each sample solution was subjected to IC to quantify the halide ions.

Irradiation Photo-irradiation of the sample solutions was performed in a Riko rotary photochemical reactor (RH440-10W-400HAP) equipped



Food Red No.3: R_1=I, R_2=H
Food Red No.104: R_1=Br, R_2=Cl
Food Red No.105: R_1=I, R_2=Cl

Fig. 1. Structure of Food Red Nos. 3, 104 and 105

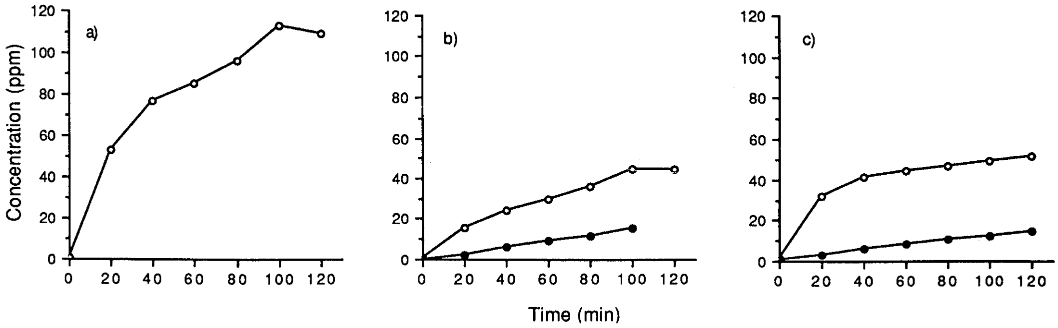


Fig. 2. Halide Ions Liberated from Food Colors under Illumination

○, iodide or bomide; ●, chloride. a) Food Red No. 3, b) Food Red No. 104, c) Food Red No. 105. Pyrex test-tubes were used. The results were obtained without the steel-wire screen. Iodide ions of 10 ppm in the solutions of Food Red Nos. 3 and 105 correspond to 1.8 and 2.0% of the total iodine bonded to the respective food colors. Chloride ions of 10 ppm in the solutions of Food Red Nos. 104 and 105 correspond to 5.8 and 7.1% of the total chloridne bonded to the respective food colors. Bromide ion of 10 ppm in the solution of Food Red No. 104 corresponds to 2.6% of the total bromine bonded to the respective food color.

TABLE I. Effects of Color Filters on the Liberation of Halide Ions

Filter			Food Red No. 3	Food Red No. 104		Food Red No. 105	
CS No.[a)]	TS band[b)]	Class	I$^-$	Cl$^-$	Br$^-$	Cl$^-$	I$^-$
None			34.65	2.70	16.42	2.52	25.03
2-58	>640	Sharp-cut red	0.37	0.01	0.51	0.06	0.35
3-70	>500	Sharp-cut yellow	17.38	0.31	5.81	0.04	22.76
3-73	>430	Sharp-cut yellow	26.33	0.34	14.66	0.11	24.95
5-58	390—430	Blue	1.75	0.20	1.85	0.07	1.69
7-54	250—395	UV transmission	5.18	1.99	5.35	1.77	9.05

Quartz test-tube were used. Each figure indicates the concentration (ppm) after irradiation for 30 min through a specified color filter and a steel-wire screen mentioned in Materials and Methods. *a)* Color specification number. *b)* Transmission band: the band which permits more than 30% transmission. The maximum transmittance of the 2-58, 3-70, 3-73, 5-58 and 7-54 filters is about 90, 90, 90, 40 and 85%, respectively.

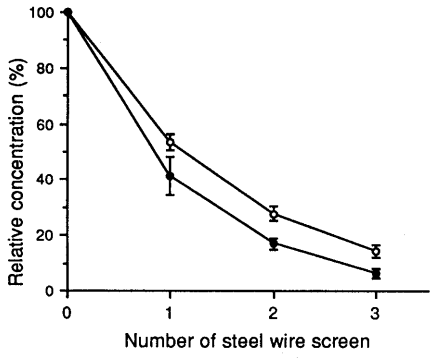


Fig. 3. Relationship between the Concentration of Halide Ions Liberated from Food Red No. 105 by Irradiation and the Number of Steel-Wire Screens

○, iodide; ●, chloride. Each point is the mean of four experiments ± standard deviation. Pyrex test-tubes were used. Irradiation was performed for 30 min.

with a 400 W high pressure mercury-vapor lamp. The principal wavelengths in the line spectrum of the lamp are 238, 250, 278, 295, 313, 366, 405, 436, 546, 578 and 1020 nm and the relative intensities of the corresponding wavelengths to the intensity at 366 nm are 15, 23, 15, 33, 65, 100, 30, 50, 68, 80 and 43%, respectively. The reactor was maintained at 25 °C. The test-tubes remained stationary in the reactor unless otherwise noted. In the experiments, the test-tubes were automatically rotated around the lamp. In most experiments, a number of steel-wire screens (100 mesh, 149 μm) were placed between the lamp and the test-tubes to attenuate the light intensity. One steel-wire screen reduces the light intensity to 48 ± 2% by lux. The number of steel-wire screens used in each experiment is specified in the table and figures. During the experiment investigating wavelength-dependency, a color filter obtained from Corning was placed between the steel-wire screen and the test-tube. The color specification numbers of the filters used in this study were 2-58, 3-70, 3-73, 5-58 and 7-54. Each experiment was repeated several times, and representative data are shown in the figures for the time-course.

Results

Irradiation Time The time-dependency of the liberation of halide ions from R3, R104 and R105 was studied. As shown in Fig. 2, the halide ion concentrations in aqueous solutions of the xanthene colors increased with irradiation time. It is noteworthy that iodide or bromide increased asymptotically to reach a plateau, but chloride increased linearly over the experimental period. No increase was observed under dark conditions even after 120 min (data not drawn).

Irradiation Intensity The R105 solution was irradiated through various numbers of steel-wire screens to alter the irradiation intensity. As shown in Fig. 3, increasing the number of the steel-wire screens led to a significant decrease in both iodide and chloride ions. However, it seems that chloride release is more sensitive to light attenuation by steel screens than iodide.

Wavelength-Dependency Five color filters, each of which had a specified transmission band, were used to select wavelength bands from the lamp. Table I shows the halide concentrations in the xanthene solutions after photo-irradiation through a color filter. The filter with the color specification number 2-58, which is classified as a sharp-cut red filter and cuts off wavelength bands shorter than 640 nm, prevented halogen liberation almost completely. The color filters 3-70 and 3-73, both of which

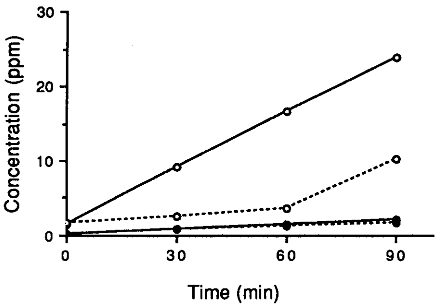


Fig. 4. Effect of Aeration on Concentration of Halide Ions Liberated from Food Red No. 105

○, iodide; ●, chloride. Pyrex test-tubes were used. Aeration with oxygen gas (—○—, —●—) or nitrogen gas (---○---, ---●---) was performed twice at 0 and 30 min. Then, additional aeration with oxygen gas was performed on all solutions at 60 min. Irradiation was performed through two steel-wire screens.

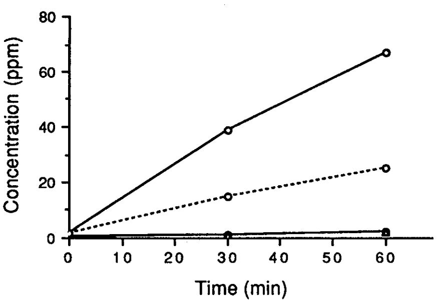


Fig. 5. Effect of Deuterium Oxide on the Concentration of Halide Ions Liberated from Food Red No. 105

—○—, iodide in D_2O; ---○---, iodide in H_2O; —△—, chloride in D_2O; ---●---, chloride in H_2O. Aeration with oxygen gas was performed at 0 and 30 min. Pyrex test-tubes and two steel-wire screens were used.

are classified as sharp-cut yellow filters, inhibited chloride liberation by more than 87%. The former, which cuts off light of wavelength shorter than 500 nm, inhibited both the liberation of iodide and bromide, while the latter, which cuts off light of wavelength shorter than 430 nm, inhibited only the liberation of iodide. The 5-58 filter, which is classified as a blue filter and transmits wavelengths from 390 nm to 430 nm, inhibited liberation of all halides by about 90%. The 7-54 filter, which is classified as a UV transmission filter, exhibited about 30% inhibition for chloride and about 70% inhibition for iodide and bromide.

Effects of Nitrogen and Oxygen The R105 solution was photo-irradiated after bubbling nitrogen or oxygen into the solution for 30 s at 30 min intervals. As shown in Fig. 4, the concentration of iodide drastically increased in the oxygenated solutions (increment: 15.0 ppm per 60 min), but increased only slightly after bubbling with nitrogen (increment: 1.9 ppm per 60 min). In contrast, little difference could be detected in the liberation of chloride in the presence of nitrogen or oxygen (increased by 1.1 and 0.9 ppm per 60 min in the oxygenated and nitrogen-aerated solutions, respectively). In order to confirm the effect of oxygenation, oxygen gas was additionally bubbled for 30 s at 60 min into the solution which had been previously purged with nitrogen gas. After bubbling oxygen through the solution, the rate of iodide liberation increased and the slope (7.4 ppm per 30 min) was almost equal to that of the solution which had been initially saturated with oxygen. (see the open circle connected to dashed line at 60 min in Fig. 4).

Effect of Singlet Oxygen The life-time of singlet oxygen is closely related to the concentration of singlet oxygen. It is well-known that the life-time of singlet oxygen is longer in D_2O[7] and shorter in aqueous NaN_3 solution,[8] than that in hydrogen oxide (H_2O). Therefore, R105 was dissolved in both solutions and photo-irradiated in order to study the effect of singlet oxygen on the liberation of halide ions.

Figures 5 and 6 show the effects of D_2O and NaN_3 on the liberation of halide ions, respectively. The concentration of iodide in D_2O is about 2.5 times higher than that in H_2O after irradiation for 30 and 60 min. On the other hand, the concentration of chloride in D_2O did not differ significantly from that in H_2O at any time. In NaN_3 solution, the concentration of liberated iodide was inversely proportional to the NaN_3 concentration. Furthermore, when nitrogen instead of oxygen, was bubbled into 0.1% NaN_3 solution to further reduce the concentration of singlet oxygen, the concentration of iodide did not differ significantly from the initial value, even after 60 min-irradiation.

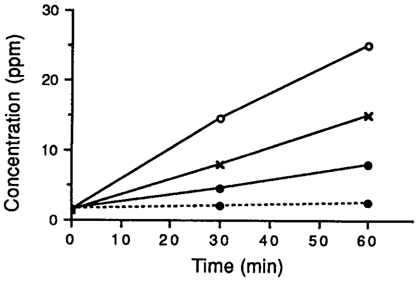


Fig. 6. Effect of Sodium Azide on the Concentration of Iodide Ions Liberated from Food Red No. 105

Pyrex test-tubes were used. The results were obtained in H_2O (—○—), 0.1% aqueous NaN_3 solution (—●—) or 0.003% aqueous NaN_3 solution (—×—) with aeration with oxygen gas at 0 and 30 min or in 0.1% aqueous NaN_3 solution with aeration with nitrogen gas (---●---) at 0 and 30 min. Irradiation was performed through two steel-wire screents.

Discussion

In our earlier studies[1b,c] performed under indoor light, we observed an increase in iodide and bromide in R105 and R104 solutions, respectively, and both of these ions should have come from the xanthene moiety of R105 and R104. Under the same conditions, however, no increase was observed in chloride, which would require cleavage of a chlorine from the benzene moiety. On the other hand, photo-irradiation of the xanthene color solutions with a high pressure mercury-vapor lamp resulted in the cleavage of the halogens bonded to both ring systems.

The amount of halides liberated depends on the time and intensity of irradiation (Figs. 2 and 3). Furthermore, detailed observations revealed that the time-course of the liberation of iodide or bromide is apparently different from that of chloride. In other words, iodide or bromide increases asymptotically to reach a plateau with the irradiation time, while chloride increases linearly. Since more than 85% of the total iodine (or bromine) is still present in the residual R105 (or R104), even after

120 min-irradiation and the amount of iodine (or bromine) in the residual R105 (or R104) is almost the same as that of chlorine in molar terms, it seems unlikely that the asymptotic increase in iodide or bromide is due to the decrease in remaining R105 (or R104) as the source of inorganic halide. Therefore, the above-mentioned findings lead to the conclusion that the mechanism of liberation of halogen atoms is quite different for the xanthene and benzene moieties. The fact that the increase in the number of steel-wire screens affected the amount of chloride liberated more than that of iodide (Fig. 3) also supports this conclusion. These differences in the mechanism of liberation are confirmed by studies of the irradiation wavelength (Table I). The liberation of chloride from the benzene moiety requires irradiation with UV light, whereas halide cleavage from the xanthene moiety proceeds on irradiation with light from the visible region of the spectrum.

The wavelength-dependency of the liberation of halide clarifies the apparent discrepancy between the results of our previous studies[1b,c] and those obtained in this study. In the former studies, aqueous solutions of food colors were not kept under UV light, while the high pressure mercury-vapor lamp used in this study, produced wavelengths in the UV region. Therefore, liberation of chloride was observed in this study.

The 3-70 color filter, which transmits light of 500 nm and above, does not promote liberation of bromide, while the 3-73 color filter which transmits at 430 nm and above does. Therefore, the liberation of bromide specifically needs irradiation by light of wavelengths from 430 nm to 500 nm. Since both of these filters do not block the photochemical liberation of iodide, the liberation of iodide and bromide require different wavelengths.

Studies on purging the solutions with nitrogen or oxygen further confirmed the fundamental difference between the liberation of halides from xanthene and benzene moieties. As shown in Fig. 4, purging with oxygen does affect halide cleavage from the xanthene portion of the molecule, but not from the phenyl group. This finding strongly suggests that dissolved oxygen is involved in the liberation of halide from the xanthene moiety.

It is well-known that xanthene colors possess photodynamic properties.[2] For example, singlet oxygen is generated from dissolved oxygen when the xanthene colors absorb visible light. Therefore, we investigated the relationship between singlet oxygen and the liberation of halides from the xanthene moiety. As shown in Figs. 5 and 6, the effects of D_2O and NaN_3 on the cleavage of iodine from R 105 are exactly what one would expect if singlet oxygen is involved. On the other hand, the presence or absence of D_2O and NaN_3 had no affect on the amount of liberated chloride; therefore, it is unlikely that singlet oxygen is involved. The amount of singlet oxygen is limited by the amount of dissolved oxygen and, therefore, the asymptotic increase in liberated iodide or bromide in Fig. 2 may be due to the limited amount of oxygen available.

The photo-excited state of the dye molecule is not involved in the cleavage of the carbon–halogen bonds since oxygen is a well-known quencher of excited molecules and photolysis of oxygen-saturated solutions results in a greater degree of halide liberation than photolysis of de-oxygenated solutions.

We can conclude from the findings described above that the liberation of halide ions from the xanthene and benzene moieties in xanthene colors arises from the irradiation with visible and UV light, respectively. Also, the liberation from the xanthene moiety involves singlet oxygen which is formed from the photochemical reaction of the xanthene molecule and the dissolved oxygen.

From the mechanistic point of view, it seems to be of interest to study in more detail the liberation of iodide (or bromide) and chloride from the benzene and xanthene moieties, respectively. However, these experiments are difficult because these derivatives are not commercially available.

Xanthene colors are generally administered to test animals as aqueous solutions to study the biological effects of these substances. Since iodide and bromide ions are biologically active, care should be taken to shield the test material from light to prevent the photochemical degradation of the xanthene colors.

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