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HPLC INVESTIGATION OF THE DEGRADATION OF SOME ARTIFICIAL AZO FOOD COLORANTS IN THE PRESENCE OF ASCORBIC ACID

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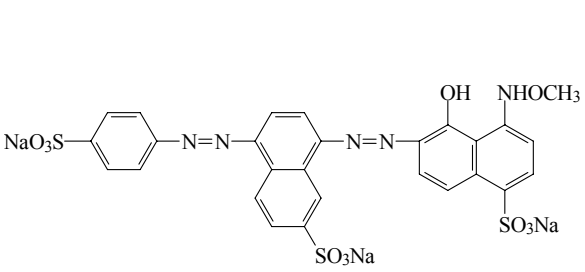
A b s t r a c t: The influence of the concentration of ascorbic acid on some azo food colorant (Brilliant Black BN, Brilliant Blue FCF, Chinoline Yellow, Indigotine, Patent Blue V and Tartazine) degradation in the solution has been studied. Rate constant (k), time of colorant half-life ($t_{1/2}$), as well as the type of the kinetic reaction have been determined. The colorants and the ascorbic acid have been determined by high performance liquid chromatography (HPLC) with diode array detector (DAD) and UV-VIS spectrometry. It was found that the higher concentration of ascorbic acid leads to the higher rate constant and lower colorants half-life. The values of the rate constants are 2.50–4.25 times higher when the colorant degradation was run in the presence of 500 mg L^{-1} of ascorbic acid than in the case of the presence of 100 mg L^{-1} ascorbic acid for Brilliant Blue FCF, Chinoline Yellow, Patent Blue V and Tartazine. From the other side, the degradation of Brilliant Black BN and Indigotine is very fast with a total degradation for 6–9 days. Therefore, the rate constants for those two food colorants are much higher than for the other investigated colorants. According to the linearity of the kinetics curves ($\ln(c/c_o)$ v.s. time) it appeared to be first order kinetics for the interval up to 4 days.

Key words: Food Colorants; Brilliant Black BN; Brilliant Blue FCF; Chinoline Yellow; Indigotine; Patent Blue V; Tartazine; ascorbic acid; degradation; kinetics rate constant; HPLC; UV-VIS spectrometry

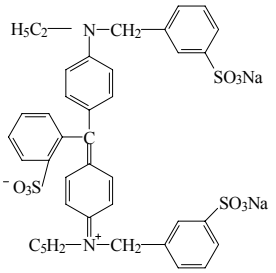
1. INTRODUCTION

The artificial food colorants are widely used in food industry, for coloring foodstuff matter, but in fact they have no any nutritional value. The use of particular food colorant in food industry depends primarily of its toxicity. However, at the same time, of major importance are also some other factors such as stability of the colorant used for coloring certain food product and whether there is a possible interaction between some component of the foodstuff matter and the colorant. There are several papers concern on the stability of some of the investigated colorants using different reducing agents or factors: by inorganic oxysulfur compounds [1], pH [2], microflora [3] or temperature [4]. Some reducing agents present in the food may react especially with azo dyes forming degradation products, which may react further with other food components forming undesirable compounds [5–13]. One of the agents, which can react with these dyes causing their degradation, is ascorbic acid [14–17]. The ascorbic acid is often used in the soft drinks as a nutritional supplement or as an antioxidant and provides a reducing environment in which azo dyes can easily be degraded. In real food, the sucrose, known as a stabilizer of ascorbic acid [14], is also present. These facts make the field of investigation of degradation of colorants used in food industry very actual.

In our previous paper [17] we presented data about degradation investigations of artificial food colorants with naphtalenic structure (Ponceau 4R, Amaranth, Allura Red, Brilliant Black BN, Carmoisine and Sunset Yellow) in the presence of ascorbic acid. In the present study, the influence of the concentration of ascorbic acid on the stability of some other azo dyes (Brilliant Blue BN, Brilliant Blue FCF, Chinoline Yellow, Indigotine, Patent Blue V, Tartazine), Fig. 1, has been presented. Rate constant (k), time of colorant half-life ($t_{1/2}$), as well as the type of the kinetic reaction have been determined. At the same time, degradation of ascorbic acid in the solutions has been also followed.



Brilliant Blue BN



Brilliant Blue FCF

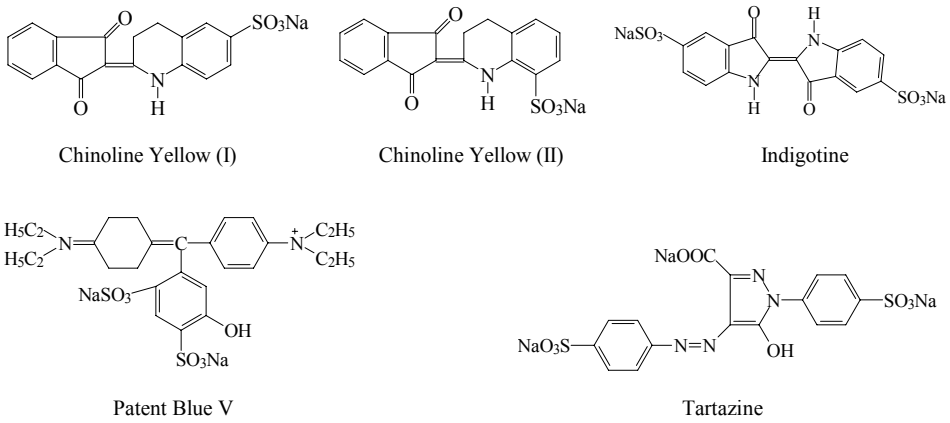


Fig. 1. Structural formulas of the investigated food colorants

2. EXPERIMENTAL

Instrumental

HPLC system Perkin-Elmer (Perkin-Elmer, USA) equipped with Diode Array Detector 235 C, and binary pump series 200 have been used.

UV-VIS Spectrophotometer, model Milton Roy (Milton Roy, France).

Liquid chromatography column was used. Stainless steel 10 cm long and 4.6 mm inside diameter, packed with 5 μm Pecosphere C18, was used (Merck, Germany).

Reagents

Mobile phase was composed of solvent A (0.01 mol L^{-1} KH_2PO_4 adjusted to pH 4.5 with *o*-phosphoric acid) and solvent B (methanol HPLC grade, Merck, Germany).

Acetate buffer (pH = 5.5) was prepared by dissolving 6.8 g of sodium acetate, adjusted with acetic acid in 1 L.

Reference food colorant standards were obtained from Etol (Slovenia) and Wurth (Austria).

Methods

The model solutions containing investigated dyes were made in acetate buffer (pH = 5.5). Concentration of added dyes in model solutions was 40 mg L^{-1}, and concentrations of the ascorbic acid were 100, 250 and 500 mg L^{-1}. The dissolved oxygen was removed from solutions by purging of helium.

The solutions were kept in dark at room temperature. The degree of degradation of ascorbic acid and food colorants was monitored within 4 weeks. The rate of ascorbic acid degradation was monitored by HPLC, and the rate of food colorant degradation was monitored simultaneously by HPLC and by visible spectrometry [18].

Gradient profile used for all colorants (except eritrozine) was as follows: 3 minutes 100 % A, 0 – 100 % B in 4 min, held at 100 % B for 8 min, returned to 100 % A and held 10 min before next injection. Gradient profile for eritrozine was: 2 minutes 100 % A, 0 – 100 % B in 1 min, 30–50 % B for 1 min, 70–100 % B for 1 min, held at 100 % B for 9 min, returned to 100 % A and held 10 min before next injection.

HPLC determination of ascorbic acid was performed at 255 nm. Wavelengths for HPLC and spectrometric determination of artificial food colorants are given in Table 1.

T a b l e 1

Wavelengths for determinations of some synthetic food colorants

Synthetic food colorant	EEC serial No	Coloring Index No	*Chemical Abstracts* No	Wavelength, nm	
				HPLC	Vis spectrophotometry
Brilliant Black BN	E 151	28440	2519-30-4	220	568
Brilliant Blue FCF	E 133	42090	2650-18-2	220	630
Chinoline Yellow	E 104	47005	8004-92-0	245	414
Indigotine	E 132	73015	860-22-0	245	615
Patent Blue V	E 131	42051	129-17-9	210	638
Tartazine	E 102	19140	1934-21-0	255	427

3. RESULTS AND DISCUSSION

In our previous investigation [17] we found that the presence of ascorbic acid influences the degradation of the food colorants with naphtalenic structure (Ponceau 4R, Amaranth, Allura Red, Carmoisine and Sunset Yellow). In this paper, the influence of the ascorbic acid on the degradation of other azo food colorant (Brilliant Black BN, Brilliant Blue FCF, Chinoline Yellow, Indigotine, Patent Blue V, Tartazine) was studied.

The rate of degradation of the investigated food colorants was studied in the presence of ascorbic acid with different concentration (100, 250 and 500 mg L^{-1}). The model solutions were previously preserved by adding of potassium sorbate (48 mg L^{-1}). The concentration of all added dyes was 40 mg L^{-1}, because this concentration appeared to be most likely used in the preparation of soft drinks. The ascorbic acid is very rapidly oxidized by dissolved molecular oxygen in the model solutions. In order to decrease the degradation of ascorbic acid oxygen was removed from the solution by purged the samples by helium instead nitrogen proposed by other authors [19]. The colorants and the ascorbic acid have been determined by high performance liquid chromatography (HPLC) with diode array detector (DAD) and UV-VIS spectrometry.

From the obtained results (Fig. 2, Table 2) it can be seen that the rate of degradation of the investigated colorants increases with the concentration of the ascorbic acid. Namely, when the degradation is followed in the presence of 100 mg L^{-1} ascorbic acid, after 21 days more than 85 % of colorants remains in solution (from 86.5 % for tartazine to 93.8 % for brilliant blue FCF). In the presence of 250 mg L^{-1} ascorbic acid, after 28 days nondegradated colorants are present from 70.5 % for tartazine to 79.2 % for chinoline yellow and brilliant blue. In the model solution with 500 mg L^{-1} ascorbic acid, the corresponding value ranges from 61.2 % for tartazine to 74.5 % for chinoline yellow. The data for the rate constants are similar to the azo dyes with one naphthalene structure [17]. However, the presence of ascorbic acid increases significantly degradation rate of Brilliant Black BN and Indigotine (Fig. 2). For example, the rate constant for Brilliant Black BN and Indigotine in the presence of 500 mg L^{-1} of ascorbic acid is 86 or 44 times higher than that for chinoline yellow, respectively (Table 2).

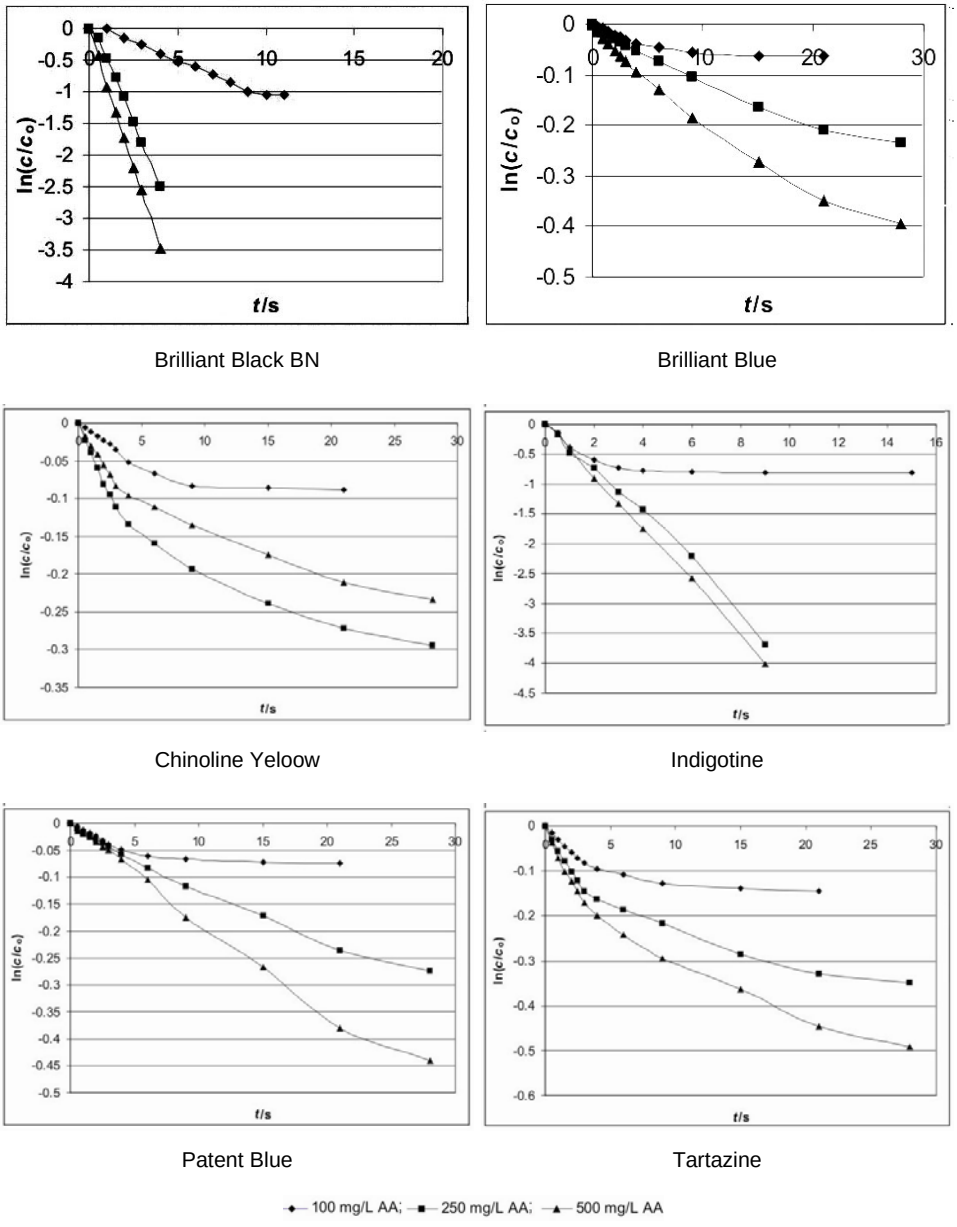


Fig. 2. Dependence $\ln(c/c_o)$ vs. time for the degradation of the investigated food colorants in the presence of 100 mg/L, 250 mg/L and 500 mg/L of ascorbic acid (AA)

According to the results for the degradation of investigated food colorants in the presence of various concentrations of ascorbic acid vs. time, rate constant (k), time of the colorants half life ($t_{1/2}$) and correlation coefficient (r) have been determined (Table 2). The values of the rate constants were calculated from the slopes of the straight lines obtained by plotting $\ln\gamma$ vs. time. This dependence has the form of the linear equation ($\ln\gamma = -kt + \ln\gamma_o$) which shows that the plot of $\ln\gamma$ versus t yields a straight line, so the reactions are first order [20].

T a b l e 2

Kinetics of degradation of the synthetic food colorants in the presence of ascorbic acid

Food coloranț	γ (ascorbic acid)/mg L^{-1}	k/d^{-1}	$t_{1/2}$/d	r
Brilliant Black BN	100	0.065	10.7	–0.998*
	250	0.64	1.1	–0.999
	500	0.86	0.8	–0.999
Brilliant Blue FCF	100	0.004	157.5	–0.992**
	250	0.009	78.5	–0.998
	500	0.015	47.1	–0.999
Chinoline Yellow	100	0.004	156.3	–0.994**
	250	0.008	87.2	–0.995
	500	0.010	68.7	–0.999
Indigotine	100	0.038	18.4	–0.996***
	250	0.340	2.0	–0.994
	500	0.440	1.6	–0.992
Pattent Blue V	100	0.004	159.0	–0.993**
	250	0.010	68.9	–0.997
	500	0.017	41.7	–0.998
Tartazine	100	0.006	109.3	–0.993**
	250	0.012	58.9	–0.996
	500	0.017	41.7	–0.998

* – Values for r are for interval for 4 days
** – Values for r are for interval for 3 days
*** – Values for r are for interval from 0 to 24 hours

As it can be seen from these results, the rate constant (k) and half-life ($t_{1/2}$) are higher by increasing the initial concentration of ascorbic acid in the model solution. Correlation coefficients for degradation in the presence of 100 mg L^{-1} of ascorbic acid, were calculated for the 3 days (except for Indigotine were it is calculated for 24 h, and for Brilliant Black BN for 4 days) period. The other kinetics parameters (k, $t_{1/2}$) refer to period of 3 weeks. All parameters for the colorant degradation in the presence of 250 and 500 mg L^{-1} of ascorbic acid were obtained for the period of 4 weeks.

As it can be seen, the higher concentration of ascorbic acid leads to the higher rate constant and lower colorants half-life. The values of the rate constants are 2.50–4.25 times higher when the colorant degradation was run in the presence of 500 mg L^{-1} of ascorbic acid than in the case of the presence of 100 mg L^{-1} ascorbic acid for Brilliant Blue FCF, Chinoline Yellow, Patent Blue V and Tartazine.

Because of the presence of two azo groups in the structure of Brilliant Black BN, degradation of this colorant is very fast with a total degradation for 6–9 days. Very similar data were obtained for Indigotine probably due to the presence of two NH groups in its structure. Therefore, the rate constants for these two food colorants are much higher than for the other investigated colorants.

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Р е з и м е

HPLC ИСПИТУВАЊА НА ДЕГРАДАЦИЈАТА НА НЕКОИ ВЕШТАЧКИ АЗО ПРЕХРАНБЕНИ БОИ ВО ПРИСУСТВО НА АСКОРБИНСКА КИСЕЛИНА

Испитувано е влијанието на присуството на аскорбинската киселина на деградацијата на некои вештачки прехранбени азо бои (Brilliant Black BN, Brilliant Blue FCF, Chinoline Yellow, Indigotine, Patent Blue V и Tartazine). Определена е константата на брзината (k) и времето на полураспаѓање ($t_{1/2}$) на овие бои. Концентрацијата на боите и аскорбинската киселина се определувани со високоефикасна течна хроматографија (HPLC) со детектор со низа од диоди (DAD) и UV-VIS-спектрометрија. Утврдено е дека зголемувањето на концентрацијата на аскорбинската киселина доведува до зголемување на константата на брзината на реакцијата на деградација и до намалување на времето на полураспаѓање на испитуваните бои. Така, утврдено е дека вредностите на константите на брзината на деградација на Brilliant Blue FCF, Chinoline Yellow, Patent Blue V и Tartazine се за 2,50 до 4,25 пати поголеми кога концентрацијата на аскорбинската киселина во растворите е 500 mg L^{-1} во однос на раствори во кои нејзината концентрација изнесува 100 mg L^{-1}. Деградацијата, пак, на Brilliant Black BN и Indigotine е значително повисока отколку онаа на другите испитувани бои при што нивното целосно разложување настанува за 6–9 дена. Според тоа произлегува дека константата на брзината на деградација на овие две бои е многукратно поголема отколку на другите испитувани бои. Врз основа на линеарноста на кинетичките криви ($\ln(c/c_o$ од t) е утврдено дека кинетика на деградација на овие бои е од прв ред во периодот до 4 дена.

Клучни зборови: прехранбени бои; Brilliant Black BN; Brilliant Blue FCF; Chinoline Yellow; Indigotine; Patent Blue V; Tartazine; аскорбинска киселина; деградација; кинетичка константа на брзината; HPLC; UV-VIS-спектрометрија

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