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Voltammetric and Spectrophotometric Studies on Tartrazine – a Food Colorant*

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Tartrazine – a food colorant – was examined by the spectrophotometric method to determine its absorption bands and the dependence of absorption on concentration. A linear relationship was obtained within the concentration range from 1×10^{-5} to 5×10^{-5} mol l^{-1}.
The reduction of tartrazine in different supporting-electrolyte solutions was examined by differential pulse voltammetry. Basing on the obtained results, a new method for the quantitative determination of this dye has been developed. A linear dependence of the voltammetric peak current on concentration has been obtained within the concentration range from 5×10^{-6} to 5×10^{-4} mol l^{-1} in the solution of Britton–Robinson's buffer of pH 4.10.

Metodą spektrofotometryczną zbadano spożywczy barwnik azowy – tartrazynę, wyznaczając jej widma absorpcyjne oraz zależność absorbancji od stężenia. Prostoliniową zależność uzyskano dla zakresu stężeń od 1×10^{-5} do 5×10^{-5} mol l^{-1}.
Metodą pulsowej woltametrii różnicowej zbadano proces redukcji tartrazyny w czterech różnych roztworach elektrolitów podstawowych. W oparciu o uzyskane wyniki opracowano warunki ilościowego oznaczania tego barwnika. Prostoliniową zależność prądu piku woltamperometrycznego od stężenia uzyskano dla zakresu stężeń od 5×10^{-6} do 5×10^{-4} mol l^{-1} w roztworze elektrolitu podstawowego, którym był bufor Brittona–Robinsona o pH 4.10.

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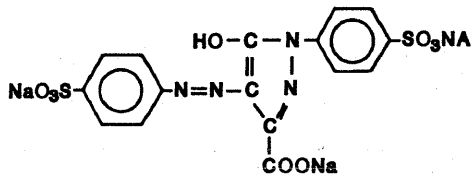
Tartrazine (C.I. 19140), symbol E-102, is one of the most common food colorant in the world. It belongs to monoazo dyes [1] and contains in its molecule acidic groups: SO_3^- and OH^- (formula I). Tartrazine occurs in the form of a fine-crystalline yellow powder and is well soluble in water, slightly more difficult soluble in some organic solvents, such as ethanol, acetone, diethyl ether and chloroform, and insoluble in oils and fats [2]. In most countries, this dye has been approved for food, medicine and cosmetic colouring.



I. Tartrazine $C_{16}H_9N_4O_9S_2Na_3$ M.W.534

The potential carcinogenic effects of azo compounds and products of their decomposition create a need for a systematic examination of the reduction processes and the process mechanisms for these compounds. Particulary, it concerns the synthetic azo dyes added to foodstuffs [1–5]. Some authors [3–5] are of the opinion that azo dyes, in the alimentary canal, are initially reduced to hydrazine compounds which are futher changed into aromatic amines, being characterized by proved carcinogenic effects. There are opinions that azo dyes react with tissue proteins which process can bring about the tumor formation [3].

Tartrazine is considered to be a dye with a low level of toxicity. Hitherto performed examinations with animals have shown, in one case, the occurrence of a mild toxicity in rats with symptoms such as loss of weight and a slow growth of erythrocytes and hemoglobin [6]. Tartrazine is also said to be an allergen which added to food can bring about intolerance and allergy [4]. A significant problem arises also from the fact that azo dyes are polluted with aromatic amines during the manufacturing process [2]. The most frequent pollutant of tartrazine is benzidine. The synthesis of tartrazine uses sulfanilic acid which in turn is prepared from aniline. Benzidine is the only possible product of aniline oxidation and is often detected as a residue. It also forms benzidine dye derivatives being health hazard pollutants [7].

The above mentioned facts create a need for developing affective methods for detection and quantitative determination of tartrazine and its metabolites in foodstuffs. So far, we have examined the acid–base properties of food azo dyes [9], their reactions with metal ions [10], polarographic reduction of brilliant black [11], orange yellow [12], cochineal red [13] and amaranth [14]. Our recent paper reports on the development of the method for detection and determination of erythrosine in foodstuffs. This study is devoted to voltammetric and spectrophotometric studies on tartrazine in pure specimens and commercial blends.

EXPERIMENTAL

Apparatus and reagents

Spectrophotomeric measurements were carried out by means of a Specord UV–VIS spectrophotometer, Carl Zeiss Jena (Germany), using 1-cm quartz cells. The absorption bands of the aqueous dye solutions under investigation were recorded within the wavelength range from 200 to 800 nm.

Voltammetric measurements were performed with a PA-3 polarograph and an XY recorder, type 4105, both of L.P. Praha (Czech Republic), using a 75 ml glass electrolytic vessel and the three electrode system. A hanging mercury drop electrode was used as the cathode, a saturated calomel electrode was the reference electrode, and a 1.5-cm^2 platinum plate constituted the counter electrode. As supporting electrolyte the following solutions were used: an universal Britton–Robinson buffer, 0.5 mol l^{-1} $NaClO_4$ or 1.0 mol l^{-1} KCl solutions prepared from analar grade reagents obtained from POCh, Gliwice. The pH values of the investigated solutions were measured by means of an universal pH-meter, type N-5122, Elwro (Poland).

The aqueous solutions of tartrazine were prepared from analar grade reagents from BASF (Germany) and Boruta-Zgierz (Poland), and distilled water with a conductivity of 0.1 μS. Two commercial blends containing tartrazine were also examined: lemon yellow A and egg yellow A of Boruta-Zgierz (Poland).

Measurement methods and experiment conditions

The stock solutions of dyes were 1×10^{-2} mol l^{-1}. Spectrophotometric measurements of tartrazine at concentration from 1×10^{-5} to 6×10^{-5} mol l^{-1} were carried out within the UV range 200–350 nm and the VIS range 350–800 nm. The absorbance measurements of the solutions under investigation were performed at 20±1°C in an aqueous system (pH = 6.5) in relation to redistilled water as a reference.

The voltammetric measurements of tartrazine solutions were carried out within the concentration range from 8.5×10^{-5} to 5.0×10^{-5} mol l^{-1} using the differential pulse voltammetric method within the potential range from +0.1 to –1.4 V. The pulse amplitude was 50 mV and scan rate was 5 mV s^{-1}. The reduction waves of tartrazine were recorded in Britton–Robinson's buffer within the pH range from 1.81 to 11.20 as well as in solutions: 0.5 mol l^{-1} $NaClO_4$, 1 mol l^{-1} $HClO_4$ and 0.2 mol l^{-1} NaOH. Prior to the measurements, pure argon was passed through the solutions for 15 min to remove oxygen and stirr the solution. The measurements were performed at 20±1°C.

RESULTS AND DISCUSSION

Figure 1 shows an absorption spectra of standard aqueous solutions of tartrazine (BASF). As it can be seen, this dye is characterized by intensive absorption bands both in the UV range (λ = 258 nm) an in VIS range (λ= 430 nm). With an increase in tartrazine concentration in the solutions under investigation, one can observe a clear linear increase in the solution absorbance, which points to the fact that the spectrophotometric method is suitable for the quantitative determination of this dye in foodstuffs. Such a relation was obtained for the concentration range from 1×10^{-5} to 5×10^{-5} mol l^{-1}.

The characteristic absorption bands and the calculated values of molar absorption coefficients are listed in Table 1. Then, the absorption spectra of aqueous solutions of the following three commercial preparations were recorded: tartrazine, egg yellow A and lemon yellow A (all of Boruta-Zgierz, Poland). The concentration being constant $c=4\times10^{-5}$ mol l^{-1}. The obtained spectra are characterized by a

considerably lower intensity than that of the spectrum of the standard tartrazine sample (BASF) of the same concentration. Using the previously found regression equations, the real content of pure tartrazine in these three samples of dye preparations was determined. The corresponding data are given in Table 2.

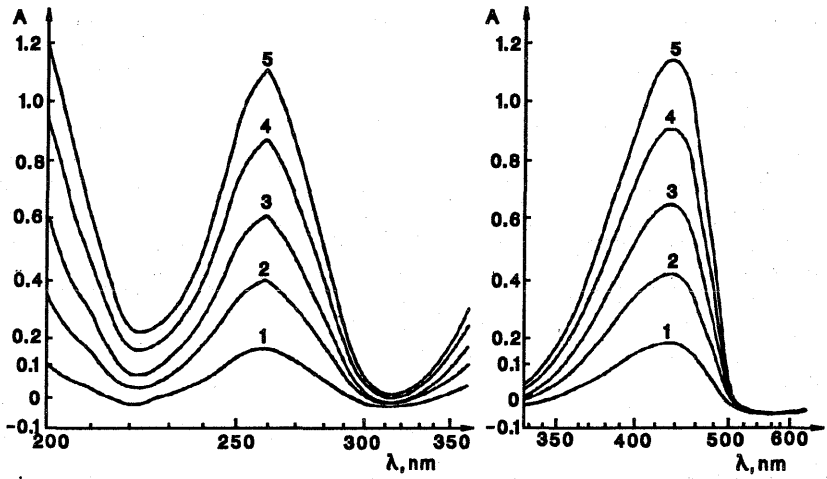


Figure 1. Absorption bands of aqueous solutions of tartrazine at concentration: 1 – 1×10^{-5} 2 – 2×10^{-5}; 3 – 3×10^{-5}; 4 – 4×10^{-5}; 5 – 5×10^{-5} mol l^{-1}; d = 1 cm

Table 1. Experimental data characteristic for absorption spectra of aqueous solutions of tartrazine from BASF (d = 1 cm)

Concentration mol l^{-1}	Band I (λ = 250 nm)		Band II (λ = 430 nm)	
	A	ε, mol l^{-1} cm^{-1}	A	ε, mol l^{-1} cm^{-1}
1×10^{-5}	0.17		0.18	
2×10^{-5}	0.40		0.43	
3×10^{-5}	0.62	2.04×10^{4}	0.66	2.16×10^{4}
4×10^{-5}	0.86		0.92	
5×10^{-5}	1.10		1.15	
Regression equation	$A = 2.32\times10^{4}c - 6.6\times10^{-2}$		$A = 2.43\times10^{4}c - 6.1\times10^{-2}$	
Regression coeficient	0.999		0.999	
Determination limit	5×10^{-6} mol l^{-1}			

Table 2. Results of determination of pure tartrazine content in three samples of commercial colouring matters obtained by spectrophotometric and voltammetric methods

Sample*	Spectrophotometry		Voltammetry	
	Absorbance (λ = 430 nm)	Content of tartrazine, %	Peak current, μA	Content of tartrazine, %
Tartrazine	0.720	80	0.205	77
Egg yellow A	0.620	69	0.215	62
Lemon yellow A	0.600	67	0.205	59

*All test samples were produced by ZPB "Boruta" Zgierz (Poland).

The voltammetric measurements of tartrazine solutions were carried out in Britton–Robinson's buffer. Figure 2 shows the obtained voltammetric curves illustrating the changes in tartrazine (BASF) reduction within the pH range from 1.81 to 11.98. In acidic medium (pH from 1.81 to 5.02), as shown in Figure 2, one voltammetric wave is observed. So, the dye is reduced in one stage. As pH of the solution increases, peak potential E_p is shifted towards more negative potentials. The determined values of the voltammetric wave potentials (E_p) for various pH are given in Table 3. The regression equations of these relationships are shown in Table 4.

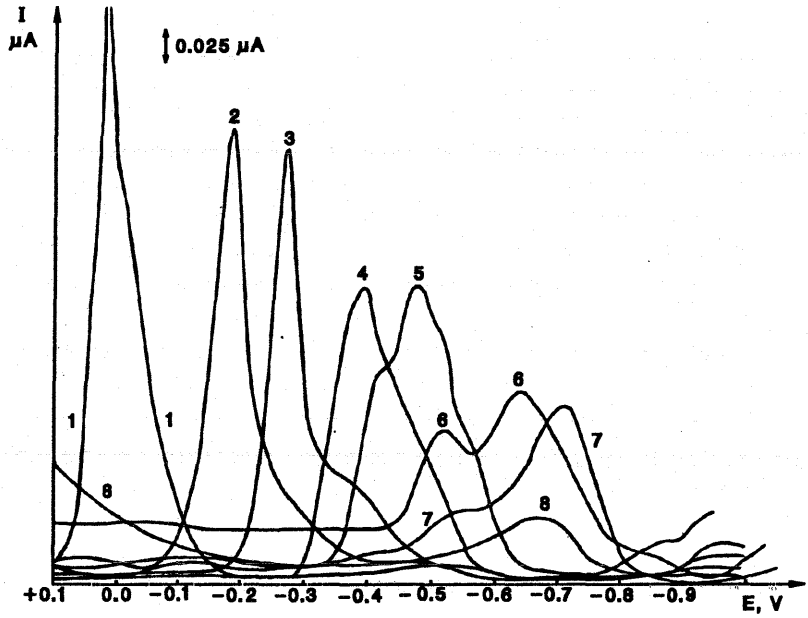


Figure 2. Curves of voltammetric reduction of tartrazine at concentration $c = 5\times10^{-5}$ mol l^{-1} in Britton–Robinson buffer at pH: 1 – 1.81; 2 – 2.87; 3 – 4.10; 4 – 5.02; 5 – 6.09; 6 – 7.96; 7 – 9.91 and 8 – 11.98

Table 3. Determined values of potentials of the voltammetric reduction wave [V] of tartrazine at varying pH of the solution

Examined sample	pH							
	1.81	2.87	4.10	5.02	6.09	7.96	9.91	
Tartrazine from BASF	+0.015	–0.190	–0.270	–0.395	–0.475	–0.645	–0.715	–0.735
Tartrazine from Boruta-Zgierz	+0.015	–0.150	–0.245	–0.320	–0.455	–0.651	–0.675	–0.725
Lemon yellow A from Boruta-Zgierz	+0.010	–0.150	–0.245	–0.270	–0.390	–0.600	–0.675	–0.730
Egg yellow from Boruta-Zgierz	+0.015	–0.151	–0.245	–0.280	–0.405	–0.615	–0.655	–0.705

Table 4. Regression equations express association of potential of voltammetric wave (E_p, V) and pH value of the solution

Dye	Range of pH	Regression equation $E_p = a \times pH + b$	Regression coeficient
Tartrazine	1.81–5.02	$E_p = -0.124\times pH + 0.214$	0.978
from BASF	6.09–9.91	$E_p = -0.058 \times pH - 0.155$	0.951
Tartrazine	1.81–5.02	$E_p = -0.105\times pH + 0.184$	0.979
from Boruta-Zgierz	7.00–10.98	$E_p = -0.028\times pH - 0.411$	0.962
Lemon yellow A	1.81–5.02	$E_p = -0.091\times pH + 0.146$	0.952
from Boruta-Zgierz	7.00–10.98	$E_p = -0.051\times pH - 0.176$	0.989
Egg yellow A	1.81–5.02	$E_p = -0.095\times pH + 0.155$	0.955
from Boruta-Zgierz	7.00–10.98	$E_p = -0.041\times pH - 0.257$	0.958

It is also seen from the data in Figure 1 that the wave height of the dye reduction is lowered with the increase in pH. Betwen pH 7.00 and pH 10.88 the wave of tartrazine reduction is transformed into two voltammetric waves. They are equal at half the height obtained in the acidic medium. In a strongly basic medium, a single wave is observed again, showing a considerably suppressed maximum. Basing on the performed measurements, we have proposed a mechanism for the tartrazine reduction (Figure 3). The reduction of this dye proceeds in two steps. The first step, consists of the reduction of azo group (–N=N–) of the tartrazine molecule (I), which results in the formation of a hydrazine compound (II); this is a two-electron transformation with participation of two protons (E': $2H^+$, 2e). Then, as the second analogous step, also two-electron E'': ($2H^+$, 2e) further reduction of the hydrazine group takes place, resulting in the formation of two molecules of aromatic amines.

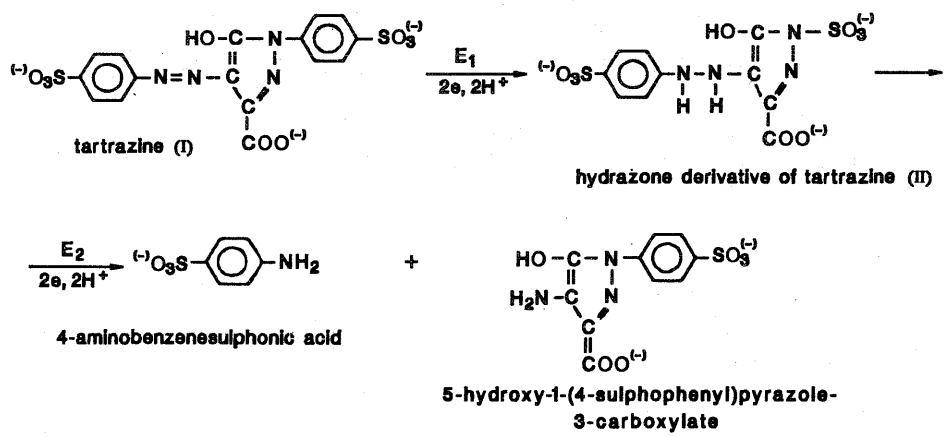


Figure 3. Proposed mechanism of voltammetric reduction of tartrazine

In the acidic medium, the protonation of azo group of the dye and further reduction processes to simple amines proceed very fast and therefore stages E' and E'' are revealed as one 4-electron transformation (curves 1–4, Figure 2). At pH above 7 the protonation of hydrazine compounds is more difficult and the reduction process proceeds at a lower rate and in two steps. Some dye molecules remain as hydrazine compound. Therefore, two waves are observed in the voltammmperograms.

Identical examinations of the dependence of voltammetric wave potentials on pH, to the above, were performed for the other tartrazine samples from Boruta-Zgierz. The reduction of these dyes proceeds in the same way as in the case of tartrazine from BASF. The detailed results of analysis of voltammetric waves for all the examined tartrazine samples are given in Tables 3 and 4.

The effect of the type of supporting electrolyte on the shape and position of the peaks of the investigated tartrazine solutions was also examined. Table 5 contains the values of peak potentials E_p [V] and peak current I [μA] for several types of supporting electrolytes: 0.5 mol l^{-1} $NaClO_4$, 1 mol l^{-1} KCl, 1 mol l^{-1} $HClO_4$ and 0.1 mol l^{-1} $HClO_4$.

Table 5. Parameters of the voltammetric reduction peaks of solutions of tartrazine at concentration 5×10^{-5} mol l^{-1} in four various supporting electrolytes

Dye	Polarographic parameters	Supporting electrolyte					
		1 mol l^{-1} $HClO_4$	0.1 mol l^{-1} $HClO_4$	1 mol l^{-1} KCl		0.5 mol l^{-1} $NaClO_4$	
				peak I	peak II	peak I	peak II
Tartrazine	E_p, V	+0.098	+0.065	–0.445	–0.590	–0.490	–0.625
from BASF	I, A	0.185	0.365	0.088	0.100	0.070	0.133
Tartrazine	E_p, V	+0.100	+0.065	–0.455	–0.595	–0.485	–0.635
from Boruta-Zgierz	I, A	0.128	0.290	0.073	0.078	0.065	0.098
Lemon yellow A	E_p, V	+0.100	+0.065	–0.455	–0.600	–0.485	–0.630
from Boruta-Zgierz	I, A	0.113	0.248	0.070	0.068	0.063	0.090
Egg yellow A	E_p, V	+0.100	+0.065	–0.455	–0.600	–0.485	–0.625
from Boruta-Zgierz	I, A	0.125	0.268	0.071	0.073	0.063	0.090

Examples of the voltammperograms recorded for tartrazine from BASF and tartrazine from Boruta-Zgierz are shown in Figures 4a and 4b. Very small differences in the values of peak potentials, about 0.01 V, were observed in all the investigated samples. The highest values of peak currents at the given potential value were obtained for tartrazine from BASF, then for tartrazine from Boruta-Zgierz and respectively lower for egg yellow A- and lemon yellow A preparations. No well developed peaks of the investigated dyes were observed in 0.2 mol NaOH.

Basing on the fundamental studies on the voltammetric reduction of tartrazine, a new method for the quantitative determination of this dye was developed, including optimal conditions of performing this determination. A linear dependence of peak current, I, on tartrazine concentration, c, was obtained in Britton–Robinson's buffer at pH 4.10. It takes place within the dye concentration range from 5×10^{-6} to 5×10^{-4} mol l^{-1}. This dependence is expressed by a regression equations in Table 6. Figure 5 shows example voltammperograms of tartrazine solutions (BASF) at pH 4.10, which were recorded within the concentration range from 0.5×10^{-5} to 3×10^{-5} mol l^{-1}.

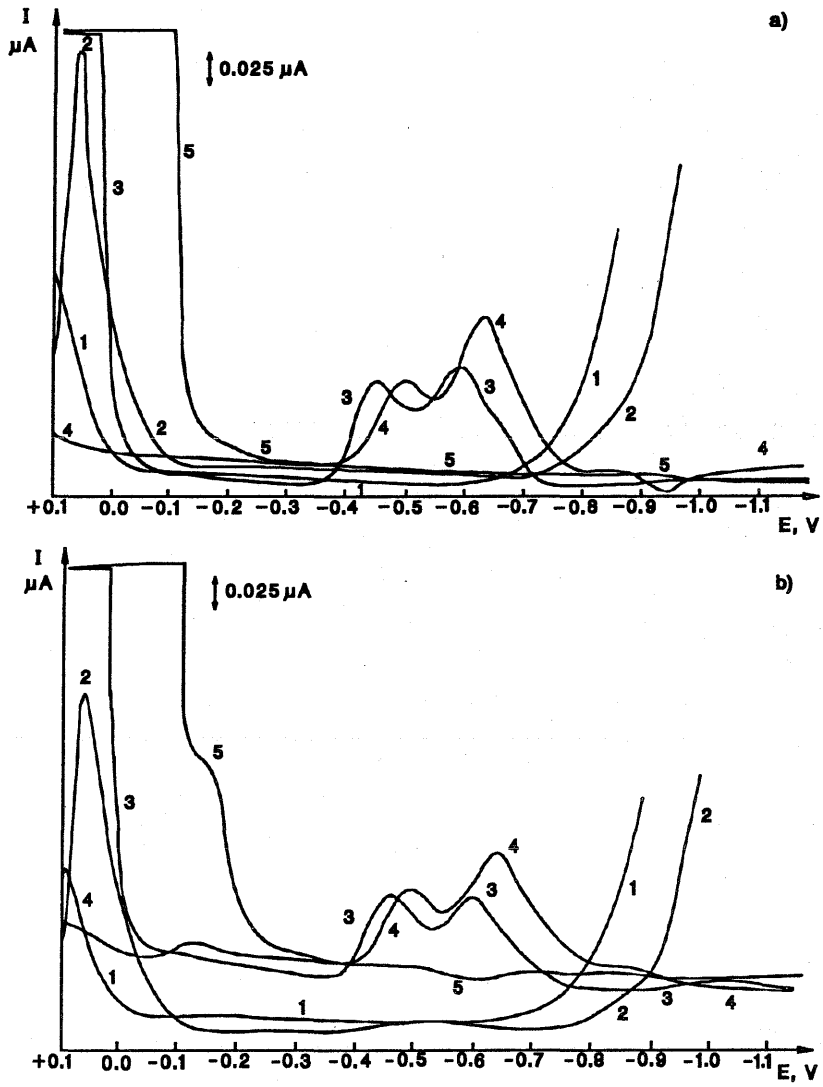


Figure 4. Some voltammperograms of tartrazine in various electrolyte solutions: 1 – 1 mol l^{-1} $HClO_4$; 2 – 0.1 mol l^{-1} $HClO_4$; 3 – 1 mol l^{-1} KCl; 4 – 0.5 mol l^{-1} $NaClO_4$ and 5 – 0.2 mol l^{-1} NaOH; a) tartrazine from BASF; b) tartrazine from Boruta-Zgierz

Table 6. Regression equations of molar concentrations (c) of tartrazine associated with current value of voltammetric wave (I); supporting electrolyte: Britton–Robinson buffer of pH 4.10

Dye concentration mol l^{-1}	Regression equation $I = f(c)$	Regression coeficient	Determination limit
5×10^{-4}–5×10^{-5}	$I = 5.26\times10^{3}c + 3.93\times10^{-2}$	0.994	–
5×10^{-5}x10-6	$I = 2.63\times10^{3}c + 1.01\times10^{-2}$	0.989	0.25×10^{-5}

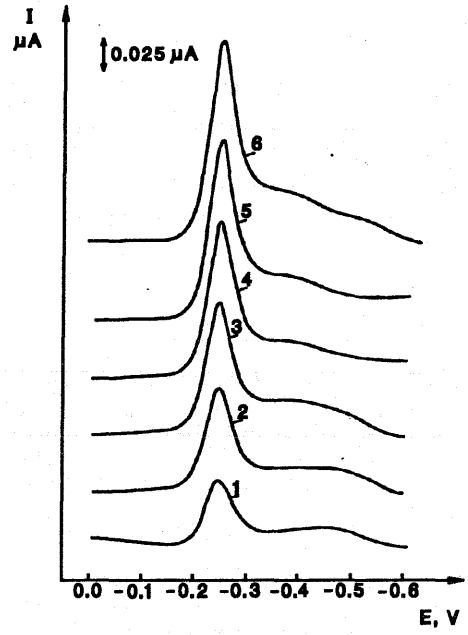


Figure 5. Tartrazine (BASF) solutions voltammperograms recorded for following dye concentrations: 1 – 0.5×10^{-5}; 2 – 1×10^{-5}; 3 – 1.5×10^{-5}; 4 – 2×10^{-5}; 5 – 2.5×10^{-5} and 6 – 3×10^{-5} mol l^{-1}. Supporting electrolyte: B–R buffer of pH 4.10; sensitiveness of the apparatus: 0.025 μA cm^{-1}; temp. 20°C

The voltammetric measurements described above were used for the determination of the tartrazine content in the following commercial preparations manufactured by Boruta-Zgierz: tartrazine, egg yellow and lemon yellow. As the voltammetric peaks of tartrazine from BASF were the highest, they were treated as the standard. Experimental data used for calculations and the results obtained by the differential pulse voltammetric method are given in Table 2. They agree with the previously obtained results by the spectrophotometric method. The highest content of pure tartrazine was found in tartrazine from Boruta-Zgierz, and then in egg yellow from Boruta-Zgierz; the lowest one in lemon yellow from Boruta-Zgierz.

Conclusions

The performed examination and measurements have shown that tartrazine can be readily identified and quantitatively determined by both spectrophotometric and voltammetric methods. In both methods, linear dependences of the solution absorbance on the dye concentration, and the voltammetric peak current on the tartrazine concentration were determined. It has been shown that the developed methods are suitable for the determination of tartrazine in commercially available dye preparations in the form of dye mixtures. The average error of the determination is ±5 for the spectrophotometric method and ±7% for the voltammetric method. The mechanism of voltammetric reduction of tartrazine has been examined, and it has been shown

that it is similar to that of azo dyes previously studied by us. It has been shown that the increase in pH of solution brings about a shift of the voltammetric reduction wave of tartrazine towards more negative potentials. In the acidic medium, the reduction of the dye to two aromatic amines proceeds rapidly and then only one wave is observed in the voltammperograms. At pH above 7, the reduction process proceeds at a lower rate, since it is partly inhibited at the stage of formation of hydrazine compounds. Then, two voltammetric waves are observed in the voltammperograms.

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