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ERIOCHROME BLACK T – A NEW ANALYTICAL REAGENT FOR SPECTROPHOTOMETRIC DETERMINATION OF SULPHANILAMIDES

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Abstract. Optimal conditions of the sulphanilamides diazonium salts interaction with *o,o'*-dihydroxysubstituted azo dye eriochrom black T have been defined. Therefrom the method of spectrophotometric determination of ten sulphanilamides in finished medicinal forms has been developed. This method is based on the azocoupling reaction of the sulphanilamide diazo salt with one of the products of the redox destruction of azo dye with formation of a colored azo compound. This method is characterized by the wide linearity range (from 3 to 64 μg/ml depending on the detectable sulphanilamide), simplicity, rapidity (duration of analysis is under 30 min), and reproducibility ($S_r \sim 0.040$).

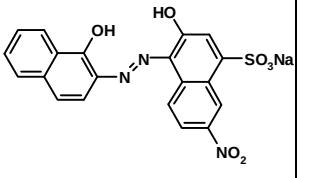
Keywords: sulphanilamides, eriochrome black T, spectrophotometry, finished dosage forms.

1. Introduction

Numerous methods of spectrophotometric determination of sulphanilamides (SA) content in finished dosage forms have been elaborated. They are based on the interaction of both SA [1-5] and SA diazonium salts [6-13] with organic reagents leading to formation of the colored analytical forms. Even though dyes are common analytical reagents in pharmaceutical analysis, they are rarely used for SA determination. In the literature the use of alizarine and its derivatives [14], which form charge transfer complexes with SA, is described. However these reactions are characterized by a low contrast. Previously we developed techniques of spectrophotometric SA determination with application of azo reagents, such as acid mono azo dye tropaeolin O [15, 16], heterocyclic azo reagents 4-(2-pyridylazo) resorcinol [17, 18], and 4-(2-thiazolylazo) resorcinol [19], which are characterized by a high sensitivity and selectivity at the presence of other classes of biologically active substances. The goal of our investigation was the search of effective analytical reagents for SA determination by the examining of azo dyes; one of them is *o,o'*-dihydroxysubstituted azo dye eriochrom black T (EBT). Structural formula and some physico-chemical properties of this azo reagent are presented in the Table 1.

Table 1

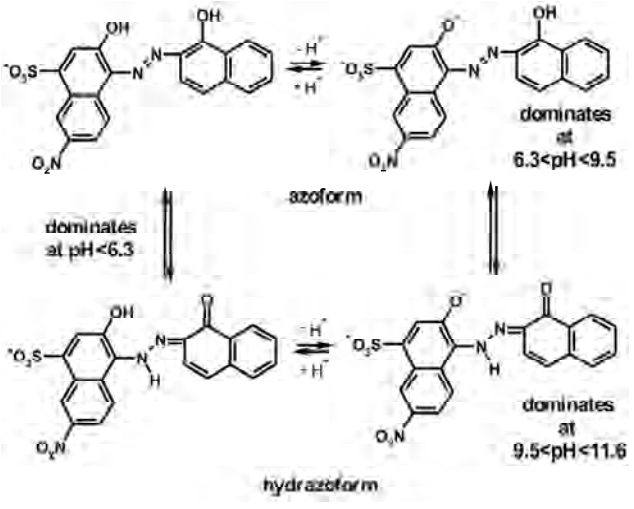
Structural formula and some characteristics of azo dye EBT (C.I. 14645, CAS No. 1787-61-7) [20-23]

Structural formula	Characteristics
	dark blue powder $pK_{H_3Ind} = 3.9$; red $pK_{H_2Ind^-} = 6.4$; red; pH < 6 $pK_{HInd^{2-}} = 11.5$; $e_{615} = 2.35 \cdot 10^4$; pH = 10; Ind^{3-} orange; pH > 12

Within the pH range from 6–7 to 12–13 the EBT molecules in solution are present in the form of dissociated ion (with one dissociated hydroxyl group), while azo form of reagent dominates at lower values of this pH range at the acid and neutral media (Scheme 1) [20-23].

In analytic chemistry EBT is more often used in the inorganic analysis. For the analysis of organic substances its application is limited. According to literature data it is known that EBT interacts with organic molecules, resulting in formation of ion associates, which are extracted by

organic solvents. In this way the following substances: diphenhydramine in pharmaceutical and biological objects [24], lansoprazole in finished dosage forms, such as capsules [25], levofloxacin in pills [26], trace amounts of nifedipine [27], amlodipine besylate [28], and promethazine [29] in pharmaceutical forms are determined. Using the EBT the following proteins such as a human and ox serum albumin [30], globulin, albumin, and lysozyme [31] are determined by the method of flow-injection analysis in the combination with Rayleigh scattering.



Sheme 1. Forms of existence of the EBT in aqueous solutions

2. Experimental

2.1. Reagents and Instruments

We studied the interaction of ten most common in medical practice SA (Table 2).

Sulphanilamides were purchased from Sigma (USA). Solutions of sulphamethoxazole, sulphamethazine, sulphamerazine, sulphadimetoxine, sulphathiazole, sulphadiazine, sulphametoxypyridazine, and sulphamonometoxine were prepared by dissolving appropriate amounts of the reagents of pharmacopoeia grade in 0.1 M sodium hydroxide solution. Solutions of sulphanilamide were prepared by dissolving appropriate amounts of the reagents of pharmacopoeia grade in 0.1 M hydrochloric acid. Solutions of sulphaguanidine were prepared by dissolving appropriate amounts of the reagents of pharmacopoeia grade in the mixture of equal amounts of 0.1 M hydrochloric acid and 96 % ethanol.

Solutions of eriohrom black T (Merck, Germany) were prepared by dissolving appropriate amounts of the reagent of analytical grade ≥ 98 % purity in the 30 % mixture of ethanol (96 %) and water.

The solutions of hydrochloric acid, sodium hydroxide, sodium nitrite, urea, sulphamic acid, and universal buffer mixture (UBM) were prepared from the chemicals of the analytical grade.

Table 2

Physical and chemical properties of SA

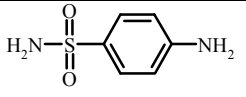
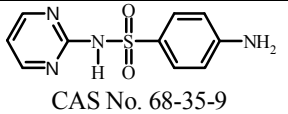
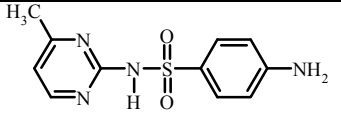
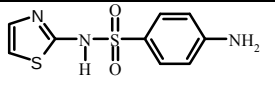
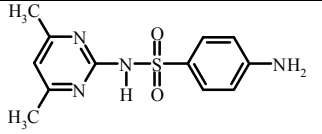
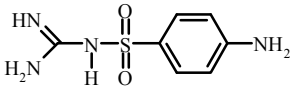
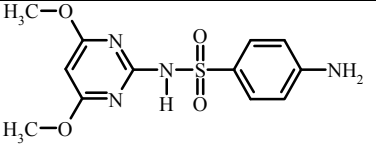
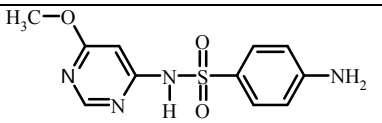
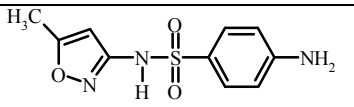
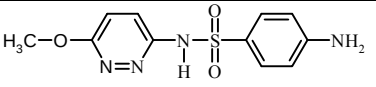
Structural formula	Name	Some characteristics [32-34]
1	2	3
 CAS No. 63-74-1	Sulphanilamide (SAM), *p*-aminobenzenesulphanilamide	M = 172.2 g/mol; pK_1 = —; pK_2 = 10.1
 CAS No. 68-35-9	Sulphadiazine (SDA), 2-(*p*-aminobenzenesulfonamido)-pyrimidine	M = 250.3 g/mol; pK_1 = 2.49; pK_2 = 6.48
 CAS No. 127-79-7	Sulphamerazine (SMR), 2-(*p*-aminobenzenesulfonamido)-4-methylpyrimidine	M = 264.3 g/mol; pK_1 = —; pK_2 = 7.00
 CAS 72-14-0	Sulfathiazole (STZ), 2-(*p*-aminobenzenesulfonamido)-1,3-thiazol	M = 255.3 g/mol; pK_1 = 2.62; pK_2 = 7.37
 CAS No. 57-68-1	Sulphamethazine (SMZ), 2-(*p*-aminobenzenesulfonamido)-4,6-dimethylpyrimidine	M = 278.3 g/mol; pK_1 = 2.65; pK_2 = 7.58

Table 2 (Continued)

1	2	3
 CAS 57-67-0	Sulphaguanidine (SGN), N-(*p*-aminobenzenesulfonamido)-aminoiminomethyl	M = 214.2 g/mol; pK_1 = 2.72 pK_2 = 11.82
 CAS No. 122-11-2	Sulphadimethoxine (SDM), 6-(*p*-aminobenzenesulfonamido)-2,4-dimethoxypyrimidine	M = 310.3 g/mol; pK_1 = 2.65 pK_2 = 6.82
 CAS 1220-83-3	Sulphamonomethoxine (SMM), 4-(*p*-aminobenzenesulfonamido)-2-methoxypyrimidine	M = 280.3 g/mol; pK_1 = 2.51 pK_2 = 7.28
 CAS No. 723-46-6	Sulphamethoxazole (SMX), 3-(*p*-aminobenzenesulfonamido)-5-methyloxazole	M = 253.3 g/mol; pK_1 = 1.74 pK_2 = 5.70
 CAS No. 000080-35-3	Sulphamethoxypyridazine (SMP), 3-(*p*-aminobenzenesulfonamido)-6-methoxypyridazine	M = 280.3 g/mol; pK_1 = — pK_2 = 7.20

UV-Vis measurements were performed with UV-Vis scanning spectrophotometers CARY.WIN–UV-VIS-50 (Varian, USA) and SPECORD M-40 (Carl Zeiss Jena, Germany) using 1 cm cuvettes. All absorbance measurements were performed at 293–298 K.

The pH value was measured by pH-meter RV 11 (Sartorius, Germany) as well as pH-meter model pH 150M (Gomelsky Plant of Measuring Devices, Belarus), equipped with a combination electrode, which combines both the glass and the reference silver chloride electrodes into one body. The required pH of each solution was adjusted using diluted HCl and NaOH solutions. Potentiometric titration was performed using a potentiometer pH 150M with a platinum indicator electrode EPL-02 and a silver chloride reference electrode EVL-1M4 (Gomelsky Plant of Measuring Devices, Belarus).

Polarographic researches were performed using an oscillopolarograph PO-5122 model 03 (Russia) with additional hardware and a three-electrode thermostated cell with an indicator mercury dropping electrode, auxiliary platinum electrode, and the reference saturated calomel electrode (linear potential range from -0.2 to -1.75 V by the defined conditions: potential sweep rate – 2.0 mV/s, the delay imposition voltage – 4.0 s). Polarograms were recorded at a room temperature (293–298 K). Dissolved oxygen was eliminated from the test solutions by means of bubbling with purified argon for 15 min.

2.2. General Procedure of SA Determination with EBT

5.0 ml of 1.0 M hydrochloric acid solution was placed into a 25 ml volumetric flask. Then a sample of solution containing 4–64 μg ml^{-1} of SA in final volume was added. Next 0.5 ml of $5.0 \cdot 10^{-2}$ M sodium nitrite solution was added into the flask. After stirring the mixture was held for 20 min at a room temperature, then 0.5 ml of 2.5 M urea solution was added, the mixture was stirred and was held for 10 min at a room temperature for destruction of nitrite-ions excess. Then 1.5 ml of $6.0 \cdot 10^{-3}$ M EBT solution as well as 2.5 ml of 0.04 M solution of UBM were added into the flask. Obtained mixture was neutralized by adding of sodium hydroxide solution so that the pH value was adjusted to pH = 8.0. Next, distilled water was added to the full volume of 25 ml. Then the solution was mixed thoroughly and the absorbance measurements (at room temperature ~ 293 K) were carried out for all blank solutions of corresponding reagents at 485 nm in 1.0 cm cuvettes. SA concentration was calculated using the methods of calibration curve and single-point standardization.

2.3. Procedure of Tablets Preparation for SA Determination

Twenty tablets were weighed and finely powdered in a porcelain mortar. The accurate amount of powder,

equal to ~ 100 mg of SA, was placed into a 100 ml volumetric flask and dissolved in 50 ml of 0.1 M NaOH. Then the solution was mixed for 10 min and 0.1 M NaOH was added to complete the volume to 100 ml. The obtained solution was mixed again and filtered through the fold filter of medium porosity. The filtrate was used for analysis.

2.4. Sample Preparation of Suppository for SA Determination

Sample of suppository containing 100 mg of SA in accordance with the content of the product, was placed into a chemical glass and 50 ml of 0.1 M sodium hydroxide was added. The mixture was heated on a water bath at the temperature of ~ 353 K for at least 10 min to complete melting of the ointment. Received mixture was filtered through a folded paper filter (white ribbon) in a volumetric flask 100 ml (glass with mixture was kept in a hot water bath to prevent solidification of the preparation till filtering was complete). The filter was washed several times with hot 0.1 M sodium hydroxide solution. The filtrate was cooled. Next, the same solvent was added to the mark of the flask. The obtained filtrate was used for analysis.

3. Results and Discussion

3.1. Spectrophotometry of SA with EBT

3.1.1. Electronic spectrums of product of SA diazonium salts interaction with EBT

During the preliminary research of SA interaction it was discovered that only diazotized SA form colored compounds with EBT. On the UV-Vis absorbance spectra of products of interaction the diminishing of absorbance maximum of azo dye itself, and the appearance and increase of the new absorbance maximum proportional to SA concentrations were observed. It was established that the excess amount of nitrite ions, which have been used for obtaining of diazonium salts, interacts with azo dye because the absorbance at EBT λ_{max} on spectra of both proper dye and products of SA diazonium salts interaction with EBT decrease. Following from this, the action of nitrite-ions on EBT is the same as the action of diazotized SA. Therefore, nitrite ions remaining unreacted with SA should be removed from the reaction mixture by means of urea.

As can be seen from absorption spectra (Fig. 1), maximum absorbance of compounds, which were formed after the SA diazonium salts interaction with EBT is at λ_{max} = 485 nm, while the absorbance maximum of azo dye EBT is at λ_{max} = 600 nm under the reaction conditions, and it decreases with a slight bathochromic shift for all products of interaction.

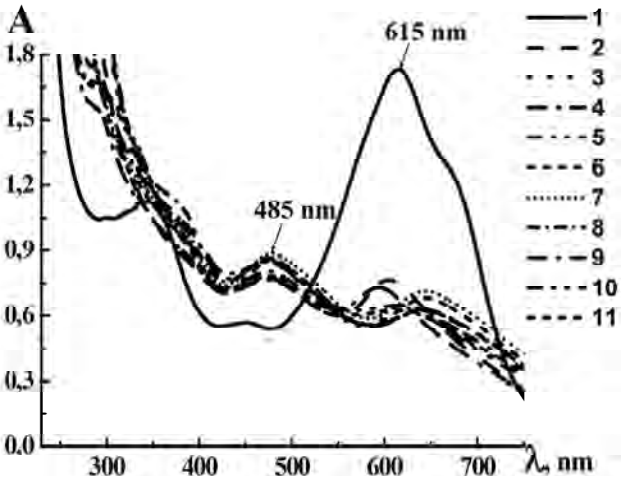


Fig. 1. Absorption spectra of solution EBT (1) and products of its interaction with SA diazonium salts aqueous solution: SAM (2), SMX (3), SMZ (4), SDM (5), SMR (6), STZ (7), SGN (8), SMP (9), SMM (10), SDA (11). C_{HCl}=1.0 M, C_{SA}=6.0·10^{-5} M, C_{NaNO_2}=8.0·10^{-4} M, C_{Urea}=4.0·10^{-2} M, C_{EBT}=3.6·10^{-4} M, C_{UBM}=4.0·10^{-3} M, pH=8.0, λ=485 nm

Obtained colored products of this reaction are most probably formed because of the redox process between diazotized SA and azo dye. Diazonium salt can oxidize azo dye and destroy it with the release of N_2 and colored degradation products, which in its turn can interact between themselves or with SA diazonium salts [35].

3.1.2. Conditions for SA diazotization

The conditions of SA diazotization and destruction of excess amount of nitrite ions for receiving maximum efficiency of products interaction of SA with azo dye have been tested. As follows from the research results, the maximum efficiency of the products of SA interaction with EBT is observed at SA diazotization in 0.5–1.0 M hydrochloric or sulphuric acids, whereas the use of phosphate and acetic acids does not allow achieving the same result. Therefore we used 1 M HCl for SA diazotization in all following experiments.

To achieve maximum analytical signal it is necessary to use excess of nitrite ions compared to SA content, and after diazotization reaction to remove remaining unreacted nitrite ions by urea or sulfamic acid. The optimal conditions of SA diazotization for obtaining of maximum analytical signal at the SA interaction with EBT are shown in Table 3.

Table 3

Optimal conditions of SA diazonium salts obtaining for maximum analytical signal at the SA interaction with EBT

Concentration of HCl	1.0 mol·l^{-1}
Amount of $NaNO_2$	> 3-fold excess to the concentration of SA
Diazotation time	20 min at 293 K
Amount of urea	> 50-fold excess to the concentration of $NaNO_2$
Reaction time	10 min at 293 K

3.2. Conditions of Interaction of Diazotized SA with EBT

3.2.1. Effect of medium acidity on the interaction of diazotized SA with EBT

In order to establish maximum efficiency of SA diazonium salts interaction with EBT, the investigations of pH effect on the analytical signal value were carried out. As follows from Fig. 2, the maximal yield of products for reaction of the most SA diazonium salts with EBT is observed in the slightly alkaline medium within the range of pH = 7.5–9.5.

According to literature data in alkaline aqueous solutions EBT has dissociated one of the OH-groups (Scheme 1), and in condition of interaction with the SA diazonium salts its azo-form dominates over hydrazo-form. The interaction between diazotized SA and azo dyes in the acidic medium does not take place as phenol and naphthol compounds are easier oxidized in alkaline medium [36, 37].

3.2.2. Effect of concentration of azo dye on interaction of SA diazonium salts with EBT

It is known that certain excess amount of reagent promotes the shift of reaction equilibrium toward the formation of reaction products that makes it possible to get the maximum yield of the colored products. For these reasons, the dependence of absorbance of the products of SA diazonium salts interaction with EBT on the excess of the reagent amount has been investigated. According to the experimental results (Fig. 3), maximal value of absorbance of the interaction products of diazotized SA with EBT is observed at 6-fold reagent excess.

At the azo group destruction in EBT molecule the formation of two particles: α-naphthol and β-naphthol containing substituents (sulfonate and nitro groups) is possible (Scheme 2).

From literature data it is known that SA diazonium salts azocouple with β-naphthol and the absorption spectra of formed products are characterized by the maximum absorbance at 477 nm [6]. The presence of the nitro group in the products of EBT destruction deactivates them as azo

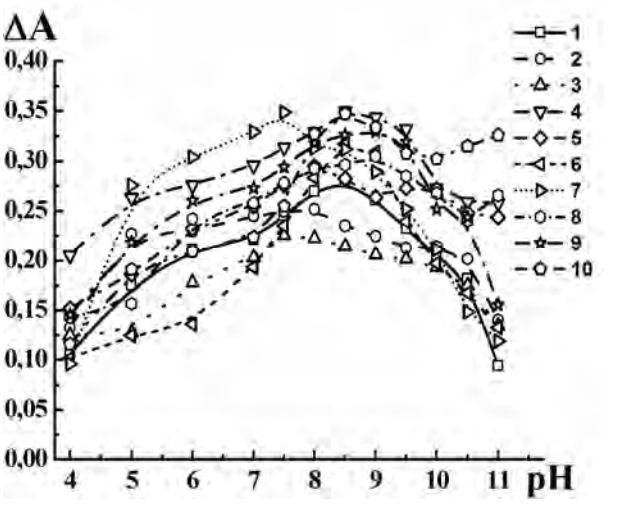


Fig. 2. Effect of pH value on EBT interaction with SA diazonium salts: SAM (1), SMX (2), SMZ (3), SDM (4), SMR (5), STZ (6), SGN (7), SMP (8), SMM (9), SDA (10). C_{HCl}=1.0 M, C_{SA}=6.0·10^{-5} M, C_{NaNO_2}=8.0·10^{-4} M, C_{Urea}=4.0·10^{-2} M, C_{EBT}=3.6·10^{-4} M, C_{UBM}=4.0·10^{-3} M, λ=485 nm

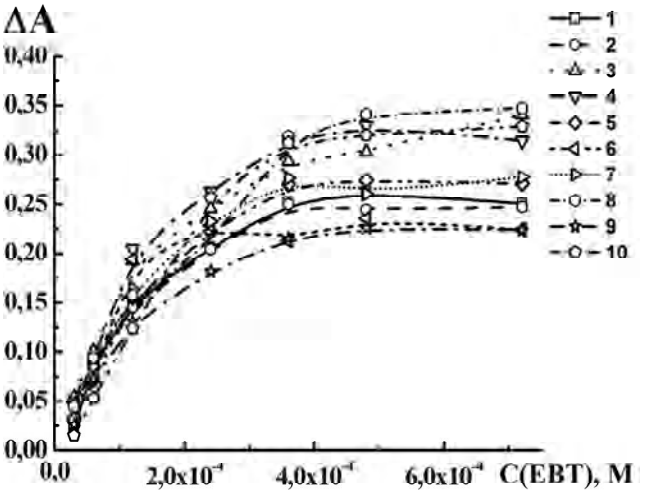
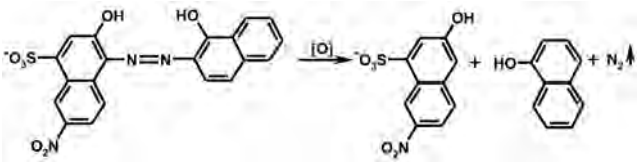


Fig. 3. Dependence of the azo reagent concentration on the absorbance of product interaction of the EBT with SA diazonium salts: SAM (1), SMX (2), SMZ (3), SDM (4), SMR (5), STZ (6), SGN (7), SMP (8), SMM (9), SDA (10) with EBT for concentration azo reagent. C_{HCl}=1.0 M, C_{SA}=6.0·10^{-5} M, C_{NaNO_2}=8.0·10^{-4} M, C_{Urea}=4.0·10^{-2} M, C_{UBM}=4.0·10^{-3} M, pH=8.0, λ=485 nm



Sheme 2. The scheme of EBT destruction

component, thus only unsubstituted α-naphthol can interact with electrophilic particles [36, 37]. We verified whether SMT diazonium salt can react with α-naphthol under the same reaction conditions as with the *o,o'*-hydroxysubstituted azo dye.

It was established that the α-naphthol reacts with SMT diazonium salt, and the product of the SMT interaction with α-naphthol is characterized by maximum absorbance at 475 nm (Fig. 4). Thus, the maximum absorbane which is observed on absorption spectrum of the products of SMT diazonium salt interaction with EBT at 470–485 nm is the maximum absorbance of compound of the SMT diazonium salt interaction with destruction product of this dye, namely with α-naphthol. This leads to an assumption about a slow oxidation of azo dye and fast azocoupling of unreacted SMT diazonium salt with products of EBT oxidation, namely, α-naphthol, to form new azo compound. Therefore a part of SA diazonium salt is used for oxidation of the dye's azo group while the rest is azocoupled with products of redox reaction. However, under the same conditions of reaction their parts in both processes are constant, which allowed developing the efficient methods of the SA determination by means of EBT.

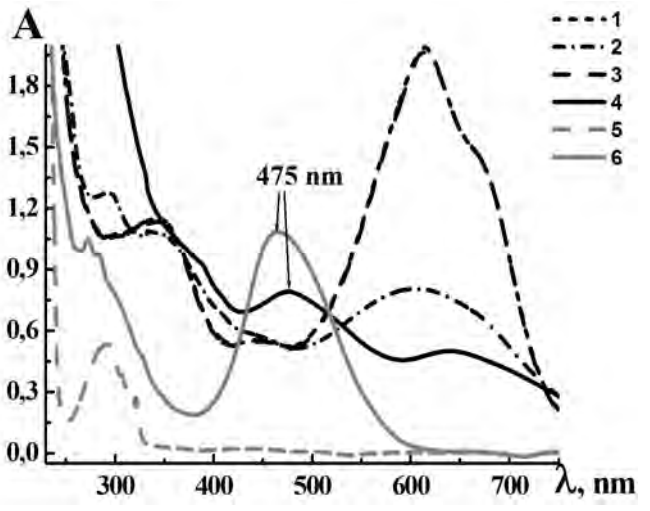


Fig. 4. Absorption spectra of the aqueous solution of the EBT, α-naphthol and products of their interaction with diazotized SMZ: EBT (1), NO_2^-+EBT (2), (NO_2^-+Urea)+ EBT (3), (SMZ+NO_2^-+Urea)+ EBT (4), (NO_2^-+Urea)+α-naphthol (5), (SMZ+NO_2^-+Urea)+ +α-naphthol (6). C_{HCl}=1.0 M, C_{SMZ}=6·10^{-5} M, C_{NaNO_2}=8.0·10^{-4}M, C_{Urea}=4.0·10^{-2} M, C_{EBT}=3.6·10^{-4}M, $C_{\alpha\text{-naphtole}}$=1.2·10^{-4} M, C_{UBM}=4.0·10^{-3} M, pH = 8.0

3.3. Potentiometry of SA with EBT

To confirm the redox interaction between SA and EBT a potentiometric redox titration of diazotized SMZ with EBT solution has been carried out (Fig. 5) in optimum conditions of their spectrophotometric determination.

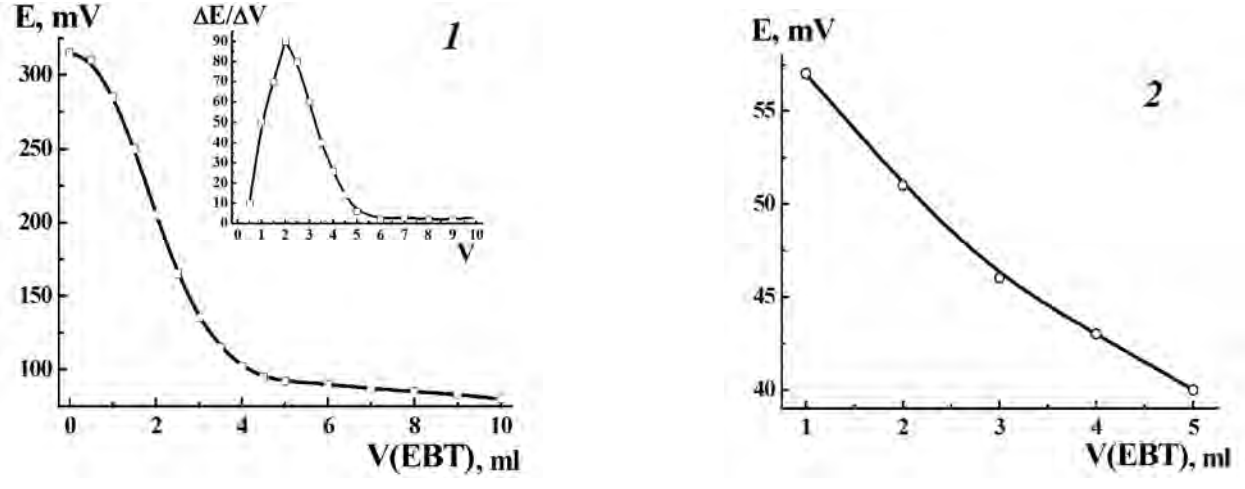


Fig. 5. Curves of the redox titration of SMZ diazonium salt (1) and blank solution (2) by the EBT solution. C_{HCl}=1.0 M, C_{SMZ}=1.75·10^{-3}M, C_{NaNO_2}=1.75·10^{-2}M, C_{Urea}=0.875 M, C_{EBT}=1.75·10^{-3}M, C_{UBM}=4.0·10^{-3} M

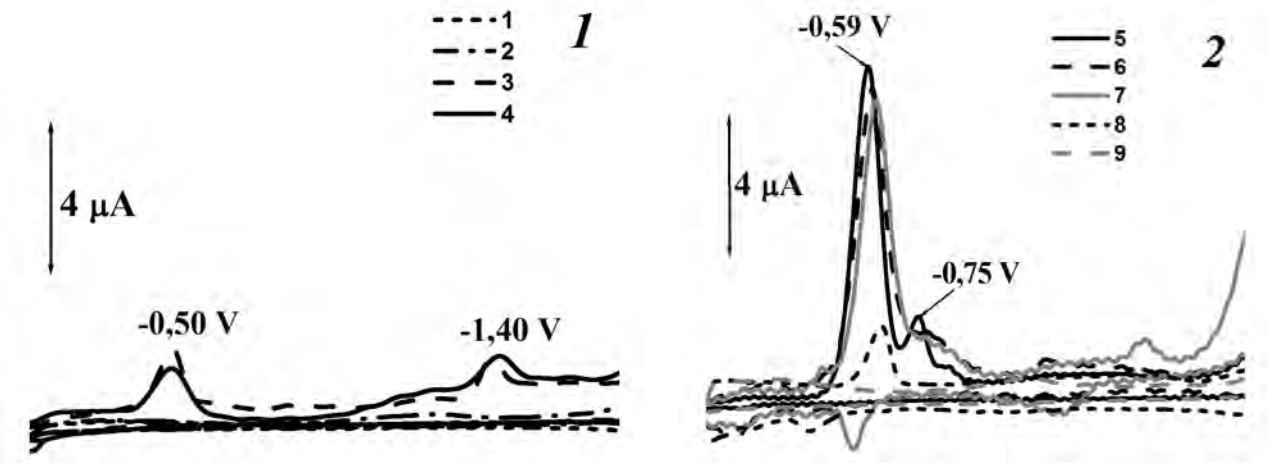


Fig. 6. Polarograms of the reduction processes: Urea (1), NO_2^- (2), SMZ (3), SMZ+NO_2^- (4), EBT (5), NO_2^-+EBT (6), (SMZ+NO_2^-+Urea)+EBT (7), α-naphthol (8), (SMZ+NO_2^-+Urea)+α-naphthol (9). C_{HCl}=1.0 M, C_{SMZ}=6.0·10^{-5}M, C_{NaNO_2}=8.0·10^{-4}M, C_{Urea}=4.0·10^{-2}M, C_{EBT}=3.6·10^{-4}M, C_{UBR}=4.0·10^{-3} M, pH = 8.0

3.4. Polarography of SA with EBT

Previously the polarographic activity of all the reagents that take part in the interaction of SA with EBT (Fig. 6 (1)) has been established.

It was established that in the conditions of a maximum efficiency of reaction products such substances as urea and nitrite ions are not polarographically active, as evidenced by the absence of peaks at the polarograms of their reduction process. With regard to the SMZ and its diazonium salt two peaks at -0.50 and -1.41 V are observed on the polarograms. In weak alkaline medium (pH = 8.0), where the interaction of SA with EBT takes place, the compounds, which contain a primary aromatic amino group (SA), or their diazonium salts can interact with each other to form azo compounds [36-40]. Peaks on polarogram correspond to reduction of formed azo groups.

The results of polarographic research of EBT and products of its interaction with SA diazonium salts are shown in Fig. 6 (2).

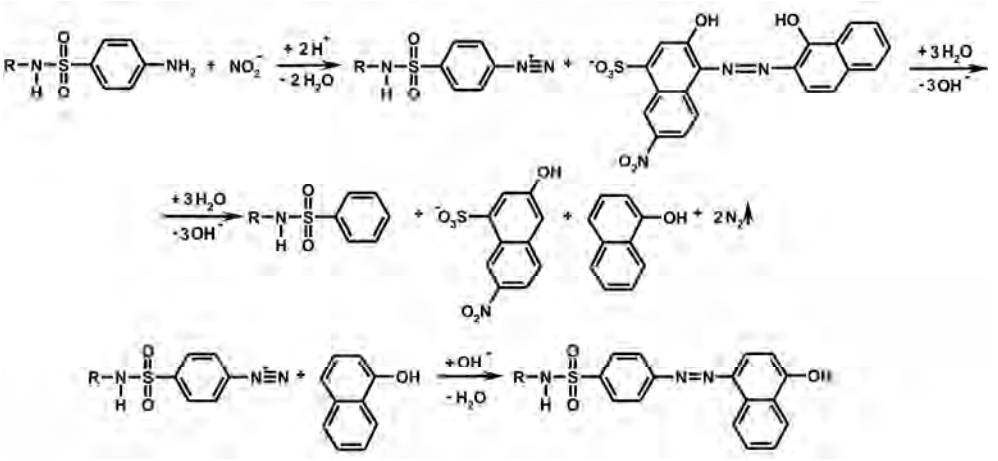
Two peaks of azo group at -0.59 and -0.75 V on polarogram of azo dye EBT redution are observed. The presence of urea does not influence the polarographic characteristics of azo dye. However, under the action of nitrite ions the peak height of azo dye reduction at -0.59 V is decreased and the peak at -0.75 V is converted into a broad shoulder, which indicates the destruction of the dye azo group. It is most probable that azo group is oxidized to azoxygroup or destroyed with the release of N_2, as follows from the literature on the oxidative properties of nitrite ions toward azo compounds [36-40]. As follows from the polarogram of azo dye reduction the excess of nitrite ions is completely destroyed under the action of urea, since polarograms at the presence as well as at the absence of nitrite ions are identical.

The polarogram curve of recovery of the diazonium salt SMT interaction product with EBT has a similar character as the polarogram of the product of the interaction of the dye with nitrite, namely the height of reduction peaks at -0.60 V is decreased and the peak at -0.75 V turns into a broad shoulder. This indicates the identical effects of both nitrite ions and SMZ diazonium salt on azo dye, namely the destruction of dye azo group under the action of these both reagents. Potential of the reduction peak of azo group of the product of SMZ diazonium salt interaction with α-naphthol is -0.55 V, however the current value of its reduction is very small, that is why the reduction peak of the EBT excess on the polarogram is much higher and masks the reduction peak of a newly formed azo compound.

The results of spectrophotometric research of the products interaction of the EBT with SA diazonium salts correspond to the results of polarographic and potentiometric research and allow to suggest the following scheme of SA interaction with EBT: oxidation of azo dyes under the action of SA diazonium salt with the following azocoupling of unreacted SA diazonium salts with α-naphthol, which is formed as a result of the azo dye destruction (Scheme 3).

3.5. Spectrophotometric and Metrologic Characteristics of SA Determination with EBT

We have investigated that the absorbance of colored products at the SA determination with EBT linearly depends on SA concentration in the solution. Validation results of spectrophotometric determination of ten SA by means of EBT are presented in Table 4.

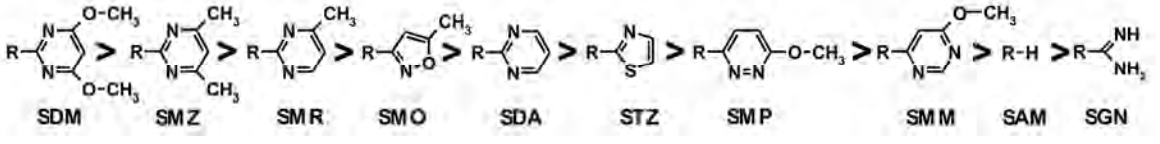


Sheme 3. Scheme of SA diazonium salts interaction with EBT

Table 4

Spectroscopic characteristics of the products of SA interaction with EBT and validation results of SA spectrophotometric determination. n = 3, P = 0.95

SA	$\varepsilon \cdot 10^{-3}$, M^{-1} cm^{-1}	Optimum photometric linear range, μg ml^{-1}	Calibration equation, C_{SA}, μg ml^{-1}	C_{LOD}, μg ml^{-1}	C_{LOQ}, μg ml^{-1}	R^2
SAM	2.2	7– 8	$\Delta A = 0.009 + 0.015 \cdot C$	2.41	7.23	0.9997
SMO	4.1	3–48	$\Delta A = 0.010 + 0.019 \cdot C$	1.05	3.15	0.9997
SMZ	4.2	3–48	$\Delta A = 0.017 + 0.018 \cdot C$	1.14	3.42	0.9995
SDM	4.8	3–48	$\Delta A = 0.014 + 0.017 \cdot C$	1.26	3.78	0.9995
SMR	4.2	3–48	$\Delta A = 0.012 + 0.017 \cdot C$	1.09	3.27	0.9994
STZ	3.3	3–48	$\Delta A = 0.013 + 0.013 \cdot C$	1.03	3.10	0.9992
SGN	1.6	14–64	$\Delta A = 0.013 + 0.014 \cdot C$	4.95	14.9	0.9998
SMP	2.8	7–48	$\Delta A = 0.011 + 0.016 \cdot C$	2.46	7.38	0.9998
SMM	2.7	7–48	$\Delta A = 0.012 + 0.015 \cdot C$	2.43	7.29	0.9997
SDA	3.4	3–48	$\Delta A = 0.011 + 0.019 \cdot C$	1.17	3.51	0.9997



Sheme 4. Series of sensitivity decrease for ten SA determinations at redox reaction with EBT

Table 5

The results of spectrophotometric SA determination with EBT in one-component medicines and veterinary drugs. C_{HCl} = 1.0 M, C_{SA} = 6.0·10^{-5} M, C_{NaNO_2} = 8.0·10^{-4} M, C_{Urea} = 4.0·10^{-2} M, C_{EBT} = 3.6·10^{-4} M, C_{UBM} = 4.0·10^{-3} M, pH = 8.0, λ = 485 nm, n = 5, P = 0.95

Determined SA (regulated content in preparation)	Amount of drug found $\bar{x} \pm \Delta x$ and relative standard deviation (S_r)	
	Pharmaceutical method (nitritometry)	Spectrophotometric method with EBT
"Intrauterine candle with sulphadimezine" suppository of the LLC "Basalt", Kyiv (excipients – polyethylene glycol, propylene glycol)		
Sulphamethazine (300 ± 30 mg/g)	298 ± 11 (0.032)	301 ± 8 (0.023)
"Streptocide" tablets of the PTC "Darnytsya" Kyiv (excipients – starch, gelatin, aerosil, calcium stearate)		
Sulphanilamide (500 ± 50 mg/tabl)	504 ± 16 (0.027)	501 ± 18 (0.031)

There is a correlation between the structure of the SA and the sensitivity of their determination with EBT. As one can see from the Scheme 4, the most sensitive are SDM and SMZ determinations, which contain six-membered heterocycles with two hetero atoms of nitrogen and two substituents of methoxy- or methyl-radicals that have +M-effect [35-37]. Less sensitive is the determination of SMR containing six-membered heterocycle with one substituent (methyl-radical), or unsubstituted SDA. STZ and SMO, whose molecules contain five-membered heterocycles with two different heteroatoms, nitrogen and Sulphur and nitrogen and oxygen, respectively, are determined with less sensitivity. The determinations of SA, which contain six-membered heterocycles with two atoms of nitrogen in the structure, but have one substituent, methoxyl radical, have lower sensitivity. SAM and SGN, whose molecules do not have heterocyclic substituents, can be determined with the lowest sensitivity.

3.6. Spectrophotometric Determination of SA in Dosage Forms

Developed methods of spectrophotometric determination of SA with EBT have been successfully used to establish the SA content in one-component drugs of various dosage forms, namely tablets and suppository. The results of SA determination in medicines are presented in the Table 5.

According to the assay results, which are presented in Table 5, the obtained SA content in medicines due to the developed technique using EBT, correlates rather well with the specified content of the SA by the manufacturer, as well as with the results obtained according to the technical documentation of investigated medicines. The value of S_r does not exceed common values of spectrophotometry errors.

4. Conclusions

1. The optimal conditions of SA diazonium salts interaction with *o*,*o′*-dihydroxysubstituted azo dye EBT have been established.
2. It was shown that the interaction of SA diazonium salts with EBT is based on the azocoupling reaction of SA diazonium salt with product of azo dye destruction. This leads to the formation of new colored azo compound.
3. The correlation between the SA structure and sensitivity of their determination with EBT has been detected. The determination sensitivity series of ten SA with azo dye has been established.
4. On the basis of carried out researches the method of spectrophotometric determination of ten SA has been elaborated.
5. The method is characterized by a wide linearity range (from 3 to 64 μg/ml depending on the detectable sulphanilamide), simplicity, rapidity, and reproducibility ($S_r \sim 0.040$).

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ЕРІОХРОМ ЧОРНИЙ Т – НОВИЙ АНАЛІТИЧНИЙ РЕАГЕНТ ДЛЯ СПЕКТРОФОТОМЕТРИЧНОГО ВИЗНАЧЕННЯ СУЛЬФАНІЛАМІДІВ

Анотація. *Встановлено оптимальні умови взаємодії діазосолей сульфаніламідів з о,о'-дигідроксизаміщеним азобарвником еріохром чорним Т на піставі чого розроблено методику спектрофотометричного визначення десяти сульфаніламідів у готових лікарських формах. В основі розробленої методики лежить реакція азосполучення діазосолі сульфаніламіду з продуктом розщеплення азобарвника, що приводить до утворення забарвленої азосполуки. Методика характеризується широкими межами лінійності (від 3 до 64 мкг/мл залежно від визначуваного сульфаніламіду), простотою, експресністю та відтворюваністю (S_r ~ 0,040).*

Ключові слова: *сульфаніламіди, еріохром чорний Т, спектрофотометрія, готові лікарські форми.*