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Spectrophotometric Determination of Sulfanilamides by a Condensation Reaction with *p*-Dimethylaminocinnamaldehyde

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Abstract—*p*-Dimethylaminocinnamaldehyde has been proposed as a reagent for the spectrophotometric determination of sulfanilamides. This reagent was shown to undergo in acetonitrile a condensation reaction with sulfanilamide, Sulfamethoxypyridazine, Sulfachloropyridazine, Sulfamethoxazole, and Sulfamethazine to form colored products. Optimal reaction conditions were found. A procedure for the spectrophotometric determination of sulfanilamide compounds with detection limit $n \times 10^{-2}$ μg/mL was developed. Determination of Sulfamethoxazole and Sulfamethazine in pharmaceuticals was carried out.

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Sulfanilamides (SA), derivatives of *p*-aminobenzenesulfonic acid, are widely used in pharmacy practice as efficient antibacterials. The safe and efficient use of these pharmaceuticals requires dosage control because of their toxicity. This feature implies the need for determination of sulfanilamides in different biological samples: biological fluids, blood plasma, and tissue sections. For this reason and on account of frequent drug adulteration, there is increasing need for rapid and reliable means for the determination of qualitative and quantitative composition of sulfanilamides in drug formulations and biological fluids.

Spectrophotometric [1–8], fluorimetric [9, 10], chromatographic [11–13], immunochemical [14–17], electroanalytical [18, 19], titrimetric [20, 21], and microbiological methods [22, 23] are used to determine sulfanilamides. The majority of spectrophotometric procedures for the determination of sulfanilamides in pharmaceuticals are based on diazotization reactions followed by coupling with 3-aminophenol [3], iminodibenzyl [4], prometazine hydrochloride (in the presence of *N*-bromosuccinimide) [5], 8-hydroxyquinoline [6], and other reagents [7, 8]. The substantial disadvantage of these procedures is their two-stage character.

The aim of this work is to study the condensation reaction of sulfanilamides with *p*-dimethylaminocinnamaldehyde (DMACA) in acetonitrile medium and to develop spectrophotometric procedure for their colorimetric determination. This reagent was previously used for the spectrophotometric determination of primary aromatic amines [24, 25].

EXPERIMENTAL

Subjects, Chemicals, and Equipment. Research subjects were sulfanilamide, Sulfamethoxypyridazine, Sulfachloropyridazine, Sulfamethoxazole, and Sulfamethazine (Sigma). Stock solutions of the sulfanilamides (0.01 M) were prepared by dissolution of weighted samples in acetonitrile. Working solutions were prepared by dilution of stock solutions immediately before use.

4-(*N*,*N*-dimethylamino)cinnamaldehyde (*p*-dimethylaminocinnamaldehyde) (DMACA) from ACROS was used as a spectrophotometric reagent. Initial solution of DMACA (0.01 M) was obtained by the dissolution of a weighted sample of the reagent in acetonitrile. The absorption spectra and optical density of solutions were recorded on an Akvilon SF-103 spectrophotometer (Russia), pH was determined on an Ekspert 001 ionometer (Russia).

RESULTS AND DISCUSSION

Optimization of Reaction Conditions for the Condensation of Sulfanilamides with DMACA. Sulfanilamides are known to enter condensation reactions with aromatic aldehydes [2]. A condensation reaction with *p*-dimethylaminobenzaldehyde to form highly colored Schiff bases is frequently used for the spectrophotometric determination of sulfanilamides. The reaction of sulfanilamides with *p*-dimethylaminocinnamaldehyde was not studied previously.

Preliminary studies showed that the condensation reaction of *p*-dimethylaminocinnamaldehyde with sulfanilamides in aqueous medium yields a poorly soluble product. On the contrary, highly colored soluble con-

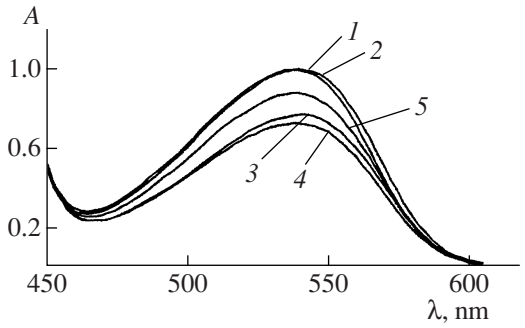


Fig. 1. Absorption spectra of products of *p*-dimethylaminocinnamaldehyde condensation with (*1*) sulfanilamide, (*2*) Sulfamethoxypyridazine, (*3*) Sulfachloropyridazine, (*4*) Sulfamethoxazole, and (*5*) Sulfamethazine in acetonitrile; $c_{SA} = 2 \times 10^{-5}$ mol/L, $c_{DMACA} = 2 \times 10^{-3}$ mol/L, c_{HCl} = 0.02 mol/L, and 10 vol % H_2O.

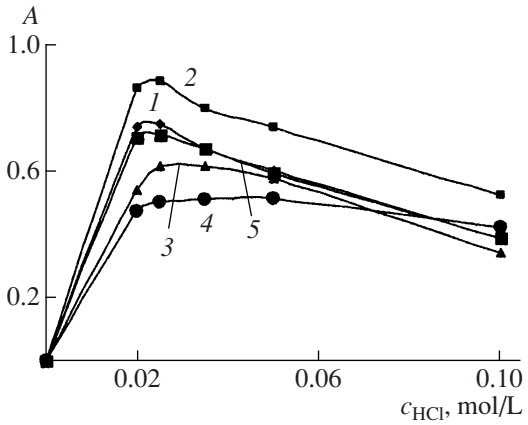
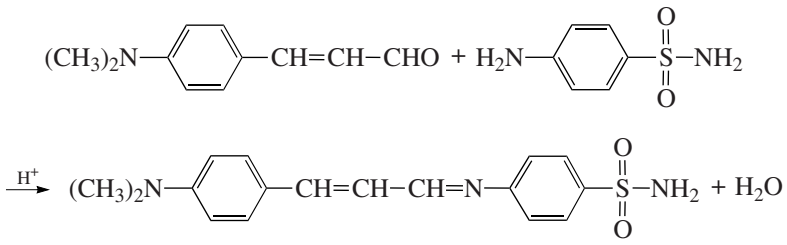


Fig. 2. Optical density vs. hydrochloric acid concentration for the products of condensation of *p*-dimethylaminocinnamaldehyde with (*1*) sulfanilamide, (*2*) Sulfamethoxypyridazine, (*3*) Sulfachloropyridazine, (*4*) Sulfamethoxazole, and (*5*) Sulfamethazine. $c_{DMACA} = 2 \times 10^{-3}$ mol/L, $c_{SA} = 2 \times 10^{-5}$ mol/L, λ_{max} = 540 nm, and 10 vol % H_2O.

densation products appear in nonaqueous solvents (methanol, acetonitrile). We have studied the reaction of sulfanilamides with DMACA in acetonitrile in more detail. Acetonitrile was selected because it was often used in foodstuff sample preparation for sulfanilamide extraction [26] and as an eluent in solid-phase extraction and HPLC. In addition, acetonitrile is less toxic than methanol.

To reveal the optimal conditions of the condensation reaction, we have studied the reaction of DMACA with sulfanilamides with varying sulfanilamide, component concentration (DMACA, HCl), and water content. The highest yield of condensation products was after 10–15 min. Figure 1 shows the absorption spectra of the products of DMACA condensation with sulfanilamide, Sulfamethoxypyridazine, Sulfachloropyridazine, Sulfamethoxazole, and Sulfamethazine; their comparison reveals no marked difference in the spectral characteristics of the condensation products. Absorption maxima are at 540 nm, while molar absorption coefficients are $(3.7–5.1) \times 10^4$; these data can be used to develop a procedure for the determination of total sulfanilamides.

By analogy with the literature data on the mechanism of the reaction of sulfanilamides with *p*-dimethylaminobenzaldehyde and aromatic amines with DMACA [2, 24, 25], one can suppose that sulfanilamides react with DMACA according to the scheme below.



Scheme

Water content in the system is a significant factor affecting the yield of colored products. For all sulfanilamides studied, optical density reached a maximal value in solutions containing 10% of water and decreased with increasing water content from 10 to 60%.

It was found that the concentrations of hydrochloric acid (Fig. 2) and DMACA (Fig. 3) affected the yield of the condensation products. The highest yield of the condensation products was observed within HCl concentrations of 0.020–0.025 M at constant DMACA concentration of 2×10^{-3} M. In choosing optimal DMACA concentration, we took into account that the time of a control experiment increased markedly as reagent concentration rose from 1×10^{-3} to 1.5×10^{-2} M.

On the basis of our studies, we found optimal conditions for the condensation of sulfanilamides with DMACA in acetonitrile: c_{HCl} = 0.02 mol/L, $c_{DMACA} = 2 \times 10^{-3}$ mol/L, 10 vol % of water, and product color development for 15 min.

Table 1. Metrological characteristics of procedures for the spectrophotometric determination of sulfanilamides with *p*-dimethylaminocinnamaldehyde

Compound	Calibration cruve equation	Range of determinable concentrations, mol/L (μg/mL)	c_{min}, mol/L (μg/mL)
Sulfanilamide	$y = 46887c$	7.8×10^{-7}–2×10^{-5} (0.12–3.4)	2.6×10^{-7} (0.04)
Sulfamethoxypyridazine	$y = 42272c$	8.4×10^{-7}–2×10^{-5} (0.24–5.6)	2.8×10^{-7} (0.08)
Sulfachloropyridazine	$y = 30213c$	1.2×10^{-6}–2×10^{-5} (0.33–5.7)	4.0×10^{-7} (0.11)
Sulfamethoxazole	$y = 27405c$	1.3×10^{-6}–2×10^{-5} (0.33–5.1)	4.4×10^{-7} (0.11)
Sulfamethazine	$y = 34196c$	1.1×10^{-6}–2×10^{-5} (0.30–5.6)	3.5×10^{-7} (0.10)

Determination of Sulfanilamides. To construct calibration curves, we prepared a series of solutions containing from 2×10^{-6} to 2×10^{-5} mol/L (0.34–5.7 μg/mL) of sulfanilamide. Hydrochloric acid (0.5 mL of 0.2 M solution), 1 mL of 0.01 M DMACA solution in acetonitrile, and acetonitrile (to 5 mL) were added sequentially to each solution. The optical density of solutions was measured at 540 nm. The metrological characteristics of the determination procedures are presented in Table 1. The detection limits calculated from 3S criterion were 40, 80, 110, 110, and 100 ng/mL for sulfanilamide, Sulfamethoxypyridazine, Sulfachloropyridazine, Sulfamethoxazole, and Sulfamethazine, respectively. Thus, the procedure allows one to determine sulfanilamides at the level of 0.4–1.0 of the maximum permissible concentration for waters. The adequacy of spectrophotometric determinations of sulfanilamides was tested for model mixtures by added/found analysis (Table 2).

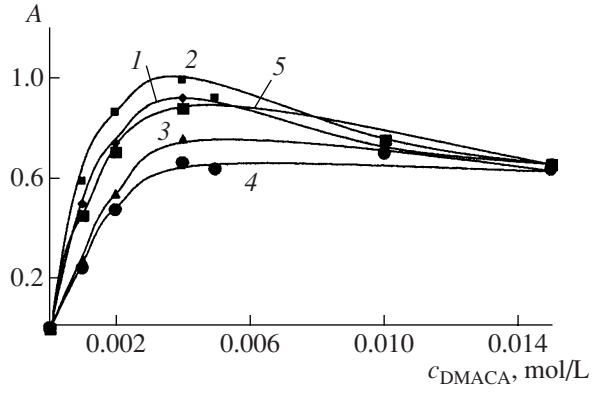


Fig. 3. Optical density vs. aldehyde concentration for the products of condensation of *p*-dimethylaminocinnamaldehyde with (*1*) sulfanilamide, (*2*) Sulfamethoxypyridazine, (*3*) Sulfachloropyridazine, (*4*) Sulfamethoxazole, and (*5*) Sulfamethazine. $c_{SA} = 2 \times 10^{-5}$ mol/L, $c_{HCl} = 0.02$ mol/L, $\lambda_{max} = 540$ nm, 10 vol % H_2O.

Determination of Sulfanilamides in Pharmaceuticals. To assess the practical utility of the spectrophotometric procedure, we determined Sulfamethoxazole in Biseptol pharmaceutical and Sulfamethazine in Zinaprim drug (which is used in veterinary). Trimetoprim, a component of drugs, was shown to be inert in the condensation with *p*-dimethylaminocinnamaldehyde.

A Biseptol tablet (0.6723 g) was powdered and dissolved in 100 mL of acetonitrile. An aliquot was taken from this solution for determination. Using addition method, it was found that the pharmaceutical contains 405 ± 60 mg of Sulfamethoxazole ($s_r = 0.06$), which agrees well with data certified by the producer (400 mg in 1 tablet).

Zinaprim as a solution for injections was diluted, and Sulfamethazine was determined by the calibration curve method. The drug contained 190 ± 20 mg of Sulfamethazine ($s_r = 0.04$), which also agrees well with the certificate (200 mg/mL).

To summarize, our studies showed a possibility to use *p*-dimethylaminocinnamaldehyde as a reagent for the spectrophotometric determination of sulfanilamides. The determination procedure is characterized by low detection limits, simplicity, and good reproducibility.

Table 2. Validation of the procedure for determination of sulfanilamides by added/found analysis ($n = 3$; $P = 0.95$)

Compound	Added, μg/mL	Found, μg/mL	s_r
Sulfanilamide	3.4	3.3 ± 0.5	0.06
Sulfamethoxypyridazine	5.6	5.4 ± 0.7	0.05
Sulfachloropyridazine	5.7	5.5 ± 0.4	0.03
Sulfamethoxazole	5.1	5.1 ± 0.4	0.03
Sulfamethazine	5.6	5.3 ± 0.5	0.04

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