

Chromatography of the Tetracyclines. A new column containing the silica gel Partisil PXS 5 was washed with water, with 50% acetonitrile, with water, and with 0.1 N  $\text{NaH}_2\text{PO}_4$  brought to pH 1.0 with nitric acid and saturated with EDTA, and, finally, it was equilibrated with a mobile phase consisting of 99.5% of 0.01 N  $\text{NaH}_2\text{PO}_4$ , pH 1.0, saturated with EDTA, and 0.5% of acetonitrile. Elution was performed at the rate of 1 ml/min at a sensitivity of 0.01, the detection of the tetracycline being performed at 355 nm.

Extraction of the Tetracyclines from Blood Plasma. To 1 ml of plasma was added a standard solution of tetracyclines and 50  $\mu\text{l}$  of an aqueous solution of cetyltrimethylammonium bromide (5 mg/ml), the mixture was carefully stirred, and the antibiotics were extracted with ethyl acetate ( $3 \times 3$  ml) for 5 min. The suspension was separated by centrifugation, the ethyl acetate layer was evaporated in vacuum, the dry residue was dissolved in 0.5 ml of 0.01 N HCl, and an aliquot (20  $\mu\text{l}$ ) was deposited directly on the column of the chromatograph.

#### SUMMARY

1. A new method is proposed for separating the antibiotics tetracycline, oxytetracycline, methacycline, and doxycycline on silica gel by high-performance liquid chromatography.
2. A new variant of the extraction of tetracyclines from plasma into an organic phase with the aid of a carrier — cetyltrimethylammonium bromide — has been developed. The method permits the determination of about 0.3  $\mu\text{g}$  of the antibiotics in 1 ml of blood plasma.

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#### INFLUENCE OF AMINES ON THE CHANGE IN THE SPECTRAL PROPERTIES OF ALKALINE SOLUTIONS OF SODA LIGNIN AND THE TRANSFORMATION OF SULFATE CELLULOSE ON PHOTOIRRADIATION

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It has been shown that in the irradiation with light of alkaline solutions of soda lignin and suspensions of sulfate cellulose additions of ethylenediamine, monoethanolamine, and hydrazine lead to a decrease in the optical density in the visible region of the absorption spectra of the samples studied.

The yellowing of lignocellulose materials under the action of sunlight is connected with photochemical reactions leading to the degradation of the components of wood. A leading role in this process is played by the chromophoric groups of the lignin, which are formed as the result of its oxidation and photochemical decomposition. The chromophores formed in the cellulose and the extraction substances amount to only 10% and less than 1%, respectively, [1]. The absorption of lignin and technical celluloses in the visible region of the spectrum is due, in the opinion of many workers, to the presence of chromophores containing carbonyl groups conjugated with aromatic rings, quinone methide groupings and diphenyl structures, and also  $\alpha, \beta$ -conjugated double bonds [2].

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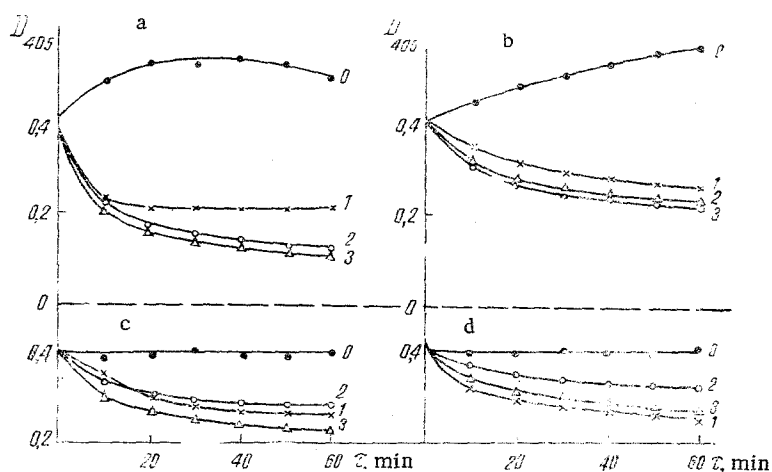


Fig. 1. Dynamics of the change in optical density ( $D_{405}$ ) of alkaline solutions of soda lignin on irradiation through various filters: a)  $\lambda_{50\%} = 195$  nm; b)  $\lambda_{50\%} = 275$  nm; c)  $\lambda_{50\%} = 330$  nm; d)  $\lambda_{50\%} = 430$  nm; 0) initial; 1) with the addition of EDA; 2) with MEA; 3) with hydrazine.

It is extremely important to find methods for solving the problem of reducing the coloration (yellowing) of cellulose without intensifying its degradation.

We have established previously that additions of certain amines in the cooking of wood chips and also during treatment under cooking conditions of isolated lignins lead to a reduction in the coloration of the solutions of lignin and of wood residue [3, 4]. Amines — in particular, triethylamine, triethanolamine, and ethylenediaminetetraacetic acid — have also been used as reducing agents in photochemical processes [5]. Consequently, we have attempted to use hydrazine, monoethanolamine (MEA) and ethylenediamine (EDA) as reducing agents in photochemical transformations of soda lignin in alkaline solutions and of the residual lignin in sulfate cellulose with the aim of reducing the coloration and preventing the degradation of the cellulose.

When alkaline solutions of Björkman lignin are irradiated in UV light, their chromophoric composition changes considerably, the increase in the optical density of the solutions in the 330–350 nm region (the region of the absorption of the oxidized phenolic elements of the lignin during photolysis) being proportional to the energy of the exciting light [6].

The change in the optical density  $D$  of solutions of soda lignin in the visible regions of the spectrum on photoirradiation is shown in Fig. 1. While on irradiation of lignin solutions without the addition of reducing agents the coloration intensifies, the addition of monoethanolamine, ethylenediamine, and hydrazine appreciably lowers the value of  $D$  relative to the initial value (the original value of the optical density for all the solutions was normalized to the value of  $D$  of the solution without additives). With an increase in the wavelength (decrease in the energy of the quanta) of the exciting light, the rate of change of  $D$  falls.

The results on the irradiation of suspensions of cellulose show that for each of the media in which irradiation was carried out its own limiting value of the degree of bleaching is reached in time (Fig. 2). The efficiency of the bleaching of the chromophores of the individual lignin rises on passing from an alkaline medium without addition of the reducing agents to media with the addition of EDA, MEA, and hydrazine, respectively.

The dynamics of the change in the degree of polymerization (DP) of the cellulose and the accumulation of carbonyl groups during photolysis is shown in Figs. 3 and 4. On comparing these results it can be observed that the processes involved in the degradation of the cellulose are accompanied by an increase in the number of carbonyl groups. The destruction of sulfate cellulose (and, correspondingly, the rise in the number of carbonyl groups) on photolysis is most pronounced in an alkaline medium with EDA. Additions of hydrazine and MEA retard these processes as compared with photolysis in an alkaline medium without a reducing agent. The action of MEA has a more complex nature. Initially, as compared with a blank

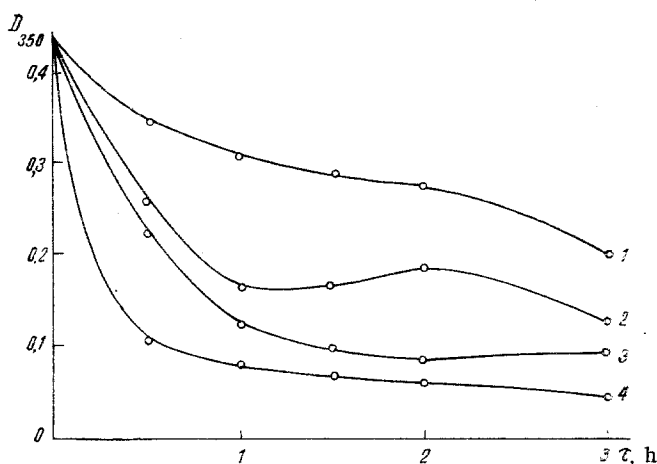


Fig. 2. Dynamics of the change in the optical density ( $D_{350}$ ) of cadoxene solutions of sulfate cellulose on irradiation ( $\lambda_{50\%} = 195$  nm): 1) irradiation in NaOH; 2) with the addition of EDA; 3) with MEA; 4) with hydrazine.

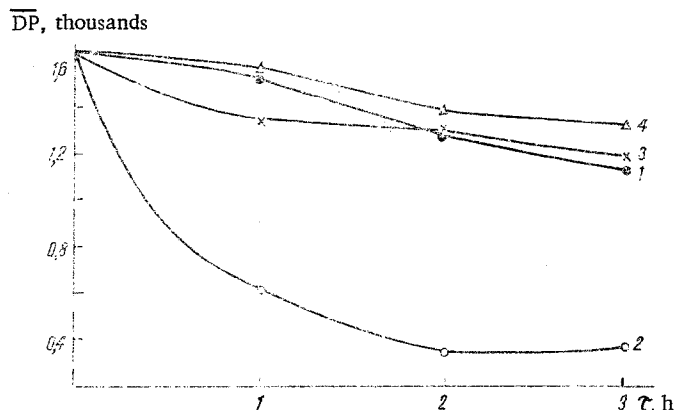


Fig. 3. Dynamics of the change in the DP of sulfate cellulose on irradiation ( $\lambda_{50\%} = 195$  nm): 1) irradiation in NaOH; 2) with the addition of EDA; 3) with MEA; 4) with hydrozine.

experiment, in MEA the degradation of the cellulose and the accumulation of carbonyl groups predominate, and then (after irradiation for approximately 1.5-2 h) these processes stabilize.

The fact that the amines that we added in the photolysis of cellulose affect the processes involved in its degradation and the accumulation of carbonyl groups differently can probably be explained by their different stabilities to the action of light and also by different mechanisms of the phototransformations.

#### EXPERIMENTAL

Solutions of soda lignin in 1 N NaOH without additives and with the addition of hydrazine, MEA, and EDA (4 M solutions) were subjected to photoirradiation. The preparation of soda lignin was obtained by the following method: Pine chips were heated in an autoclave in 1 N NaOH at 160°C for 3 hours. The lignin was precipitated from the lye by acidification and was washed with water to neutrality, after which it was placed in a desiccator and dried over  $P_2O_5$ . Analysis of the functional groups of the material obtained showed that it contained the following, %:  $[CO] = 4.12$ ;  $[COOH] = 2.45$ ;  $[OH]_{phen} = 4.18$ ;  $[OH]_{aliph. tot} = 5.1$ ;  $[OH]_{prim.} = 2.53$ ;  $[OH]_{sec.} = 2.57$ ;  $[OCH_3] = 13.5$ . The analysis was performed by standard methods [7].

Irradiation was performed with light from a DRL-1000 medium-pressure mercury lamp through liquid cut-off filters [8] and through quartz glass (thickness 10 mm). As the wavelength at which the maximum change in optical density was observed we took the value of  $\lambda$  at 50% transmission of the filter. The  $\lambda_{50\%}$  values of the filter used were: for quartz 195 nm, for

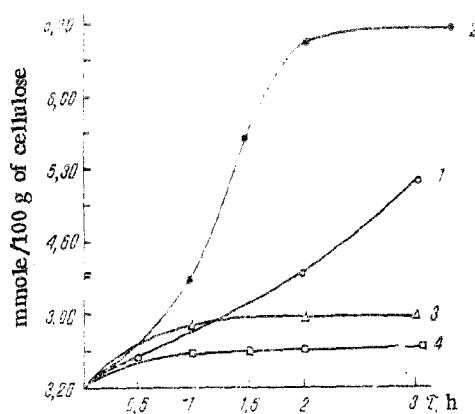


Fig. 4. Dynamics of the accumulation of carbonyl groups on the irradiation of sulfate cellulose ( $\lambda_{\text{exc}} = 195$  nm): 1) irradiation in NaOH; 2) with the addition of EDA; 3) with MEA; 4) with hydrazine.

carbon tetrachloride 275 nm, for  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (57 g/liter) 330 nm, and for nitrobenzene 430 nm. The photometry of the solutions in the visible region of the spectrum (after irradiation for 10 minutes) was carried out on an Eppendorf spectrophotometer,  $\lambda = 405$  nm,  $C_{\text{lignin}} = 0.1$  g/liter). In the UV region, the spectra were measured on an SF-16 spectrophotometer.

Unbleached sulfate cellulose (1% weight) was placed in a 1 N NaOH solution and 4 M alkaline solutions of hydrazine, MEA, and EDA. Irradiation was carried out in the full light of the mercury lamp at a distance of 7.5 cm from the cell containing the sample, with continuous stirring by a magnetic stirrer. After irradiation the cellulose was filtered off, washed with water to neutrality, and pressed out. The decantates were dried at  $100^\circ\text{C}$  for 12 h. The change in the coloration of the residual lignin was checked by measuring the UV absorption spectra of cadoxene solutions of the cellulose, and the degree of degradation from the change in viscosity. The amount of carbonyl groups in the cellulose was determined by Sabolks' method [9].

#### SUMMARY

1. Additions of ethylenediamine, monoethanolamine, and hydrazine as reducing agents in the photolysis of lignin and of technical cellulose lead to the decoloration of the colored structural elements of the lignin. The efficacy of reducing the coloration of lignocellulose materials is determined by the type of radiation and the type of additive.

2. In the presence of monoethanolamine and hydrazine the reduction in the coloration of sulfate cellulose on photolysis is not accompanied by an appreciable intensification of its degradation.

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