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Photometric Determination of Urea by Inhibition of Methyl Orange Oxidation by Bromate Ion

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ABSTRACT

Gayfulina R.R. Photometric Determination of Urea by Inhibition of Methyl Orange Oxidation by Bromate Ion – Chelyabinsk: SUSU, ET-451, 2017. – 43 p., 19 fig., 13 tables, 18 references.

A kinetic method (the initial rate method) was studied in order to optimize the reaction conditions for methyl orange reaction with potassium bromate, used for determination of urea as an inhibitor in acidic medium.

The aim of the study is optimization of the photometric determination of urea by inhibition of methyl orange oxidation by bromate ion and its recovery from cosmetic products.

In order to achieve the research aim the following objectives have been met:

– the literature review in the research area;
– the influence of variables;
– the analysis of urea-containing cosmetic creams;
– the calculation of metrological characteristics.

Optimal conditions for determination: 0.257 M HCl, $6 \cdot 10^{-5}$ M methyl orange, $1.3 \cdot 10^{-3}$ M $KBrO_3$. The calibration graph is linear in the range (0.2-1.2) $\cdot 10^{-5}$ M urea concentration, which corresponds to (12–72) μg/mL. The optimized method was used to determine urea in cosmetic creams; the error in determination of known amount did not exceed 4.8 %, the systematic errors were not significant.

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INTRODUCTION

Urea (NH_2CONH_2) is an important constituent of many products of pharmaceutical, food and fertilizer industries and its determination is required for quality control of the products as well as for monitoring ground and river water. It is the final product of protein and amino acid metabolism. Determination of urea is required for clinical and pathological monitoring [1].

Urea is an important endogenous product of mammalian metabolism. This may partly explain why it has not been rigorously studied with toxicological tests. Nevertheless, urea appears to cause little or no toxicity to most mammalian species (ruminants are an exception) and humans at reasonable dose levels. Although urea generally has a low acute ecotoxicity to organisms, its well-documented indirect and long-term effects to the ecosystems, *e.g.* eutrophication, groundwater pollution, soil acidification, and ammonia emissions to air, should be considered [2].

Urea-containing creams are used as topical dermatological products to promote rehydration of the skin. Urea 40 % is indicated for psoriasis, xerosis, onychomycosis, ichthyosis, eczema, keratosis, keratoderma, corns, and calluses. If covered by an occlusive dressing, 40 % urea preparations may also be used for nonsurgical debridement of nails. Urea 40 % dissolves “the intercellular matrix” of the nail plate. Only diseased or dystrophic nails are removed, as there is no effect on healthy portions of the nail. This drug is also used as an earwax removal aid [3].

The aim of our study is to investigate the method of kinetic photometric determination of urea by inhibition of methyl orange oxidation by bromate ion, to find optimal conditions of urea determination, to analyze samples of cosmetic products and to calculate the metrological characteristics of urea analysis by the studied method.

1 LITERATURE REVIEW

The kinetic method of analysis is based on the measurement of the rate of the chemical reaction, but it can be realized in various modifications, such as the fixed-time method, the fixed-concentration method, the initial rate method.

A useful scheme for classifying chemical kinetic methods of analysis is shown in Figure 1.1. Methods are divided into two main categories [4].

Figure 1.1 – Classification of chemical kinetic methods of analysis.

Every chemical reaction occurs at a finite rate and, therefore, can potentially serve as the basis for a chemical kinetic method of analysis. To be effective, however, the chemical reaction must meet three conditions.

First, the rate of the chemical reaction must be fast enough that the analysis can be conducted in a reasonable time, but slow enough that the reaction does not approach its equilibrium position while the reagents are mixing. As a practical limit, reactions reaching equilibrium within 1 s are not easily studied without the aid of specialized equipment allowing for the rapid mixing of reactants.

A second requirement is that the rate law for the chemical reaction must be known for the period in which measurements are made. In addition, the rate law should allow the kinetic parameters of interest, such as rate constants and concentrations, to be easily estimated.

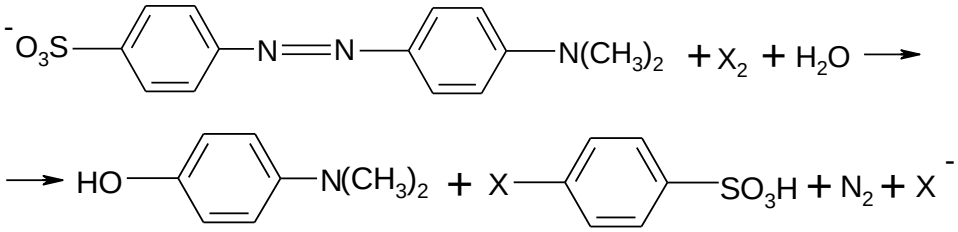
A final requirement for a chemical kinetic method of analysis is that it must be possible to monitor the reaction's progress by following the change in concentration for one of the reactants or products as a function of time [4].

Various ways of analytical signal detection are possible; one of the most widespread techniques is spectrophotometrical signal detection. For example, it is in pharmaceutical analysis, due to its inherent simplicity, economic advantage, and wide availability in most quality control laboratories. The application of these methods offers some specific

advantages over classical spectrophotometry, such as improved selectivity due to the measurement of the evolution of the absorbance with the reaction time [4].

Using organic dyes, it is possible to increase the sensitivity of kinetic photometric determination and carry out the measurement in the visual light. For example, a sensitive kinetic spectrophotometric method has been developed for rapid determination of trace quantities of iodate. The method is based on the accelerating effect of iodate on the reaction of bromate and hydrochloric acid, with decolorizing methyl orange as the product of the reaction. It goes very fast in acidic medium, but the presence of hydrazine conveniently slows it. The reaction is monitored by measuring the decrease of absorbance at 525 nm [5]. The chemical bases of the method is described by the following chemical reactions (Scheme 1.1), the produced chlorine and bromine react with methyl orange and decolorize it:

$$2BrO_3^- + 10\ Cl^- + 12\ H^+ \rightarrow Br_2 + 5\ Cl_2 + 6\ H_2O.$$



(X = Cl; Br).

Scheme 1.1

The initial rate method is based on determining the tangent of the slope angle of the kinetic curves (tgα) at known concentrations of the substance being determined. In this case, tgα characterizes the rate of the indicator reaction .The calibration graph is plotted in coordinates: tgα as the function of concentration of the analyte.

The initial rate method is applicable for various types of reactions. Some difficulties can arise only when complex multi-stage reactions are used. Since a number of experimental measurements are used in the initial rate method, the probability of an outlier (loss of the result) is small here, and the accuracy of the determination exceeds the accuracy of the determination by the remaining methods, such as the fixed-time method, the fixed concentration method, and others [6].

Kinetic methods of analysis are based on the rate at which a chemical or physical process, involving the analyte, occurs. Chemical kinetic methods are based on the rate at which a chemical reaction, involving the analyte, proceeds. Either the integrated or differential form of the rate law may be used. When using an integral method, the concentration of analyte, or a reactant or product stoichiometrically related to the analyte, is determined at one or more points in time following the reactions initiation. The initial concentration of analyte is then determined using the integral form of the reaction rate law [7].

Different methods such as potentiometric, fluorometric, enzymatic, amperometric, spectrophotometric and colorimetric methods have also been reported for the

determination of urea and no single technique is dominant in all areas, because of the diversity of applications. At present methods are often categorized as direct and indirect methods.

1. The term indirect usually refers to the enzyme degradation of urea prior to detection.

2. Direct procedures have been defined as those resulting in a colored product that does not include prior degradation, but this category is often expended to include miscellaneous methods that do not involve enzymatic degradation or colorimetric, such as manometric, infrared or ultraviolet/visible absorbance measurements [2].

The enzymatic methods for determining urea are quite numerous and all consist of two stages. The first stage is common for all urease methods. Its essence lies in the fact that under the action of urease hydrolysis of urea to ammonia occurs. At the second stage, the concentration of ammonia formed at the first stage is determined. Depending on the methods used to record the ammonia concentration, the enzymatic (urease) methods for the determination of urea can be divided into the following groups.

Colorimetric at the end point

In this group of methods, in the second stage, ammonia reacts with the chromogenic complex to form a colored compound that is photometric at a wavelength of 580 to 600 nm. The intensity of the color is directly proportional to the concentration of urea in the test material [9].

Methods using conjugated enzyme reactions

In these methods for determining urea, the level of ammonia in the second stage is determined using the combined enzymatic reactions. The most popular method for determining urea in serum or urine is based on the use of glutamate dehydrogenase as an indicator enzyme. It should be noted that many enzymes contained in serum are able to compete with glutamate dehydrogenase for coenzyme nicotinamide adenine dinucleotide $NADH_2$ and interfere with the results of the study. In addition, ammonia, which can be contained in the composition of reagents, can lead to false overestimation of the results [10].

Urea is hydrolysed in presence of urease to produce ammonia and CO_2 (Scheme 1.2):

$$(NH_2)_2CO + H_2O + 2\ H^+ \rightarrow \quad 2\ NH_4^+ + CO_2.$$

Scheme 1.2

The ammonia produced combines with 2 – oxoglutarate and NAD(H) in presence of glutamate dehydrogenase GLDH to yield glutamate and NAD [11] (Scheme 1.3):

Analytical sensitivity expressed as detection limit: 5 mg/dL (0.83 mmol/L). The lower detection limit represents the lowest measurable urea activity that can be distinguished from zero [11].

$$2\ NH_4^+ + \text{2-Oxoglutarate} + 2\ NADH \rightarrow H_2O + 2\ NAD^+ + \text{Glutamate.}$$

Scheme 1.3

Another possibility of the produced ammonium ion determination is the reaction with α-ketoglutarate in a reaction catalysed by GLDH with simultaneous oxidation of NADH to NAD^+ (Scheme 1.4) [12]:

$$2\ NH_4^+ + \alpha\text{-ketoglutarate} + 2\ NADH \rightarrow H_2O + 2\ NAD^+ + \text{L-Glutamate.}$$

Scheme 1.4

The decrease in absorbance due to the decrease of NADH concentration in unit time is proportional to the urea concentration.

Measuring range: from detection limit 1 mg/dL to linearity limit 350 mg/dL. The obtained results did not show systematic differences when compared with other commercial reagents. The results of the performance characteristics depend on the analyzer used [12].

For the determination of small quantities of urea in biological fluids and microdialysate the luminometric kinetic method has been suggested. The method is based on the use of urease that breaks down urea in ATP-dependent reactions. The rate of hydrolysis of ATP is proportional to the urea content in the sample. A similar approach was used for real-time monitoring of the efficiency of hemodialysis [10].

To date, various urea assay systems have been designed based on different physicochemical principles, such as conductance, potentiometry, voltammetry, colorimetric and spectrometric methods. Among these protocols, fluorescent sensing strategy is superior due to its high sensitivity, nondestructive nature, low background noise and wide dynamic ranges [13].

Electrochemical methods for the determination of urea are widely used. In this group of methods, the reaction rate is estimated from the change in the electrical conductivity of the medium during the urea hydrolysis reaction by urease. The CO_2 and ammonia formed in the urease reaction increase the electrical conductivity of the reaction mixture. With the kinetic method of analysis, both serum and urine samples can be analyzed [9].

To record the urease response rate, ion-selective electrodes are used in some analyzers. In the potentiometric method, an ammonium-selective electrode with immobilized urease is used. This principle has been used in various analyzers. Potentiometric methods for the determination of urea are economical, accurate and fast, but require special equipment (ion-selective analyzer or block) [9].

Methods using the technology of "dry chemistry» for determining the concentration of urea in the blood serum consists in using a reaction between ammonia and a pH-indicator. This approach is used in the technology of "dry chemistry" in the form of test strips with subsequent visual evaluation of the results using reflective photometry. It is

believed that methods using the technology of "dry chemistry" are highly accurate, as they do not experience significant interference from outside connections. The main disadvantage of these methods is their "closeness", i.e., strict adaptation to certain analyzers [10].

A simple but effective fluorescent platform for urea sensing with pH-sensitive grapheme quantum dots (GQDs) as the signal output was described in [13]. The fluorescence of GQDs is greatly decreased with the addition of urea due to the increase of pH caused by the urease-catalyzed hydrolysis of urea (Figure 1.2). There is a good linear relationship between the fluorescence intensity of the GQDs and the urea concentration in the range of 0.1–100 mM. The fluorescent sensing system has been successfully used for the assay of urea in human serum.

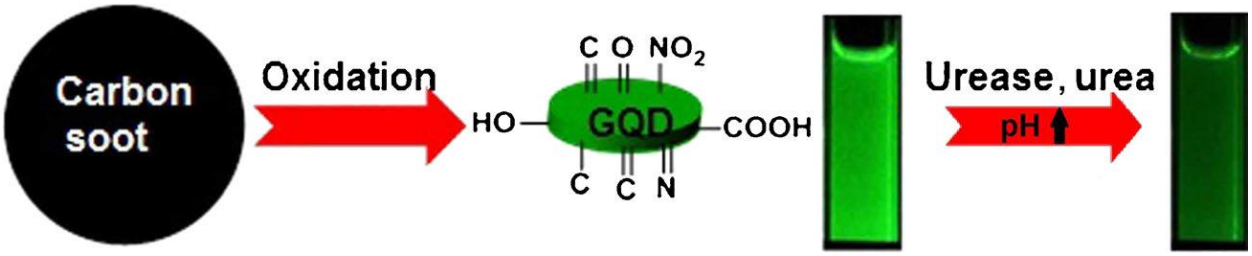


Figure 1.2 – The graphical representation for fabrication of pH-sensitive GQDs and working principle for urea sensing

By the progress of thermometric methods and the increased sensitivity of both chemical and instrumental techniques, the amounts of material for the tests could substantially be reduced during the past decade, and hence the potential of biochemical applications was increased. Also, continuous analyses and computerized data processing became feasible.

Trischler developed a procedure to determine urea in fertilizers. It is based on oxidation performed with excess hypobromite, reduction of the excess with sodium sulfite and measurement of the heat of reaction. Halász and co-workers [14] developed a method for the joint determination of urea and ammonium salts in fertilizers, based on the fortunate coincidence that the heat of reaction for the two compounds with hypobromite is very similar (Scheme 1.5):

$$(NH_2)_2CO + 3\ BrO^- \leftrightarrow N_2 + 3\ Br^- + 2\ H_2O + CO_2,$$

$$\Delta H = -280.9\ \text{kJ/mol};$$

$$2\ NH_3 + 3\ BrO^- \leftrightarrow N_2 + 3\ Br^- + 3\ H_2O,$$

$$\Delta H = -270.5\ \text{kJ/mol}.$$

Scheme 1.5

It seems promising to determine urea and creatinine by the catalytic electrochemical oxidation of analytes with the chronoamperometric detection of the signal. The major problem of the chronoamperometric analysis of urea and creatinine in multicomponent solutions and real samples refers to its insufficient selectivity toward the analytes. The problem can be solved using additional steps of the separation and preconcentration of the target components [15].

Of great interest is the direct spectrophotometric determination of urea. For the detection of urea, yellow-green coloration is obtained when the solution reacts with p-dimethylaminobenzaldehyde (DMABA) in the presence of HCl, the detection limit is 2 mg / L. The formation of the yellow urea compound with DMABA is the basis of the method for determining the mass fraction of amide nitrogen in fertilizers, the range of determined contents is 20–46 % by weight (160–320 mg of urea were introduced into flasks with a capacity of 100 ml to construct the calibration curve) [16].

There are systems for the determination of reductants based on the fact that there is a reaction of oxidation of methyl orange and the presence of nitrogen-containing compounds with reducing properties slow down this reaction. Therefore, kinetic determination is possible.

There is a possibility of iodate and periodate determination by different kinetic behaviours of the analytes, such as their consecutive reactions with iodide-starch system at 291, 354 and 585 nm; using the same reaction with iodide in acidic media it is possible to determine periodate-bromate and iodate-bromate mixtures simultaneously, by the H-point standard addition method . Using organic dyes, such as methyl orange, it is possible to increase the sensitivity of kinetic photometric determination and carry out the measurement in the visual light, for example, in the reaction with pyrogallol red at 470 nm, the kinetic data for iodate and periodate determination is processed by principle component artificial neural network [8]. The method for oxidizing agent determination can be modified for reducing agent determination, as it is based on the same reaction (Scheme 1.1). Hydrazine was described in this context [5], but urea has similar properties and can be determined from the discoloration of methyl orange.

The construction industry uses determination of urea in concrete mixtures. Rapid photometric method of determining urea in the “on site” mode provides a range of determined concentrations from 20 to 200 mg/kg in conversion to urea. The detection limit is 6 mg/kg with the sample mass 20 g. The time of one determination does not exceed 10 min. The relative error is ±25 % (P = 0.95) [17].

With the fast development of dairy processing industry in recent years, safety of dairy products was challenged by illegal adulterants such as urea, starch, whey, dextrin and melamine. Urea is a natural ingredient of milk in the range of 18.00–40.00 mg/dL, the permissible level of urea in milk should be less than 70.00 mg/dL. When urea content in milk excesses the permissible range, many health problems may be caused, which include ulcer, cancers, indigestion, acidity and malfunctions of kidney. Recently in 2016, a nonlinear chemical fingerprint method was reported in analysis of urea in milk and milk powder.

Urea content in milk powder was maintained in the range of 0–40 mg/g. A limit of detection (LOD) based on the signal-to-noise ratio of 3 in response to blank sample was $7.8 \cdot 10^{-3}$ mg/g [18].

Some of described [2] methods are time-consuming and suffer from lack of selectivity and short linear dynamic range, require complicated and expensive instruments, or use reagents that are not commercially available. For example, the Kjeldahl method, that is the official method for determination of urea in dermatologic formulations and cosmetics in the Pharmacopoeia of Serbia, requires about 3 h for the determination of urea at each sample [2].

The kinetic spectrophotometric method is one of the most attractive approaches for determination of urea. Its advantage is that only a spectrophotometer is required as the main instrument, and such reagents as methyl orange and potassium bromate are used.

Methyl orange, such as many acid dyes, is prone to oxidation to form colorless products in an acid medium, thus providing a suitable analytical approach for the indirect assay of inorganic ions, organic compounds, and pharmaceuticals. The produced bromine and chlorine react with methyl orange and this reaction causes decolorization of the solution (Scheme 1.1).

The authors of the paper [2] developed a sensitive, simple, low-cost, fast (requiring only 10 min), and relatively selective method for the determination of urea based on its inhibiting effect on the reaction of bromate with hydrochloric acid, was used to monitor the reaction spectrophotometrically at 505 nm.

The inhibited decolorization of methyl orange by the reaction products was monitored spectrophotometrically by observing the change in the absorbance of the reagents solution at 505 nm. The change in the absorbance with time was measured for 1–20 min from the initiation of addition of the last drop of the bromate solution. All the solutions were kept in a thermostate at 20 °C.

After optimum conditions for the investigated reaction were determined, changes in the absorbance were observed over time at $6.0 \cdot 10^{-4}$ mol/L methyl orange, 0.230 mol/L HCl, and $1 \cdot 10^{-4}$ mol/L $KBrO_3$ in the presence of urea. The absorbance increases linearly with urea concentration (r = 0.9998). The systems obey Beer's law for 0.0600–0.900 mol/L. The calculated apparent molar absorbance values are found to be $4.537 \cdot 10^3$ dm^3/ mol·cm and the Sandell's sensitivity is 0.013 mg/cm^2. The variables affecting the rate of the reaction were investigated. The relative standard deviation for five-replication determination of 0.0600 mol/L urea was 2.1 % and the detection limit of the method is 0.34 ng/mL [2].

We studied the method suggested by the paper [2] in order to optimize, the conditions, dispensing with thermostating, investigating other ways of sample preparation and calculating the metrological characteristics of modified determination.

2 EXPERIMENTAL

Instruments used : KFK-2; centrifuge CM-6M ELMI.

A standard solution of urea 0.010 M was prepared by dissolving 0.0600 g of analytical- grade reagent urea in distilled water and diluting to the mark in a 100-mL volumetric flask. The working solution was freshly prepared.

A stock solution of potassium bromate 0.001 M was prepared by dissolving 0.1670 g of analytical grade reagent $KBrO_3$ in distilled water and diluting to the mark in a 100-mL volumetric flask. Working solutions were prepared daily by precise diluting in distilled water.

Hydrochloric acid solutions 0.856 M were prepared by appropriate dilution of the concentrated acid HCl, prepared as follows: 18 mL of the initial concentrated hydrochloric acid with ρ = 1.184 g/cm^3 was brought to the mark in a 250-mL volumetric flask. This hydrochloric acid solution was standardized with a solution of sodium tetraborate from a standard titer 0.1000 N with the use of the methyl orange indicator.

A solution of methyl orange $6 \cdot 10^{-4}$ M was prepared by dissolving 0.0190 g of $C_{14}H_{14}N_3SO_3Na$ in distilled water and diluting to the mark in a 100-mL volumetric flask.

The procedure of urea determination was as following: a suitable aliquot of a working solution, of urea with the concentration of $1 \cdot 10^{-2}$ M, 3 mL of hydrochloric acid with the concentration of 0.856 M, 1 mL of methyl orange solution with the concentration of $6 \cdot 10^{-4}$ M were added; the solution was diluted to a volume of 5 mL with distilled water. Then 1.3 mL of potassium bromate solution with the concentration of $1 \cdot 10^{-2}$ M was added and the resulting solution was diluted with water to the 10-mL mark. The time of reaction was measured from the start of adding the last drop of the bromate solution; the absorbance control began 1 min later, until complete discoloration of the solution. The solution was thoroughly mixed and a part of it was transferred to the cuvette for measurement. Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. After plotting all the kinetic curves the initial rates were calculated from the experimental measurements. The slope coefficients (calculated with the use of the least-square method) are negative, as the absorbance decreases in the case of methyl orange discoloration. For graphic representation of initial rates we have chosen to use the absolute amount of slope coefficients (| |).

The procedure of urea determination in cosmetic creams was as following: to a 250-mg sample of cosmetic cream 100 mL of water measured by a volumetric flask was added, the two-phase system was thoroughly mixed and left to stand for a day (not always exactly 24 h). After that, the solution was filtered and each filtrate sample was centrifuged in a centrifuge CM-6M ELMI separately at 2000 rpm for 10 min. A 2-mL aliquot of a filtrate sample was added to a 10-mL graduated test-tube, 3 mL of hydrochloric acid with the concentration of 0.856 M, 1 mL of methyl orange solution with the concentration of $6 \cdot 10^{-4}$ M were added; the solution was diluted to a volume of 5 mL with distilled water. Then 1.3 mL of potassium bromate solution with the concentration of $1 \cdot 10^{-2}$ M was added and the resulting solution was diluted with water

to the 10-mL mark. The time of reaction was measured from the start of adding the last drop of the bromate solution; the absorbance control began 1 min later, until complete discoloration of the solution. The solution was thoroughly mixed and a part of it was transferred to the cuvette for measurement. Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. After plotting all the kinetic curves the initial rates were calculated from the experimental measurements. The slope coefficients (calculated with the use of the least-square method) are negative, as the absorbance decreases in the case of methyl orange discoloration. For graphic representation of initial rates we have chosen to use the absolute amount of slope coefficients (| |).

3 RESULT AND DISCUSSION

3.1 Kinetic curves

There is a reaction of bromate with methyl orange, the oxidation leads to the dye discoloration and the presence of urea inhibits the process of discoloration, which becomes slower.

A 1 mL aliquot of urea solution with the concentration of $1 \cdot 10^{-2}$ M, (1.8–2.4) mL of hydrochloric acid with the concentration of 0.828 M, was added to a 10-mL graduated test tube. (The HCl solution was prepared as follows: 8 mL of the initial concentrated hydrochloric acid with ρ = 1.179 g/cm^3 was brought to the mark in a 100-mL volumetric flask. This hydrochloric acid solution was standardized with a solution of sodium tetraborate from a standard titer 0.1000 N with the use of the methyl orange indicator.) Then 1 mL of methyl orange solution with the concentration of $6 \cdot 10^{-4}$ M was added; the solution was diluted to a volume of 5 mL with distilled water. Then 1 mL of potassium bromate solution with the concentration of $1 \cdot 10^{-2}$ M was added and the resulting solution was diluted with water to the 10-mL mark. The blank experiment was prepared in the same way, without urea. Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. The solution was thoroughly mixed and a part of it was transferred to the cuvette for measurement. The time of reaction was measured from the start of adding the last drop of the bromate solution until complete discoloration of the solution, the absorbance (A) control began 1 min after zero point (the time needed to dilute the solution to exact 10 mL with distilled water, to fill a glass cuvette, wipe its sides and place it in the cell holder). The data are given in Table 3.1.

Table 3.1 – Time-dependences of absorbance

t, min	A	t, min	A	t, min	A	t, min	A
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} = 0.149 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
1	1.523	9	1.215	17	0.770	25	0.284
2	1.495	10	1.155	18	0.699	26	0.230
3	1.420	11	1.097	19	0.638	27	0.180
4	1.398	12	1.046	20	0.585	28	0.134
5	1.387	13	1.000	21	0.523	29	0.094
6	1.309	14	0.921	22	0.475	30	0.061
7	1.301	15	0.879	23	0.398	31	0.036
8	1.252	16	0.824	24	0.342	32	0.022
C_{urea} = 0 M; C_{HCl} = 0.149 M; $C_{MO} = 6 \cdot 10^{-5}$ M; C $KBrO_3 = 1 \cdot 10^{-3}$ M							
1	1.221	7	0.978	13	0.648	19	0.276
2	1.174	8	0.928	14	0.568	20	0.207
3	1.143	9	0.886	15	0.481	21	0.164
4	1.097	10	0.829	16	0.446	22	0.114
5	1.051	11	0.787	17	0.329	23	0.068

Completed Table 3.1

t, min	A	t, min	A	t, min	A	t, min	A
C_{urea} = 0 M; C_{HCl} = 0.149 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
6	1.036	12	0.732	18	0.301	24	0.036
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} = 0.166 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
1	1.537	9	1.125	17	0.585	25	0.081
2	1.537	10	1.092	18	0.508	26	0.051
3	1.522	11	1.000	19	0.438	27	0.032
4	1.408	12	0.900	20	0.366	28	0.023
5	1.387	13	0.882	21	0.296	29	0.019
6	1.303	14	0.804	22	0.237	30	0.018
7	1.284	15	0.728	23	0.174	–	–
8	1.187	16	0.657	24	0.122	–	–
C_{urea} = 0 M; C_{HCl} = 0.166 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
1	1.136	6	0.856	11	0.495	16	0.105
2	1.092	7	0.796	12	0.415	17	0.051
3	1.046	8	0.721	13	0.319	18	0.023
4	0.995	9	0.649	14	0.252	–	–
5	0.921	10	0.570	15	0.164	–	–
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} = 0.182 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
1	1.523	8	1.125	15	0.515	22	0.033
2	1.523	9	1.046	16	0.423	23	0.022
3	1.456	10	0.959	17	0.337	24	0.015
4	1.398	11	0.872	18	0.259	25	0.013
5	1.318	12	0.788	19	0.184	–	–
6	1.284	13	0.697	20	0.119	–	–
7	1.208	14	0.605	21	0.071	–	–
C_{urea} = 0 M; C_{HCl} = 0.182 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
1	0.244	3	0.141	5	0.051	7	0.013
2	0.190	4	0.092	6	0.023	8	0.0096
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} = 0.198 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
1	0.917	4	0.602	7	0.323	10	0.090
2	0.824	5	0.508	8	0.244	11	0.043
3	0.740	6	0.420	9	0.155	12	0.018
C_{urea} = 0 M; C_{HCl} = 0.198 M; $C_{MO} = 6 \cdot 10^{-5}$ M; C $KBrO_3 = 1 \cdot 10^{-3}$ M							
1	1.046	4	0.745	7	0.358	10	0.018
2	0.958	5	0.620	8	0.222	–	–
3	0.854	6	0.495	9	0.097	–	–

Examples, taken for high and low concentrations of hydrochloric acid, are shown in Figure 3.1.

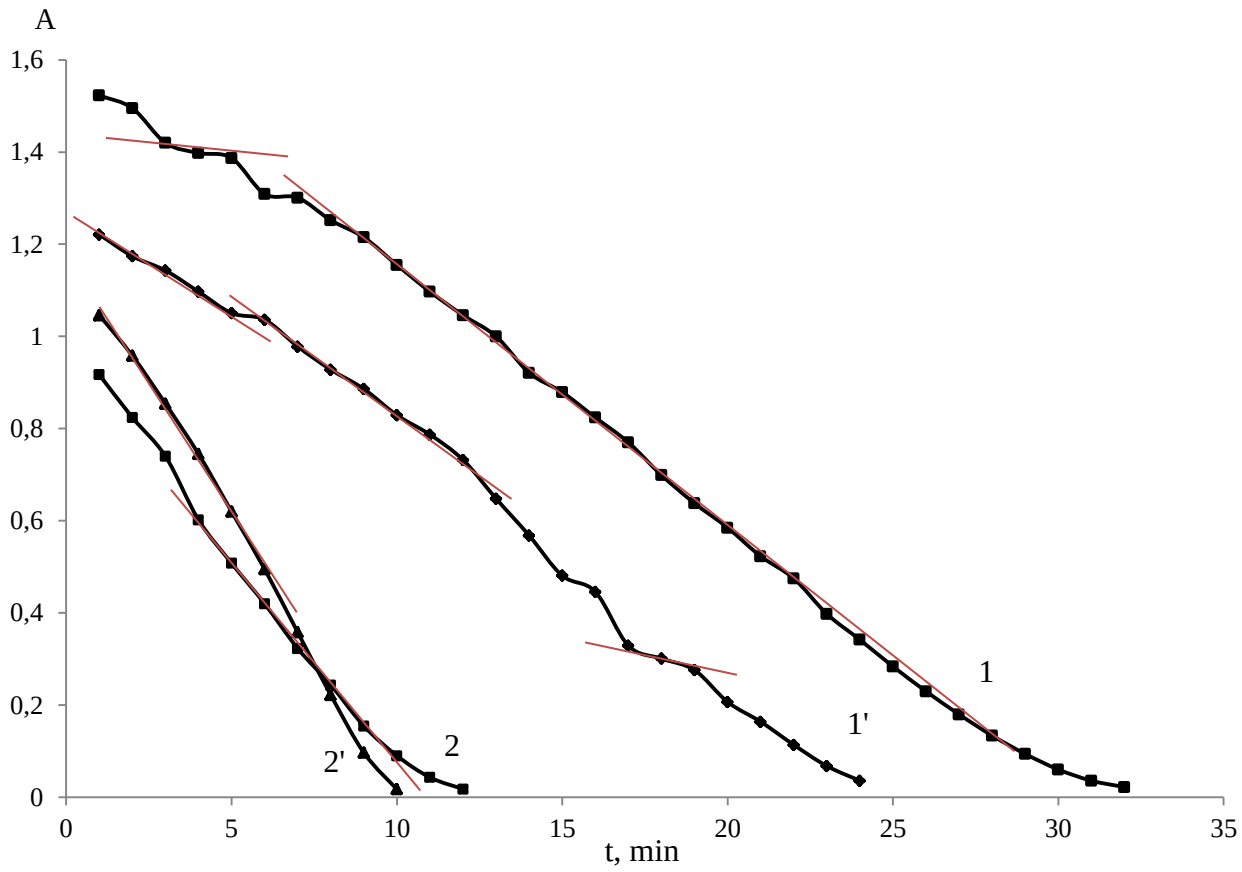


Figure 3.1 – Kinetic curves of methyl orange discoloration; $C_{MO} = 6 \cdot 10^{-5}$ M, $C_{KBrO_3} = 1 \cdot 10^{-3}$ M; 1, 2 – $C_{urea} = 1 \cdot 10^{-3}$ M, 1', 2' – $C_{urea} = 0$; 1, 1' – $C_{HCl} = 0.149$ M, 2, 2' – $C_{HCl} = 0.198$ M

The presence of urea also leads to the appearance of multistage kinetic curves. The look of kinetic curves depends on the conditions of the experiment. Further in the experimental measurement we analyzed the time dependences and choose the linear parts of the curves for rate calculation separately.

The greater is the concentration of hydrochloric acid, the sooner methyl orange is oxidized. The time needed for complete discoloration of the dye at acid concentration 0.149 M is 24 min (32 min in the presence of urea), while at the HCl concentration 0.198 M it is only 10 min (12 min in the presence of urea).

Therefore, optimization of conditions is needed (specifically, increasing the acid concentration makes the kinetic curves shorter, and their look becomes smoother). The initial rates at the conditions chosen for the calibration are given as an example in Figure 3.2 (for urea concentration $1 \cdot 10^{-3}$ M and $1 \cdot 10^{-2}$ M).

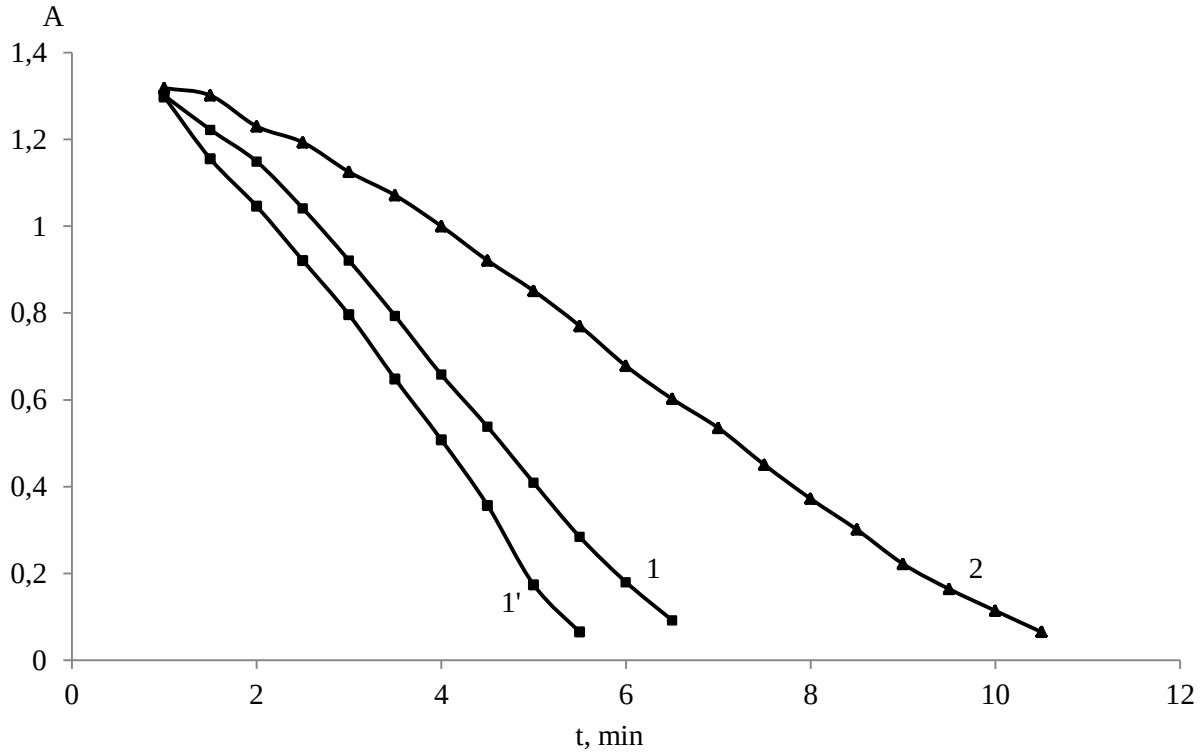


Figure 3.2 – Kinetic curves of methyl orange discoloration; C_{HCl} = 0.257 M, C_{KBrO_3}= $1.3 \cdot 10^{-3}$ M, $C_{MO} = 6 \cdot 10^{-5}$ M; 1 – $C_{urea} = 1 \cdot 10^{-3}$ M, 2 – $C_{urea} = 1 \cdot 10^{-2}$ M, 1’ – C_{urea} = 0 M

With increasing concentration of urea the reaction of methyl orange discoloration by bromate is inhibited to a greater degree (10.5 min compared to 6.5 min).

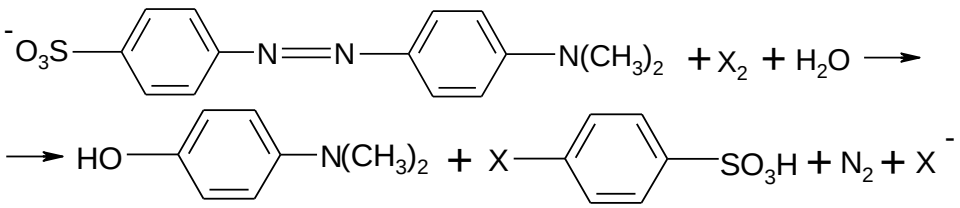
3.2 Influence of acidity

The reaction proceeds in acidic medium, and with an increase in the acid concentration the reaction rate increases, as has been shown in Section 8.1. We investigated two acids: hydrochloric acid and sulfuric acid.

$$2BrO_3^- + 10\ Cl^- + 12\ H^+ \rightarrow Br_2 + 5\ Cl_2 + 6\ H_2O.$$

Sheme 3.1

The produced chlorine and bromine react with methyl orange and decolorize it (Scheme 3.2):



(X = Cl; Br).

Scheme 3.2

Two solutions of sulfuric acid H_2SO_4 were prepared as follows: 12.5 mL and 25 mL of initial concentrated sulfuric acid with ρ = 1.830 g/cm^3 were taken to be diluted with distilled water to the mark in 100-mL volumetric flasks. The sulfuric acid solution was standardized with the solution of sodium tetraborate from a standard titer 0.1000 N with the use of the methyl orange indicator. An aliquot of 1 mL of urea solution with the concentration of $1\cdot10^{-2}$ M, 3 mL of sulfuric acid solution with concentration of 2.12 M and 5 mL of its solution with concentration of 4.24 M, 1 mL of methyl orange with concentration of $6\cdot10^{-4}$ M was added in to a 10-mL graduated test tube, diluted up to a volume of 5 mL with water. Then 1 mL of potassium bromate solution with the concentration of $1\cdot10^{-2}$ M was added and the resulting solution was diluted with water to the 10-mL mark. The blank experiment was prepared in the same way, without urea. The time of reaction was measured from the start of adding the last drop of the bromate solution; the absorbance control began 1 min later, until complete discoloration of the solution. The solution was thoroughly mixed and a part of it was transferred to the cuvette for measurement. Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. The data are given in Table 3.2.

Table 3.2 – Time-dependences of absorbance in the presence of H_2SO_4

t, min	A	t, min	A	t, min	A	t, min	A
$C_{urea} = 1\cdot10^{-3}$ M; $C_{H_2SO_4}$ =0.635 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1	1.318	9	1.096	17	0.860	25	0.593
2	1.301	10	1.055	18	0.833	26	0.560
3	1.222	11	1.045	19	0.798	27	0.523
4	1.222	12	1.000	20	0.770	28	0.481
5	1.200	13	1.000	21	0.744	29	0.444
6	1.155	14	0.958	22	0.701	30	0.409
7	1.142	15	0.921	23	0.661	–	–
8	1.108	16	0.903	24	0.623	–	–
$C_{urea} = 0$ M; $C_{H_2SO_4}$ =0.635 M ; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1	1.309	8	1.046	15	0.795	22	0.488
2	1.260	9	1.022	16	0.744	23	0.452
3	1.222	10	1.000	17	0.710	24	0.408
4	1.167	11	0.958	18	0.677	25	0.372
5	1.155	12	0.914	19	0.629	26	0.337
6	1.108	13	0.880	20	0.585	27	0.300
7	1.096	14	0.824	21	0.535	–	–
$C_{urea} = 1\cdot10^{-3}$ M; CH_2SO_4 =0.847 M; $C_{MO} = 6\cdot10^{-5}$ M; C $KBrO_3$= $1.3\cdot10^{-3}$ M							
1	1.301	9	1.161	17	1.004	25	0.796
2	1.301	10	1.155	18	0.991	26	0.770
3	1.284	11	1.143	19	0.959	27	0.733
4	1.237	12	1.108	20	0.921	28	0.712

Continued Table 3.2

t, min	A	t, min	A	t, min	A	t, min	A
5	1.222	13	1.097	21	0.914	29	0.677
6	1.222	14	1.071	22	0.880	30	0.654
7	1.222	15	1.046	23	0.847	–	–
8	1.187	16	1.041	24	0.824	–	–
C_{urea} = 0 M; $C_{H_2SO_4}$ =0.847 M ; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1	1.222	9	1.022	17	0.775	25	0.495
2	1.161	10	1.000	18	0.744	26	0.456
3	1.155	11	0.958	19	0.710	27	0.420
4	1.143	12	0.936	20	0.674	28	0.378
5	1.097	13	0.903	21	0.638	29	0.356
6	1.097	14	0.876	22	0.602	30	0.328
7	1.071	15	0.839	23	0.553	–	–
8	1.046	16	0.809	24	0.530	–	–
$C_{urea} = 1\cdot10^{-3}$ M; $C_{H_2SO_4}$ = 2.118 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1	1.102	6	1.065	11	1.022	16	0.991
2	1.102	7	1.051	12	1.008	17	0.966
3	1.096	8	1.051	13	1.004	18	0.959
4	1.092	9	1.046	14	1.000	19	0.959
5	1.081	10	1.036	15	1.000	20	0.951
C_{urea} = 0 M; $C_{H_2SO_4}$ = 2.118 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1	1.045	6	0.991	11	0.940	16	0.886
2	1.032	7	0.966	12	0.924	17	0.886
3	1.008	8	0.959	13	0.921	18	0.880
4	1.000	9	0.959	14	0.914	19	0.860
5	1.000	10	0.955	15	0.903	20	0.854

The absolute value of absorbance is lower compared to the solution acidified with HCl, the color change is very slow. At lower concentrations, the process is not noticeable at all. Apparently the stepwise oxidation reaction of methyl orange in the presence of H_2SO_4 goes differently than in the presence of hydrochloric acid (*ref.* Section 3.1).

A fresh solution of hydrochloric acid was prepared as follows: 18.0 mL of the initial concentrated hydrochloric acid with $\rho = 1.184$ g/cm^3 were taken and dissolved in water in a 250-mL volumetric flask. The hydrochloric acid solution was standardized with the solution of sodium tetraborate from a standard titer 0.1000 N with the use of the methyl orange indicator. A 1 mL aliquot of a urea solution with the concentration of $1\cdot10^{-2}$ M, (1.4–3.7) mL of hydrochloric acid with the concentration of 0.856 M, 1 mL of methyl orange solution with a concentration of $6\cdot10^{-4}$ M, were added to a 10-mL graduated test tube to dilute to volume 5 mL with distilled water. Then 1 mL of potassium bromate solution with the concentration of $1\cdot10^{-2}$ M was added and the resulting solution was

diluted with water to the 10-mL mark. The blank experiment was prepared in the same way, without urea. The time of reaction was measured from the start of adding the last drop of the bromate solution; the absorbance control began 1 min later, until complete discoloration of the solution. The solution was thoroughly mixed and a part of it was transferred to the cuvette for measurement. Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. The data are given in Table 3.3.

For illustrative purpose, examples are shown in Figure 3.3.

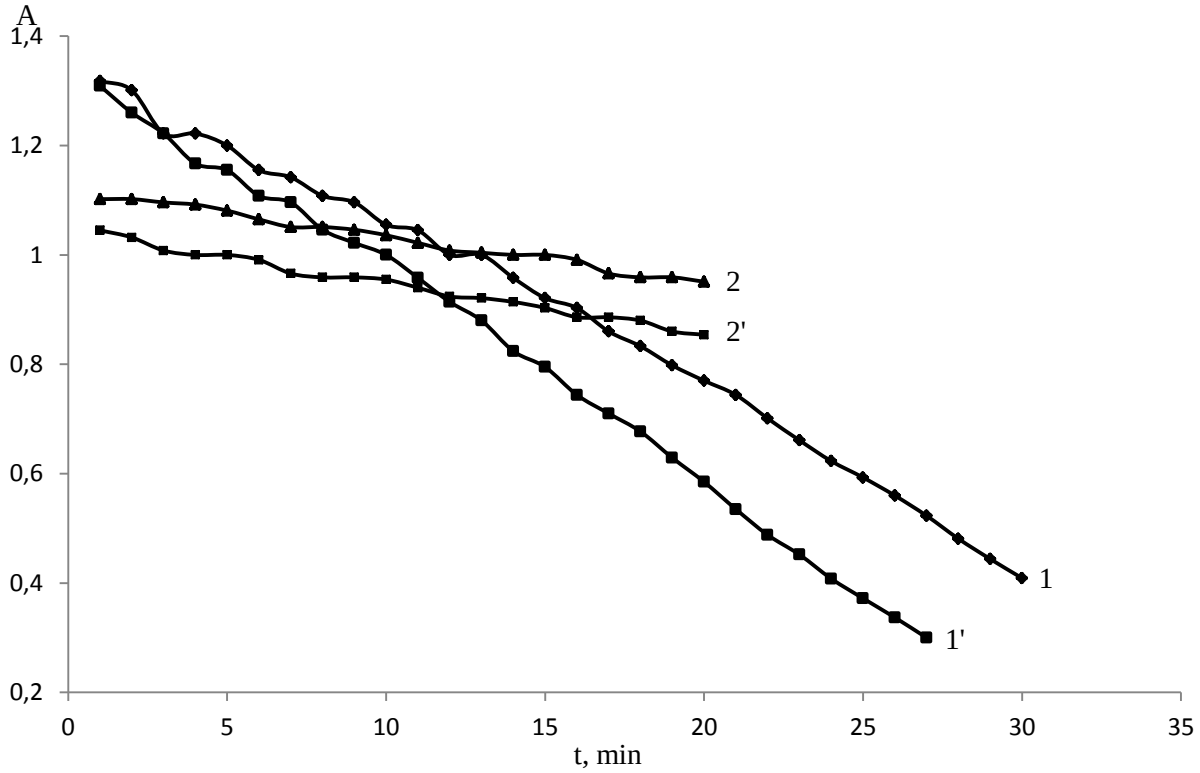


Figure 3.3 – Kinetic curves of methyl orange discoloration in the presence of sulfuric acid. C_{KBrO_3} = $1.3 \cdot 10^{-3}$ M, C_{MO} = $6 \cdot 10^{-5}$ M; 1, 1’ – $C_{H_2SO_4}$ = 0.635 M, 2. 2’ – $C_{H_2SO_4}$ = 2.118 M; 1, 2 – C_{urea} = $1 \cdot 10^{-3}$ M, 1’, 2’ – C_{urea} = 0

Table 3.3 – Time – dependences of absorbance in the presence of HCl

t, min	A	t, min	A	t, min	A	t, min	A
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} = 0.119 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
2	2.000	14	1.824	26	1.658	90	0.868
6	1.958	18	1.745	30	1.538	94	0.853
10	1.921	22	1.700	34	1.523	–	–
C_{urea} = 0 M; C_{HCl} = 0.119 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
2	2.045	18	1.824	34	1.523	72	0.809
6	2.000	22	1.722	61	1.000	–	–
10	2.000	26	1.700	65	0.934	–	–
14	1.958	30	1.602	69	0.886	–	–

Completed Table 3.3

$C_{urea} = 1 \cdot 10^{-3}$ M; $C_{HCl} = 0.205$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
2	1.700	6	1.155	10	0.658	14	0.143
4	1.398	8	0.921	12	0.372	–	–
$C_{urea} = 0$ M; $C_{HCl} = 0.205$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
2	1.408	6	0.978	10	0.444	14	0.036
4	1.222	8	0.745	12	0.230	–	–
$C_{urea} = 1 \cdot 10^{-3}$ M; $C_{HCl} = 0.316$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
1	1.658	3	0.733	5	0.032	–	–
2	1.187	4	0.292	–	–	–	–
$C_{urea} = 0$ M; $C_{HCl} = 0.316$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1 \cdot 10^{-3}$ M							
1	1.523	2	0.921	3	0.398	4	0.009

At very low acid concentrations, the discoloration time is very high, even in 94 min complete discoloration was not achieved. At the HCl concentration of 0.3 M, it is 4 to 5 min, and therefore it becomes inconvenient to monitor the reaction rate.

For illustrative purpose, examples are shown in Figure 3.4.

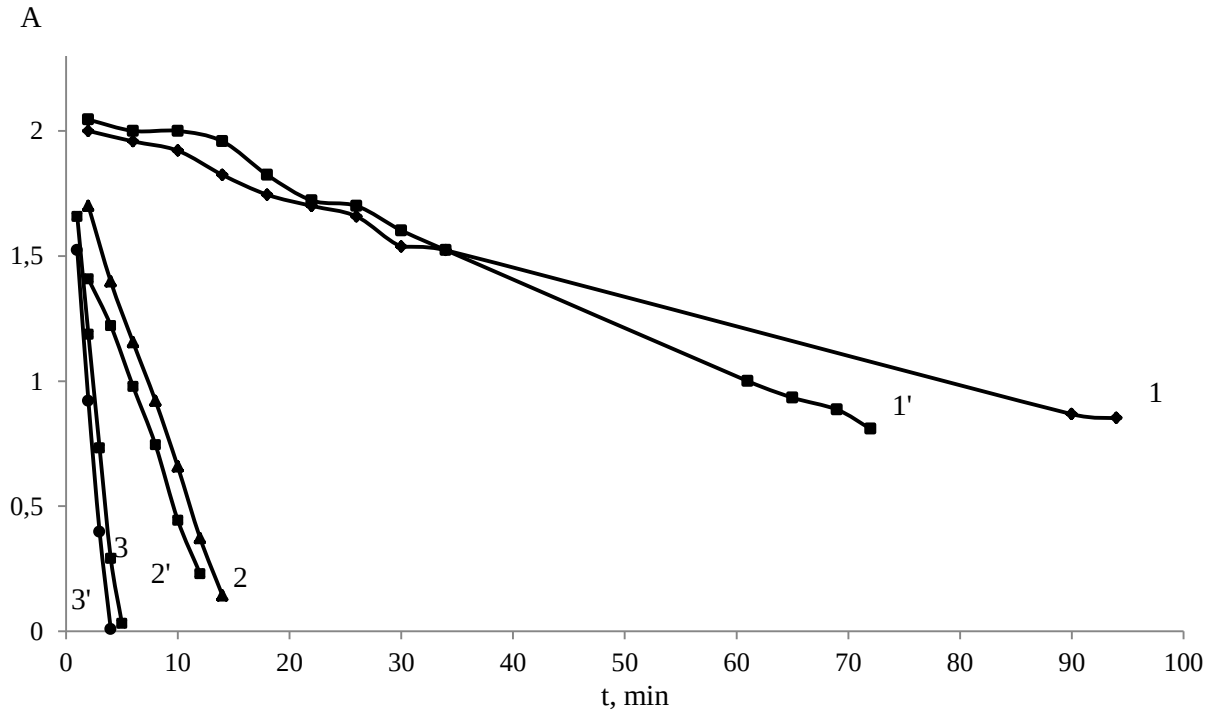


Figure 3.4 – Kinetic curves of methyl orange discoloration in the presence of hydrochloric acid. $C_{KBrO_3} = 1 \cdot 10^{-3}$ M, $C_{MO} = 6 \cdot 10^{-5}$ M; 1, 2, 3 – $C_{urea} = 1 \cdot 10^{-3}$ M, 1', 2', 3' – $C_{urea} = 0$; 1, 1' – $C_{HCl} = 0.119$ M, 2, 2' – $C_{HCl} = 0.205$ M, 3, 3' – $C_{urea} = 0.316$ M

After plotting all the kinetic curves the initial rates were calculated from the experimental measurements. The slope coefficients (calculated with the use of the least-

square method) are negative, as the absorbance decreases in the case of methyl orange discoloration. For graphic representation of initial rates we have chosen to use the absolute amount of slope coefficients (| |). The results are shown in Figure 3.5.

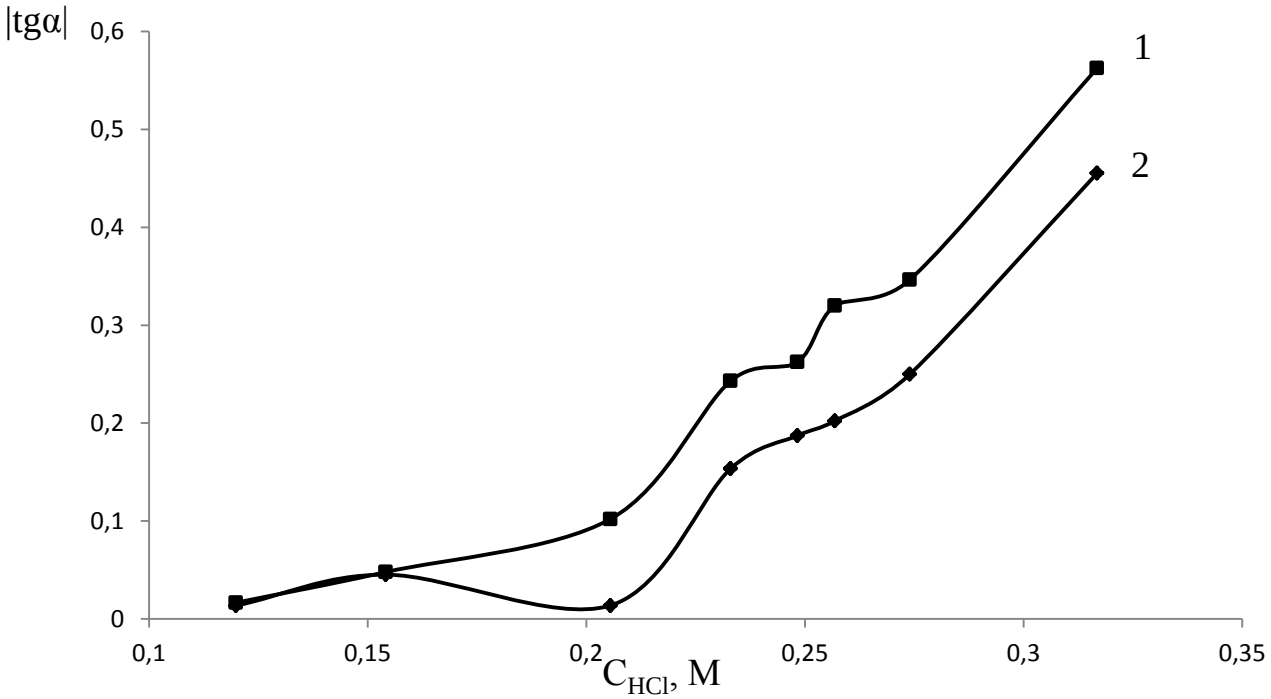


Figure 3.5 – Effect of hydrochloric acid concentration on initial rates; $C_{KBrO_3} = 1.3 \cdot 10^{-3}$ M, $C_{MO} = 6 \cdot 10^{-5}$ M; 1 – $C_{urea} = 0$, 2 – $C_{urea} = 1 \cdot 10^{-3}$ M

At concentrations of hydrochloric acid lower than 0.154 M, urea does not affect the rate of methyl orange decomposition. The greatest difference between a blank experiment and a solution in which urea is present, is observed at the concentration of hydrochloric acid of 0.257 M. This value was chosen for further experimentation. Previously, 0.233 M was suggested as the optimal acid concentration [2].

3.3 Influence of potassium bromate concentration

The effect of bromate concentration in the reaction system was studied in the range $(3 \cdot 10^{-4} – 0.02)$ M.

A 1 mL aliquot of urea solution with the concentration of $1 \cdot 10^{-2}$ M, 3 mL of hydrochloric acid with the concentration of 0.856 M, 1 mL of methyl orange solution with the concentration of $6 \cdot 10^{-4}$ M, were added; the solution was diluted to a volume of 5 mL with distilled water. Then (0.3–2) mL of potassium bromate solution with the concentration of $1 \cdot 10^{-2}$ M was added and the resulting solution was diluted with water to the 10-mL mark. The blank experiment was prepared in the same way, without urea. The time of reaction was measured from the start of adding the last drop of the bromate solution; the absorbance control began 1 min later, until complete discoloration of the solution. The solution was thoroughly mixed and a part of it was transferred to the

cuvette for measurement. Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. The data are given in Table 3.4

Table 3.4 – Time-dependences of absorbance

t, min	A	t, min	A	t, min	A	t, min	A
$C_{urea} = 1 \cdot 10^{-3}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 3 \cdot 10^{-4}$ M							
1	2.000	7	1.553	13	0.921	19	0.292
2	2.000	8	1.523	14	0.824	20	0.215
3	2.000	9	1.398	15	0.721	21	0.136
4	2.000	10	1.301	16	0.620	–	–
5	1.721	11	1.155	17	0.495	–	–
6	1.689	12	1.020	18	0.398	–	–
$C_{urea} = 0$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 3 \cdot 10^{-4}$ M							
1	2.000	6	1.420	11	0.770	16	0.180
2	1.920	7	1.301	12	0.678	17	0.076
3	1.721	8	1.208	13	0.538	–	–
4	1.699	9	1.000	14	0.420	–	–
5	1.523	10	0.921	15	0.275	–	–
$C_{urea} = 1 \cdot 10^{-3}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.2 \cdot 10^{-3}$ M							
2	1.602	4	1.022	6	0.481	8	0.060
3	1.301	5	0.721	7	0.222	–	–
$C_{urea} = 0$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.2 \cdot 10^{-3}$ M							
2	1.408	4	0.744	6	0.114	–	–
3	1.096	5	0.443	–	–	–	–
$C_{urea} = 1 \cdot 10^{-3}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 2 \cdot 10^{-3}$ M							
1	1.658	3	0.958	5	0.222	–	–
2	1.347	4	0.553	6	0.032	–	–
$C_{urea} = 0$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 2 \cdot 10^{-3}$ M							
1	1.523	2	1.046	3	0.508	4	0.046

At low concentrations of potassium bromate, the oxidation reaction of methyl orange is slow, it takes 17 min, and in the presence of urea it takes 21 min. The reaction proceeds stepwise, as can be seen from the look of kinetic curves. Differences in light absorption of the inhibited and free reaction are well noticeable with increasing bromate concentration. At the concentration of potassium bromate above $1.2 \cdot 10^{-3}$ M, the rate of decolorization becomes acceptable for analytical determination, especially as urea slows it down. The initial reaction rates were calculated with the use of the least square method; they are shown in Figure 3.7.

The results that are presented in Figure 3.7 show that an increase in the bromate concentration within the range ($3 \cdot 10^{-4}$ – $1.1 \cdot 10^{-3}$) M leads to a decrease in the initial reaction rate.

Examples of the effect of high and low concentrations of potassium bromate are given in Figure 3.6.

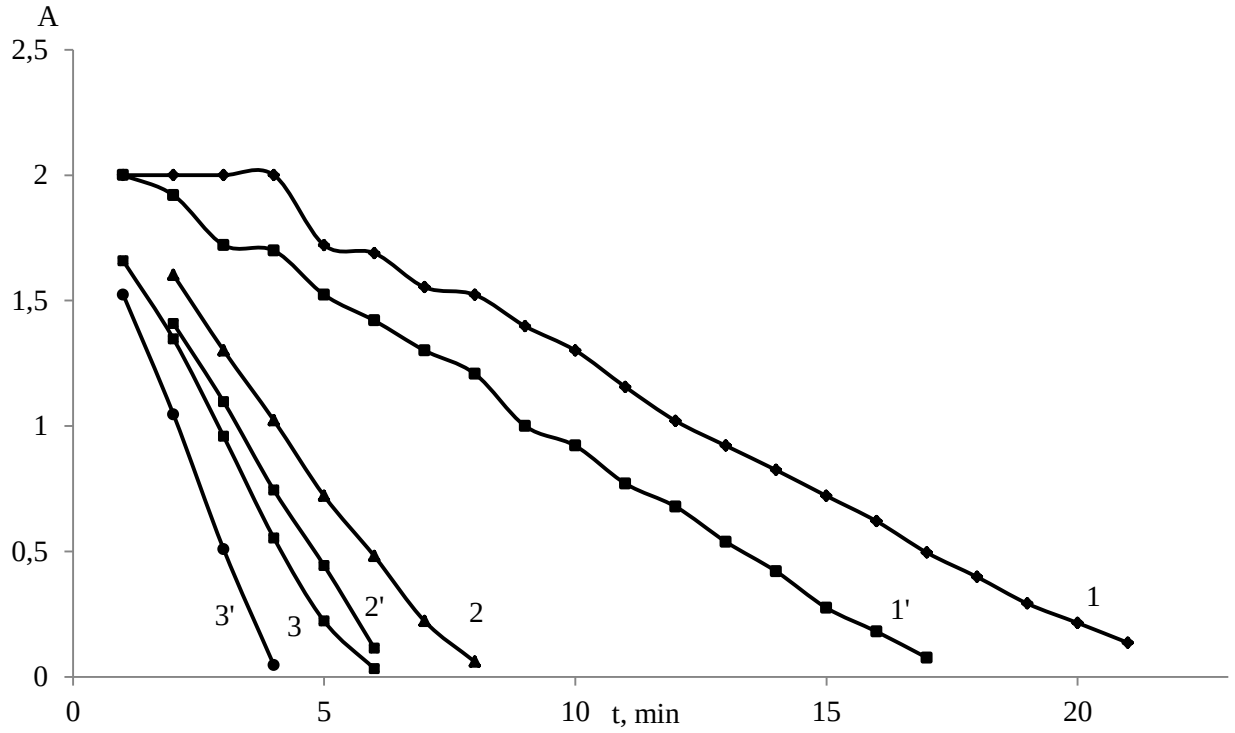


Figure 3.6 – Kinetic curves of methyl orange discoloration in the presence of potassium bromate; C_{HCl} =0.257 M, $C_{MO} = 6 \cdot 10^{-5}$ M; 1, 2, 3 – $C_{urea} = 1 \cdot 10^{-3}$ M, 1', 2', 3' – C_{urea} = 0 M; 1, 1' – $C_{KBrO_3} = 3 \cdot 10^{-4}$ M, 2, 2' – $C_{KBrO_3} = 1.2 \cdot 10^{-3}$ M, 3, 3' – $C_{KBrO_3} = 2 \cdot 10^{-3}$ M

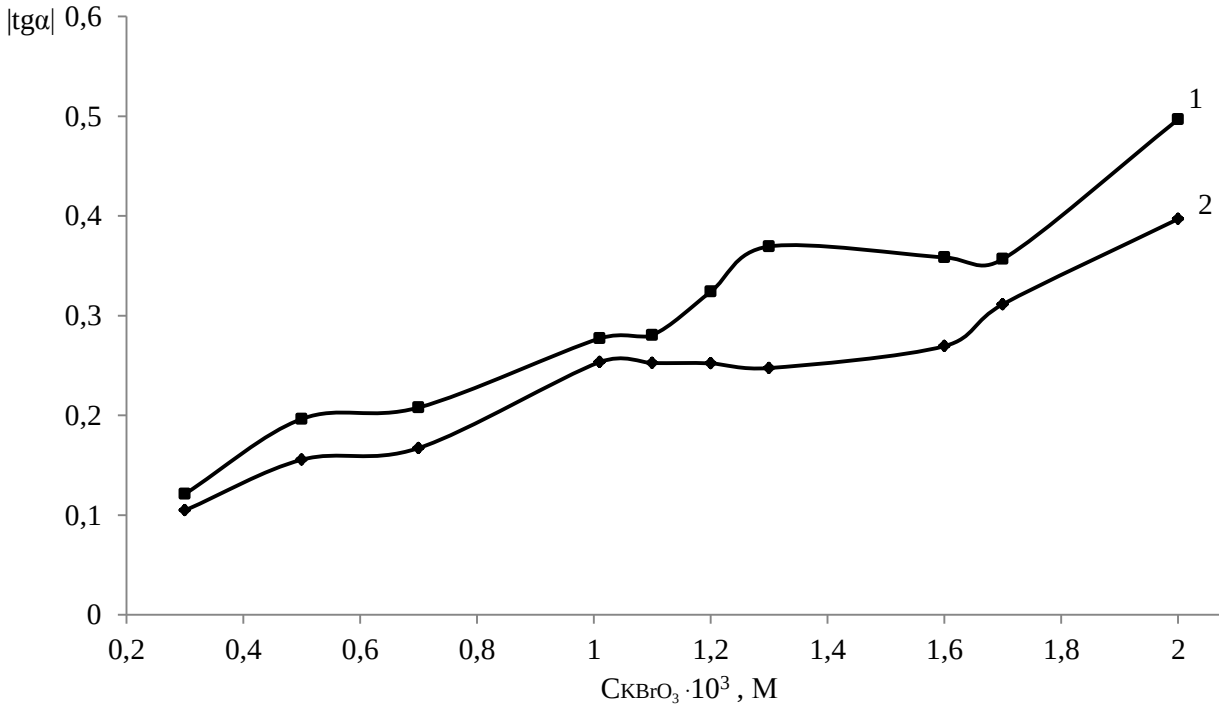


Figure 3.7 – Effect of potassium bromate concentration on initial rates; C_{HCl} =0.257 M, $C_{MO} = 6 \cdot 10^{-5}$ M; 1 – C_{urea} = 0, 2 – $C_{urea} = 1 \cdot 10^{-3}$ M

The increase in bromate concentrations also contributes to a quicker reaction in the presence of urea in the system, though it is still slower than the reaction in the absence of urea. The differences in initial rates become especially prominent in the range (1.3–1.6) · 10^{-3} M. Thus, the concentration of $1.3 \cdot 10^{-3}$ M was selected for the experiment. At even higher concentrations of bromate, the discoloration occurs so rapidly that it is impossible to obtain an analytical signal. Previously, $1 \cdot 10^{-3}$ M was suggested as the optimal potassium bromate concentration [2].

3.4 Influence of methyl orange concentration

Table 3.5 – Effect of methyl orange concentration on initial rates

$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} =0.257 M; $C_{KBrO_3} = 3 \cdot 10^{-4}$ M				C_{urea} = 0 M; C_{HCl} =0.257 M; $C_{KBrO_3} = 3 \cdot 10^{-4}$ M			
$C_{MO} \cdot 10^5$, M	tgα	$C_{MO} \cdot 10^5$, M	tgα	$C_{MO} \cdot 10^5$, M	tgα	$C_{MO} \cdot 10^5$, M	tgα
1.8	0.1425	6.0	0.2475	1.8	0.2388	6.0	0.3695
3.0	0.1860	7.2	0.2752	3.0	0.3004	7.2	0.3357
4.2	0.2445	8.4	0.3036	4.2	0.2878	8.4	0.3784
5.4	0.2533	9.6	0.3078	5.4	0.3327	9.6	0.4072

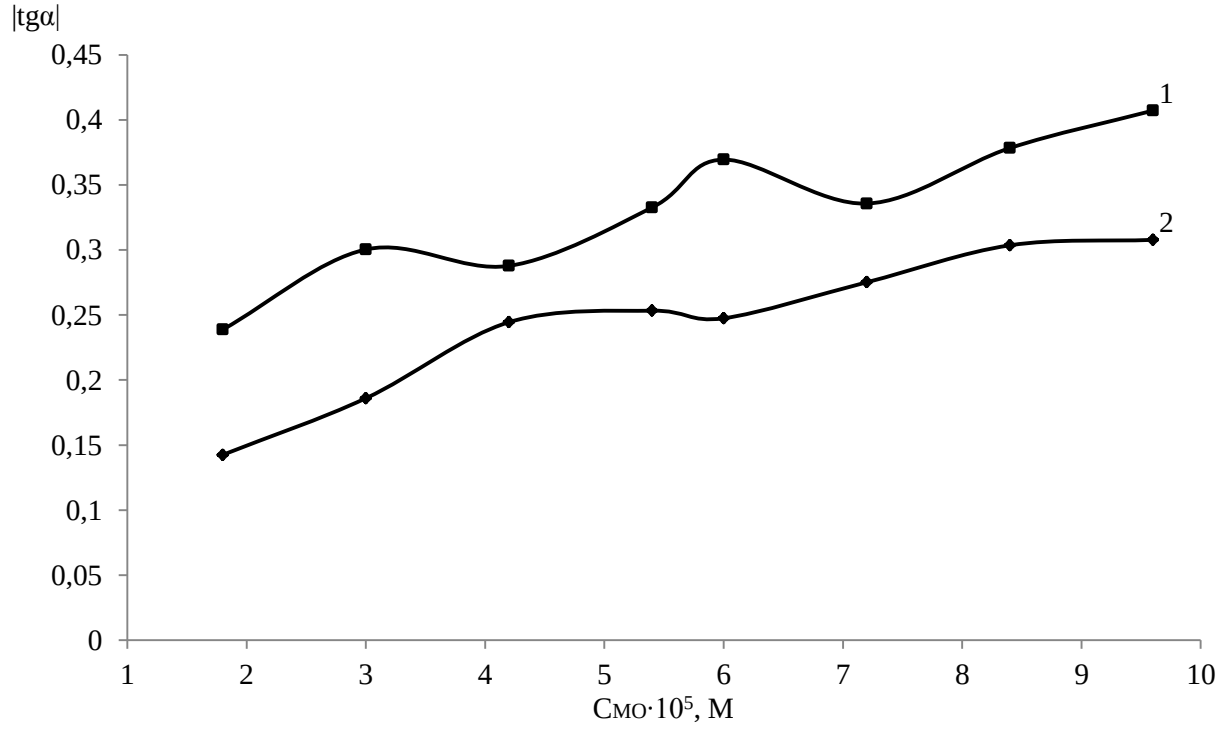


Figure 3.8 – Effect of methyl orange concentration on initial rates; C_{HCl} =0.257 M, $C_{KBrO_3} = 1.3 \cdot 10^{-3}$ M; 1 – C_{urea} = 0, 2 – $C_{urea} = 1 \cdot 10^{-3}$ M

The effect of methyl orange concentration on the reaction system was studied in the range ($1.8\cdot10^{-5}$ – $1.2\cdot10^{-4}$) M methyl orange in the presence of 0.257 M hydrochloric acid and $1.3\cdot10^{-3}$ M potassium bromate both with and without addition of urea. The data are shown in Table 3.5.

The obtained results show that, as the concentration of methyl orange increases from the lowest studied value within the interval ($1.8\cdot10^{-5}$ – $6.0\cdot10^{-4}$) M, the slope coefficients increase. The difference between initial rates of inhibited and free reactions is the highest of all at the methyl orange concentration of $6.0\cdot10^{-5}$ M; this concentration is chosen for the experiment. The authors [2] suggest the same value.

The initial reaction rates were calculated from kinetic curves with the use of the least-square technique, they are shown in Figure 3.8.

3.5 Influence of urea concentration

Determination of urea is dependent on its ability to inhibit the discoloration of methyl orange by bromate ion; it is possible because the greater is concentration of urea, the slower the reaction runs. The examples of kinetic curves can be seen in Section 3.1 (Figure 3.2).

Table 3.6 – Influence of urea concentration

t, min	A	t, min	A	t, min	A	t, min	A
C_{urea} = 0 M; C_{HCl} = 0.257 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1	2.000	3	1.259	5	0.523	–	–
2	1.658	4	0.921	6	0.162	–	–
$C_{urea} = 1\cdot10^{-5}$ M; C_{HCl} = 0.257 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.260	2.0	1.000	3.0	0.648	4.0	0.260
1.5	1.155	2.5	0.821	3.5	0.456	4.5	0.092
$C_{urea} = 1\cdot10^{-5}$ M; C_{HCl} = 0.257 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.260	2.0	1.000	3.0	0.648	4.0	0.260
1.5	1.155	2.5	0.821	3.5	0.456	4.5	0.092
$C_{urea} = 8\cdot10^{-5}$ M; C_{HCl} = 0.257 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.301	2.5	0.917	4.0	0.387	–	–
1.5	1.208	3.0	0.745	4.5	0.211	–	–
2.0	1.076	3.5	0.556	5.0	0.076	–	–
$C_{urea} = 8\cdot10^{-4}$ M; C_{HCl} = 0.257 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.125	2.5	0.733	4.0	0.276	–	–
1.5	1.022	3.0	0.577	4.5	0.137	–	–
2.0	0.886	3.5	0.420	5.0	0.036	–	–
$C_{urea} = 2\cdot10^{-4}$ M; C_{HCl} = 0.257 M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.301	2.5	0.846	4.0	0.416	5.5	0.071
1.5	1.222	3.0	0.721	4.5	0.289	–	–

Continued Table 3.6

t, min	A	t, min	A	t, min	A	t, min	A
$C_{urea} = 2\cdot10^{-4}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
2.0	0.956	3.5	0.538	5.0	0.172	5.5	0.071
$C_{urea} = 6\cdot10^{-4}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.301	2.5	0.978	4.0	0.582	5.5	0.180
1.5	1.222	3.0	0.850	4.5	0.444	6.0	0.092
2.0	1.108	3.5	0.721	5.0	0.310	–	–
$C_{urea} = 1\cdot10^{-3}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.301	2.5	1.041	4.0	0.658	5.5	0.284
1.5	1.222	3.0	0.921	4.5	0.538	6.0	0.180
2.0	1.149	3.5	0.793	5.0	0.409	6.5	0.092
$C_{urea} = 1.2\cdot10^{-3}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.318	3.0	0.987	5.0	0.495	7.0	0.065
1.5	1.260	3.5	0.870	5.5	0.367	–	–
2.0	1.187	4.0	0.744	6.0	0.244	–	–
2.5	1.097	4.5	0.610	6.5	0.140	–	–
$C_{urea} = 2\cdot10^{-3}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.346	3.0	1.041	5.0	0.850	7.0	0.137
1.5	1.292	3.5	0.921	5.5	0.458	7.5	0.076
2.0	1.222	4.0	0.810	6.0	0.347	–	–
2.5	1.125	4.5	0.700	6.5	0.237	–	–
$C_{urea} = 1\cdot10^{-2}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.318	4.0	1.000	7.0	0.535	10.0	0.114
1.5	1.301	4.5	0.921	7.5	0.450	10.5	0.065
2.0	1.230	5.0	0.851	8.0	0.372	–	–
2.5	1.193	5.5	0.770	8.5	0.301	–	–
3.0	1.125	6.0	0.678	9.0	0.222	–	–
3.5	1.071	6.5	0.602	9.5	0.164		
$C_{urea} = 5\cdot10^{-3}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.398	3.5	1.096	6.0	0.638	8.5	0.194
1.5	1.376	4.0	1.008	6.5	0.545	9.0	0.131
2.0	1.310	4.5	0.914	7.0	0.444	–	–
2.5	1.222	5.0	0.824	7.5	0.356	–	–
3.0	1.155	5.5	0.745	8.0	0.268	–	–
$C_{urea} = 1.5\cdot10^{-2}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6\cdot10^{-5}$ M; $C_{KBrO_3} = 1.3\cdot10^{-3}$ M							
1.0	1.398	4.0	1.136	7.0	0.716	10.0	0.292
1.5	1.376	4.5	1.086	7.5	0.638	10.5	0.218
2.0	1.319	5.0	1.000	8.0	0.568	11.0	0.161
2.5	1.301	5.5	0.940	8.5	0.509	–	–
3.0	1.230	6.0	0.870	9.0	0.414	–	–
3.5	1.187	6.5	0.796	9.5	0.366	–	–

Completed Table 3.6

t, min	A	t, min	A	t, min	A	t, min	A
$C_{urea} = 2 \cdot 10^{-2}$ M; $C_{HCl} = 0.257$ M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-3}$ M							
1.0	1.398	4.0	1.125	7.0	0.775	10.0	0.377
1.5	1.346	4.5	1.097	7.5	0.699	10.5	0.310
2.0	1.310	5.0	1.031	8.0	0.640	11.0	0.244
2.5	1.284	5.5	0.962	8.5	0.576	11.5	0.187
3.0	1.222	6.0	0.892	9.0	0.506	12.0	0.136
3.5	1.187	6.5	0.830	9.5	0.434	–	–

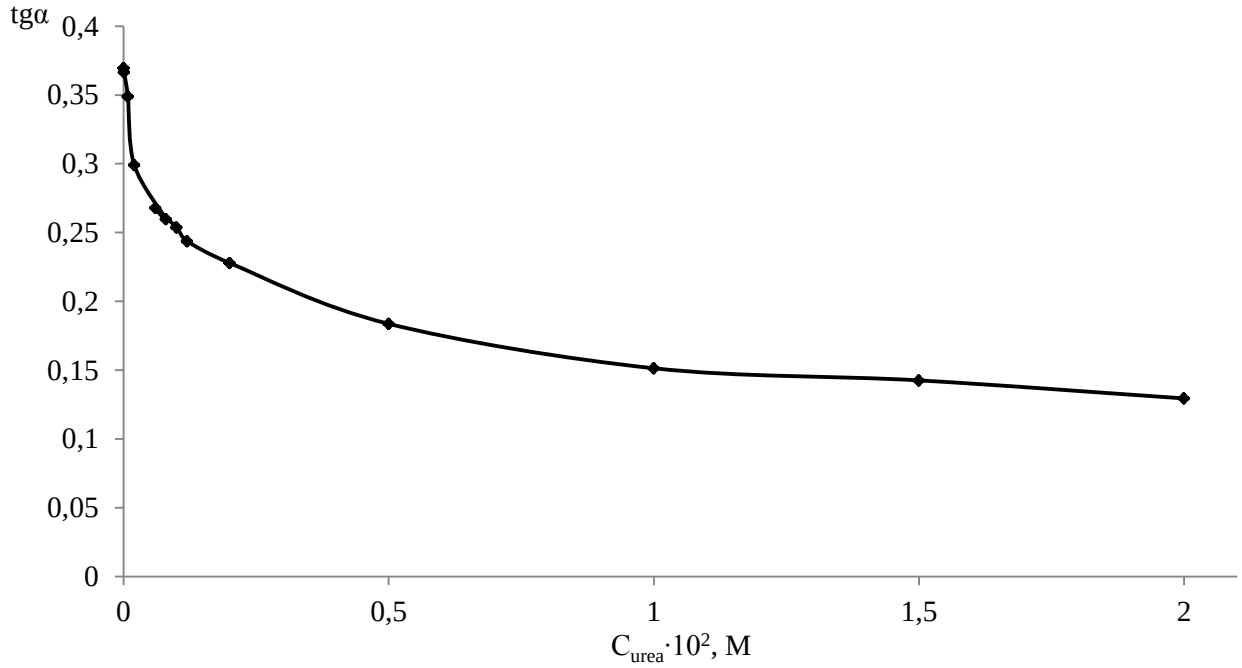


Figure 3.9 – Effect of urea concentration on the initial rate; C_{HCl} =0.257 M, $C_{KBrO_3} = 1.3 \cdot 10^{-3}$ M, $C_{MO} = 6 \cdot 10^{-5}$ M

On the kinetic curves the linear parts have been found, the initial rates have been calculated with the use of the least square method. The results are shown in Figure 3.9.

The greater is the concentration of urea, the less is the initial rate, although it does not go to zero anyway.

In the literature [18] it is said that it is possible to carry out determination with the help of curvilinear dependence. So it seems and should be done at high concentrations of urea. The straight-line part of the concentration dependence is a calibration graph, which is shown in Figure 3.10.

The graph does not pass through zero, but it is rather a rule for kinetic methods. The calibration graph is linear in the interval of $(0.2–1.2) \cdot 10^{-5}$ M of urea concentration, which corresponds to (12–72) μg/mL.

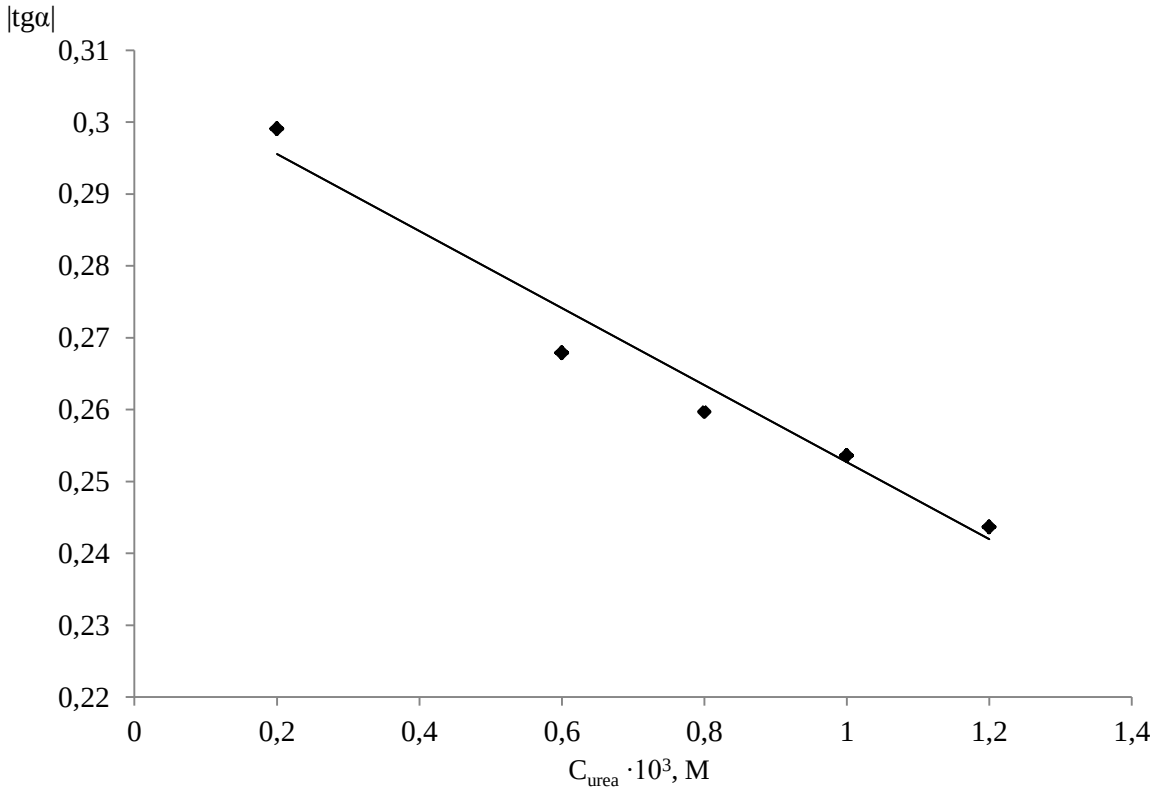


Figure 3.10 – Calibration curve; C_{HCl} = 0.257 M, C_{KBrO_3}= $1.3 \cdot 10^{-3}$ M, C_{MO} = $6 \cdot 10^{-5}$ M; linear regression: y=53.602x + 0.3063, R^2 = 0.9685; Δa = 18.98, Δb = 0.016

3.6 Metrological characteristics

At optimal conditions 4 replicate analyses were carried out. A 1 mL aliquot of urea solution with the concentration of $1 \cdot 10^{-2}$ M, 3 mL of hydrochloric acid with the concentration of 0.856 M, 1 mL of methyl orange solution with the concentration of $6 \cdot 10^{-4}$ M were added; the solution was diluted to a volume of 5 mL with distilled water. Then 1.3 mL of potassium bromate solution with the concentration of $1 \cdot 10^{-2}$ M was added and the resulting solution was diluted with water to the 10-mL mark. The time of reaction was measured from the start of adding the last drop of the bromate solution; the absorbance control began 1 min later, until complete discoloration of the solution. The solution was thoroughly mixed and a part of it was transferred to the cuvette for measurement Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. The data are given in Table 3.7.

After plotting all the kinetic curves the initial rates were calculated from the experimental measurements. The slope coefficients (calculated with the use of the least-square method) are negative, as the absorbance decreases in the case of methyl orange discoloration. For graphic representation of initial rates we have chosen to use the absolute amount of slope coefficients (| |).

We evaluated the metrological characteristics by performing 4 replicate analyses of solutions with the same urea content introduced, namely $1 \cdot 10^{-3}$ M. The results are shown in Table 3.8.

Table 3.7 – Time-dependences of absorbance

t, min	A	t, min	A	t, min	A	t, min	A
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} =0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.0	1.318	2.5	1.094	4.0	0.721	5.5	0.356
1.5	1.292	3.0	0.958	4.5	0.593	6.0	0.244
2.0	1.187	3.5	0.839	5.0	0.468	6.5	0.148
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} =0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.0	1.318	2.5	1.055	4.0	0.668	5.5	0.292
1.5	1.284	3.0	0.939	4.5	0.545	6.0	0.218
2.0	1.161	3.5	0.796	5.0	0.420	6.5	0.099
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} =0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.0	1.347	2.5	1.097	4.0	0.733	5.5	0.392
1.5	1.301	3.0	0.991	4.5	0.602	6.0	0.237
2.0	1.292	3.5	0.854	5.0	0.468	6.5	0.136
$C_{urea} = 1 \cdot 10^{-3}$ M; C_{HCl} =0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.0	1.347	2.5	1.022	4.0	0.620	5.5	0.230
1.5	1.252	3.0	0.893	4.5	0.488	6.0	0.131
2.0	1.155	3.5	0.765	5.0	0.357	6.5	0.051

Table 3.8 – Evaluation of metrological characteristics of urea determination

tgα	C_i, $M \cdot 10^{-3}$	$\bar{}_i$, $M \cdot 10^{-3}$	S_r, $M \cdot 10^{-5}$	$\Delta C \cdot 10^{-4}$	$(\Delta C/C)100$ %	δ, %
0.2429	1.18					
0.2494	1.06	1.08	7.57	1.21	11.2	8.0
0.2496	1.06					
0.2528	1.00					

As can be seen from the table, the repeatability of the results of determination of urea was expressed by 11.2 % error, while the error of determination was compared to $1.0 \cdot 10^{-3}$ M concentration introduced into the system (accuracy) at the level of 8.0 %.

3.7 Analysis of urea-containing cosmetic creams

Urea is determined in creams and other cosmetic products, where it serves to soften the skin. As one of the major soluble substances of the skin, urea has a growing importance in dermatological therapy and cosmetics. Urea is of significance for hydration of some skin layers. Normal skin contains approximately 1 % urea. Furthermore, urea may be incorporated as an active ingredient in moisturizers due to its humectant properties. In the paper [2] a method was suggested for determination of urea in cosmetic creams and other products.

Urea in cosmetic products previously was determined by a complicated method, including extraction into chloroform, with subsequent re-extraction into water.

Meanwhile, urea is highly soluble in water and when the cream comes into contact with water, its hydrophobic base remains unchanged, while urea passes into water. If the experiment is carried out by the author's [2] method, a stiff emulsion is formed, but if the cream is kept in contact with water for a long time (several hours, up to 24 h), the urea contained in it is transferred into water due to diffusion.

The determination of urea content has been investigated by our optimized procedure in two cosmetic creams:

1) the "Organic foot care" cosmetic product with the urea content of 16.4 %; its composition includes: Aqua, **urea**, paraffinum liquidum, hydrogenated palm oil, glycerin, cetearyl alcohol, triethanolamine, isopropyl palmitate, lactic acid, citric acid, stearyl alcohol, ceteareth-6, glyceryl stearate, PEG-100 stearate, ceteareth-20, hydrogenated castor oil, *Abies Sibirica* needle extract, *Pinus Sibirica* seed oil, *Chelidonium Majus* extract, phenoxyethanol, methylparaben, ethylparaben, propylparaben, DMDM hydantoin, *Juniperus Communis* wood oil, parfum, d-limonene, butylphenyl methylpropional, geraniol.

2) the "Beloruchka" cosmetic product, in which the urea content was not specified by the manufacturers; its composition includes: Aqua, sorbitol, caprylic/capric triglycerides, paraffinum liquidum, cetearyl alcohol (and) potassium cetyl phosphate, cyclopentasiloxane, octocrylene, dimethicone, hydrogenated vegetable oil, petrolatum, *Helianthus Annuus* (sunflower) seed oil, , PPG-15 stearyl ether, butyl methoxydibenzoylmethane, **urea**, acrylates/vinyl isodecanoate crosspolymer, parfum, polysorbate 20, *Prunus Amygdalus Dulsis* (sweet almond) seed oil, allantoin, *Brassica Campestris* (rapeseed) seed oil (and) BHT (and) BHA (and) propyl gallate (and) propylene glycol (and) monoglycerides (and) citric acid, tocopheryl acetate, titanium dioxide (and) silica, methylchloroisothiazolinone (and) methyl isothiazolinone, carbomer, disodium EDTA, citric acid, sodium hydroxide.

To a 250-mg sample of cosmetic cream 100 mL of water measured by a volumetric flask was added, the two-phase system was thoroughly mixed and left to stand for a day (not always exactly 24 h). After that, the solution was filtered and each filtrate sample was centrifuged in a centrifuge CM-6M ELMI separately at 2000 rpm for 10 min. A 2-mL aliquot of a filtrate sample was added to a 10-mL graduated test-tube, 3 mL of hydrochloric acid with the concentration of 0.856 M, 1 mL of methyl orange solution with the concentration of $6 \cdot 10^{-4}$ M were added; the solution was diluted to a volume of 5 mL with distilled water. Then 1.3 mL of potassium bromate solution with the concentration of $1 \cdot 10^{-2}$ M was added and the resulting solution was diluted with water to the 10-mL mark. The time of reaction was measured from the start of adding the last drop of the bromate solution; the absorbance control began 1 min later, until complete discoloration of the solution. The solution was thoroughly mixed and a part of it was transferred to the cuvette for measurement. Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. The data are given in Tables 3.9 (for sample **1** "Organic foot care") and 3.12 (for sample **2** "Beloruchka").

Table 3.9 – Time-dependences of absorbance for sample **1**

t, min	A	t, min	A	t, min	A	t, min	A
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.5	1.187	3.0	0.824	4.5	0.462	6.0	0.167
2.0	1.045	3.5	0.700	5.0	0.346	6.5	0.108
2.5	0.928	4.0	0.585	5.5	0.252	–	–
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.0	1.318	3.0	0.959	5.0	0.469	7.0	0.114
1.5	1.284	3.5	0.824	5.5	0.347	–	–
2.0	1.187	4.0	0.721	6.0	0.260	–	–
2.5	1.071	4.5	0.578	6.5	0.184	–	–
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.0	1.346	3.0	1.000	5.0	0.523	7.0	0.143
1.5	1.301	3.5	0.886	5.5	0.409	7.5	0.097
2.0	1.167	4.0	0.770	6.0	0.318	–	–
2.5	1.097	4.5	0.638	6.5	0.222	–	–
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.0	1.284	2.5	0.959	4.0	0.602	5.5	0.252
1.5	1.160	3.0	0.847	4.5	0.481	6.0	0.161
2.0	1.097	3.5	0.726	5.0	0.357	6.5	0.097
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M							
1.0	1.301	2.5	1.022	4.0	0.662	5.5	0.309
1.5	1.222	3.0	0.921	4.5	0.553	6.0	0.208
2.0	1.125	3.5	0.796	5.0	0.420	6.5	0.125

After plotting all the kinetic curves the initial rates were calculated from the experimental measurements. The slope coefficients (calculated with the use of the least-square method) are negative, as the absorbance decreases in the case of methyl orange discoloration. For graphic representation of initial rates we have chosen to use the absolute amount of slope coefficients (| |).

We evaluated the metrological characteristics by performing 5 replicate analyses of aliquots of a solution obtained from the cosmetic-cream sample with the urea content of 16.4 %. The results are shown in Table 3.10.

Table 3.10 – Evaluation of metrological characteristics of urea in cosmetic-cream determination for sample **1**

tgα	X_i, %	$\bar{X}_i$, %	S, %	ΔX	(ΔX/X)100 %	δ, %
0.2334	16.34	15.60	0.60	0.86	5.5	4.8
0.2382	15.26					
0.2386	15.14					
0.2390	15.14					
0.2351	15.98					

Thus, the result of analytical determination of the urea content in the "Organic foot care" cosmetic product shows that it amounts to (15.6 ± 0.9) %.

If it is necessary to evaluate the accuracy of the method or methodology, an analysis of a standard sample is carried out – namely, a sample with a precisely specified content of the component to be determined (the true value of the determined value μ). Let the quantitative analysis of the standard sample be carried out by the quantitative method: n replicate determinations are made and the mean value $\bar{}_i$, standard deviation S, variance $V = S^2$, are calculated. Comparison of the mean with the true value of μ should solve the question whether the discrepancy between $\bar{}_I$ and μ is significant or not significant. If it is significant, there is a systematic error in determination. To do this evaluation, we used the Student's t-test, proceeding as follows.

The Student's t-test experimental value is calculated by the formula (1):

$$\frac{|\bar{\quad} \quad|}{\quad} \sqrt{\quad}$$

The value of t_{calc} is compared to the tabulated critical value t_{tabl} of the Student's function for a given confidence level P and a given number of degrees of freedom $f = n - 1$. In our case, the number of degrees of freedom $f = 4$, the confidence level $P = 0.95$, the tabulated value $t_{p,\,f} = 3.188$.

$$\frac{|\bar{\quad} \quad|}{\quad} \sqrt{\quad} \qquad \frac{\qquad\qquad}{\qquad} \sqrt{\quad}$$

$$2.98 < 3.188$$

Since $t_{exp} < t_{p,f}$, the discrepancy between the result of the analysis and the true value is insignificant, that is, the determination does not contain systematic errors. Comparing the mean of the urea determination of sample **1** to its true value from the manufactures, we got the error 4.8 %, which is due to random factors.

Next we determined the urea content in a sample of the "Beloruchka" cosmetic product, for which the urea content was not specified by the manufacturers. Therefore, in order to evaluate the accuracy of the determination, we used the value obtained according to the method of the authors of the work [2].

The 250-mg sample of the "Beloruchka" cosmetic cream was dissolved in 50 mL of chloroform in a separatory funnel and shaken for 15–20 minutes. The resulting system is not a true solution, but a thick emulsion. Then 40 mL of distilled water was added, after which the solution was divided into two phases. Chloroform and water phases were separated, the aqueous phase was filtered through a filter to remove insolubles, and then diluted to 100 mL with distilled water. After that, the solution was filtered and each filtrate sample was centrifuged in a centrifuge CM-6M ELMI separately at 2000 rpm for 10 min. A 2-mL aliquot of a filtrate sample was added into a 10-mL graduated test-tube, 3 mL of hydrochloric acid with the concentration of 0.856 M, 1 mL of methyl orange solution with the concentration of $6 \cdot 10^{-4}$ M were added; the solution

was diluted to a volume of 5 mL with distilled water. Then 1.3 mL of potassium bromate solution with the concentration of $1 \cdot 10^{-2}$ M was added and the found solution was diluted with water to the mark 10-mL. The time of reaction was measured from the start of adding the last drop of the bromate solution; the absorbance control began 1 min later, until complete discoloration of the solution. The solution was thoroughly mixed and a part of it was transferred to the cuvette for measurement. Inhibition of the reaction was monitored by a photocolorimeter KFK-2 by observing the change in absorbance of the solution at 490 nm. The data are given in Table 3.11.

Table 3.11 – Time-dependency of re-extract absorbance

t, min	A	t, min	A
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M			
1.0	1.397	5.0	0.721
1.5	1.376	5.5	0.602
2.0	1.301	6.0	0.1481
2.5	1.222	6.5	0.347
3.0	1.155	7.0	0.230
3.5	1.046	7.5	0.113
4.0	0.959	8.0	0.041
4.5	0.839	–	–

The tgα value has been obtained; the amount and the confidence interval has been got the from calibration curve (Section 3.5). Another 250-mg sample of cosmetic cream "Beloruchka" has been prepared by adding of 100 mL of water measured by a volumetric flask, thoroughly mixing the two-phase system and standing for a day. All the rest of the procedure was the same as for the sample 1 of the "Organic foot care" cosmetic product. The data are given in Table 3.12.

The tgα value has been obtained; the amount and the confidence interval has been got the from calibration curve (Section 3.5). We evaluated the metrological characteristics by performing 5 replicate analyses of aliquots of a solution obtained from the cosmetic-cream sample. The results are shown in Table 3.13.

Table 3.12 – Time-dependences of absorbance for sample **2**

t, min	A	t, min	A	t, min	A
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M					
1.0	1.376	3.5	0.951	6.0	0.347
1.5	1.318	4.0	0.839	6.5	0.222
2.0	1.222	4.5	0.721	7.0	0.108
2.5	1.155	5.0	0.602	–	–
3.0	1.046	5.5	0.475	–	–

Completed Table 3.12

C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO_3} = 1.3 \cdot 10^{-2}$ M					
1.0	1.398	3.5	0.744	6.0	0.357
1.5	1.376	4.0	0.620	6.5	0.230
2.0	1.301	4.5	0.495	7.0	0.114
2.5	1.222	5.0	1.000	–	–
3.0	1.097	5.5	0.854	–	–
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO3} = 1.3 \cdot 10^{-2}$ M					
1.0	1.376	3.5	0.921	6.0	0.301
1.5	1.301	4.0	0.801	6.5	0.180
2.0	1.222	4.5	0.678	7.0	0.075
2.5	1.125	5.0	0.568	–	–
3.0	1.046	5.5	0.444	–	–
C_{urea} = X M; C_{HCl} = 0.257 M; $C_{MO} = 6 \cdot 10^{-5}$ M; $C_{KBrO3} = 1.3 \cdot 10^{-2}$ M					
1.0	1.376	3.5	0.921	6.0	0.321
1.5	1.301	4.0	0.796	6.5	0.230
2.0	1.222	4.5	0.678	7.0	0.114
2.5	1.155	5.0	0.563	–	–
3.0	1.046	5.5	0.432	–	–

Table 3.13 – Evaluation of metrological characteristics of urea in cosmetics-creams determination for sample **2**

tgα	X_i, mg	$\bar{}_i$, mg	S_r, mg	ΔX	(ΔX/X)100%	δ, %
0.2354 0.2480 0.2447 0.2472 0.2362	39.64 32.43 32.43 30.93 39.34	35.00	4.2	5.2	16.8	1.2
0.2431 (CH_3Cl)	35.44	35.44	0.66	2.8	7.9	–

The average weighted two variances are calculated as by the formula (2).

$$\frac{-\cdot\cdot}{\qquad\qquad}$$

where f_1 – number of degrees of freedom for the sample **2**

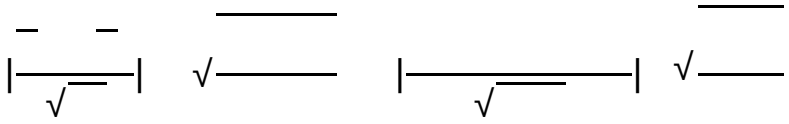
f_2 – number of degrees of freedom for the sample with CH_3Cl

– standard deviation for the sample **2**

– standard deviation for the sample with CH_3Cl

If the discrepancy between the variances is insignificant, then it is possible to compare the mean values of the two samples using the average weighted value of the variances, taking into account that the variance obtained with a larger number of degrees of freedom contributes more to the averaged value.

The value of t_{exp} is compared with t_{crit}, i.e. with the value of the Student's coefficient with the number of degrees of freedom $f = f_1 + f_2 = 4 + 2 = 6$ and the chosen trust probability. The confidence level $P = 0.95$, the tabulated value $t_{p,f} = 2.45$



$$0.38 < 2.45$$

Since $t_{exp} < t_{p,f}$, the discrepancy between the result of the analysis and the true value is insignificant, that is, the determination does not contain systematic errors. Comparing the mean of the urea determination of sample **2** to its true value, we got the error 1.2 %, which is due to random factors.

4 CONCLUSION

1. Optimal conditions were chosen for the reaction of decolorization of methyl orange by potassium bromate in order to determine urea: hydrochloric acid concentration equals 0.257 M, methyl orange concentration is $6 \cdot 10^{-5}$ M, and potassium bromate concentration is $1.3 \cdot 10^{-3}$ M.
2. The calibration curve for urea determination is linear in the range $(0.2–1.2) \cdot 10^{-5}$ M urea concentration, which corresponds to (12–72) μg/mL, the error of reproducibility is note above 11.2 %, the error in determining the introduced amount is 8.0 %.
3. The presence of urea in two cosmetic products has been investigated and the accuracy has been evaluated by comparison to the manufactures label and the results of arbitration analysis; the errors are 4.8 % and 1.2 %, respectively; the systematic errors in both cases are not significant.

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РЕФЕРАТ

Гайфулина Р.Р. Фотометрическое определение мочевины по ингибированию окисления метилоранжа бромат-ионом – Челябинск: ЮУрГУ, ЕТ-451, 2017. – 43 с., 19 ил., 13 табл., библиогр. список – 18 наим.

Был исследован кинетический метод (метод тангенсов) для оптимизации условий проведения реакции метилоранжа с броматом калия, применяемый с целью определения мочевины в кислой среде.

Цель работы – фотометрическое определение мочевины по ингибированию окисления метилоранжа бромат-ионом и её извлечение из косметических продуктах.

Для достижения цели НИР решены следующие задачи:

– проведен литературный обзор по проблеме исследования;

– исследовано влияние переменных;

– проведен анализ косметических средств, содержащих мочевину;

– рассчитаны метрологические характеристики.

Оптимальные условия определения: 0.257 М HCl, $6 \cdot 10^{-5}$ М метилоранж, $1.3 \cdot 10^{-3}$ М $KBrO_3$. Градуировочный график линеен в интервале (0.2-1.2) $\cdot 10^{-5}$ М концентраций мочевины, что соответствует (12–72) мкг/мл. Оптимизированный метод был использован для определения мочевины в косметических кремах; погрешность определения известного количества не превышала 4,8 %, систематические ошибки незначимы.