MODELING AND OPTIMIZING OF EFFECTIVE FACTORS ON KINETIC SPECTROPHOTOMETRIC DETERMINATION OF VITAMIN B_{12}

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A new, accurate, sensitive, and reliable kinetic spectrophotometric method for the determination of vitamin B_{12} in biological samples and pharmaceutical formulations has been developed. A central composite design (CCD) of the response surface methodology was used in our research to investigate the effect of reaction time and temperature and the concentration of reagents including orange G, bromate, and sulfuric acid on the assay of vitamin B_{12}. Vitamin B_{12} shows a strong inhibitory effect on the oxidation of orange G by bromate in acidic media. The wide linear dynamic range, low detection limit, and short time analysis introduce the proposed method as a new strategy for the determination of vitamin B_{12} in biological and pharmaceutical samples. Also, linear polynomial mathematical models were obtained for the determination of vitamin B_{12}. Under optimum experimental conditions, the calibration curve was linear over the range 1.0–200.0 μg/mL. The limit of detection was 0.56 μg/mL. Statistical comparison of the results with the reference methods shows excellent agreement and indicates no significant difference in accuracy and precision.

Keywords: *vitamin B_{12}, kinetic spectrophotometry, central composite design, response surface methodology, biological sample, pharmaceutical formulation, orange G-bromate reaction system.*

МОДЕЛИРОВАНИЕ И ОПТИМИЗАЦИЯ ЭФФЕКТИВНЫХ ФАКТОРОВ КИНЕТИЧЕСКОГО СПЕКТРОФОТОМЕТРИЧЕСКОГО ОПРЕДЕЛЕНИЯ ВИТАМИНА B_{12}

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Разработан точный, чувствительный и надежный кинетический спектрофотометрический метод определения витамина B_{12} в биологических образцах и фармацевтических препаратах. Для изучения влияния времени и температуры реакции, реагентов (оранжевого G), концентрации бромноватой и серной кислот при анализе характеристик витамина B_{12} использован центрально-композиционный план методологии поверхности отклика. Витамин B_{12} оказывает сильное ингибирующее действие на процесс окисления оранжевого G броматом в кислой среде. Широкий линейный динамический диапазон, низкий предел обнаружения и скорость анализа характеризуют предлагаемый метод как новую стратегию определения витамина B_{12} в биологических и фармацевтических образцах. Созданы линейные полиномиальные математические модели для определения витамина B_{12}. Для оптимальных экспериментальных условий калибровочная кривая линейная в диапазоне 1.0–200.0 мкг/мл. Предел обнаружения витамина B_{12} 0.56 мкг/мл. Статистические сравнения результатов с эталонными методами показывают отличное согласие и не указывают на существенную разницу в точности и прецизионности.

Ключевые слова: *витамин B_{12}, кинетическая спектрофотометрия, центрально-композиционный план, методология поверхности отклика, биологический образец, фармацевтический препарат, реакционная система оранжевого G-бромата.*

Introduction. Vitamins strengthen the immune system, enable the body to use other nutrients, complement enzymes, detoxify the organism, and reduce the risk of cardiac infarction [1]. They are essential for our energy production and influence metabolism reactions. Even low vitamin contents have a substantial effect. Nevertheless, under certain circumstances vitamin deficiency can occur [2–5].

Vitamin B_{12} ($C_{63}H_{88}CoN_{14}O_{14}P$) is water-soluble. It is found naturally in some foods and s available as a dietary supplement, playing an important role in the functioning of the brain and nervous system and in the formation of red blood cells [6, 7]. The requirement for vitamin B_{12} is low and the storage capacity in humans is high, so the deficiency is rare in healthy populations. However, certain population groups, such as infants, the elderly, vegetarians or vegans, can be prone to deficiency [8]. This can be controlled in vegetarian groups by supplementation. Deficiency, particularly in the elderly, is often a result of inadequate metabolism and not related to dietary intake; the need for analysis of foods is therefore low. In specific population groups, such as vegetarians, intake may rely on supplementation, and in others, such as infants, the fortification of infant formula and follow-on foods is important [8, 9]. Analysis may be required in fortified foods or supplements, primarily to confirm label declarations. Methods for the analysis of vitamin B_{12} include microbiological assay [10], polarography [11], spectrophotometry [12], radio-ligand binding [13], and various chromatographic techniques [14].

Kinetic methods have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometric methods [15, 16]. The literature is still poor in analytical assay methods based on kinetics for the determination of vitamin B_{12} in dosage forms [17–19]. The application of these methods offers some specific advantages such as improved selectivity, avoiding the interference of the colored and/or turbidity background of the samples, and possibility to avoid the interference of other active compounds present in the commercial product if they resist the reaction conditions established for the proposed kinetic method [20–24]. Therefore, there is a need for another kinetic approach to estimate trace amounts of vitamin B_{12} in real samples with different matrices. The objective of the present study was to develop a precise, accurate, and validated kinetic spectrophotometric method by the application of a new reaction system in biological samples and pharmaceutical formulations.

The response surface method was used to model data from experiments using the "reasonable design method." The response surface method involved building a model of a surface using continuous variables [25]. It was used to analyze optimal conditions in systems with multifactor interaction analysis including different factors. It was widely used in different scientific fields to solve problems with a multivariate experimental design and methods of statistical analysis [26, 27]. However, no previous study has used the response surface methodology to determine optimal methods for the kinetic spectrophotometric determination of vitamin B_{12}. In the present study, we used the method of central composite design to develop and analyze a method for the determination of vitamin B_{12}. The mathematical model was established using the response surface methodology, and then its validity was verified. The parameters for the optimal determination of vitamin B_{12} were determined using the response surface method. The results show that vitamin B_{12} is strongly affected by the oxidation of orange G by bromate in acidic media. The proposed method has great value in its application to the analysis of vitamin B_{12}.

Experimental. All the reagents used were of analytical grade or higher; they included vitamin B_{12} (Sigma), sulfuric acid 98% (Merck), potassium bromate (Merck), and orange G (Merck). Vitamin B_{12} injection solution was purchased from Exir Pharmaceutical Co (Tehran, Iran).

A double beam Unique UV-Vis spectrophotometer (T80+, UK) with 1 cm matched glass cells was used to measure the absorbance. A thermostat water bath (Hieldolph, Germany) was used to keep the temperature of all the solutions at the working temperature level (25±0.1°C). A stopwatch was used to record the reaction time.

After the initial kinetic spectrophotometric studies of the reaction system, the reagent concentrations (except vitamin B_{12}) were judiciously chosen for the analytical procedure. The inhibited reaction was studied spectrophotometrically by monitoring the change in the absorbance of the reaction mixture at 478.5 nm. To a series of 10 mL volumetric flasks, 1 mL of 6.6×10^{-4} mol/L orange G solution, 3 mL of 4.0 mol/L sulfuric acid solution, and 1 mL of 100.0 μg/mL of vitamin B_{12} solution were added. The solutions were mixed and diluted with water. Then 1 mL of 5.0×10^{-2} mol/L bromate solution was added, and the volume was adjusted to

the mark with water. The time measurement started just after adding the last drop of the oxidant solution. The solution was thoroughly mixed, and a portion of it was transferred to a glass cell. The absorbance of the inhibited reaction (ΔA_s) was measured against water at 478.5 nm, 25°C, and a time interval of 30–360 s. The measurement in the absence of vitamin B_{12} was repeated to obtain the values for the uninhibited reaction (ΔA_b). Finally, the difference in the absorbance was considered as the response ($\Delta A = \Delta A_s - \Delta A_b$). Under optimum experimental conditions, the calibration curve was constructed by plotting the response against vitamin B_{12} concentration in vitamin B_{12} working standard solutions.

Experimental design and data analyses. Based on the previous studies, we did not use the one-factor-at-a-time technique for analysis. To determine the optimal reaction conditions, a five-factor central composite design (CCD) was used (Table 1), and 50 experimental runs were performed. The variables used to represent the determination of vitamin B_{12} included reaction time (30–540 s), reaction temperature (15–40°C), orange G concentration (33.0–99.0 μmol/L), bromate concentration (1.5–10.0 mmol/L), and sulfuric acid concentration (0.2–1.6 mol/L). The results were analyzed using the response surface methodology and a second-order polynomial equation:

$$Y = \alpha_0 + \sum_{i=1}^{n} \alpha_i x_i + \sum_{i=1}^{n} \alpha_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j=i+1} \alpha_{ij} x_i x_j. \quad (1)$$

Here, Y is the response; X_i and X_j are independent variables; α_0 is an offset term; α_i is the parameter for linear effects; α_{ij} are the coefficients for the first-order interaction effect, and α_{ii} are the coefficients describing the squared effect [27, 28]. The design of experiments, building of regression models, and data analysis were performed with Design Expert software (Version 12, Stat-Ease Inc., Minneapolis, MN, USA). To determine the ideal conditions for the determination of vitamin B_{12}, analyses of variance (ANOVAs) were used, followed by regression analyses and plotting the response surfaces. In addition, factorial analyses helped to test for two-factor interactions using two-way ANOVAs, followed by one-way ANOVAs using mean separation techniques after determining whether interaction or no-interaction occurred. Three-dimensional surface charts showing the responses of two independent variables were produced from the data using the response surface methodology. Other ANOVAs or regression analyses led to linear equations and their variables. In all analyses, $p < 0.05$ was considered significant. When the optimal conditions for reactions were predicted, the tests were repeated three times to check for consistency [29, 30].

TABLE 1. The Range of Independent Variables and the Observed Responses in the Experimental Design

Factors	Name	Unit	Low level	High level
A	Orange G concentration	μmol/L	33.0	99.0
B	Bromate concentration	mmol/L	1.5	10.0
C	Sulfuric acid concentration	mol/L	0.2	1.6
D	Temperature	°C	15	40
E	Time	S	30	540

	Factors					Response
Run	*A*	*B*	*C*	*D*	*E*	Absorbance
1	99.00	5.75	0.90	27.50	285.00	0.25
2	66.00	5.75	0.90	27.50	285.00	0.30
3	79.87	3.96	0.61	22.24	177.79	0.21
4	66.00	5.75	0.90	15.00	285.00	0.19
5	79.87	7.54	1.19	22.24	177.79	0.31
6	79.87	3.96	0.61	22.24	392.21	0.42
7	66.00	5.75	0.90	40.00	285.00	0.22
8	79.87	3.96	0.61	32.76	177.79	0.23
9	52.13	3.96	0.61	32.76	177.79	0.20
10	66.00	5.75	0.20	27.50	285.00	0.13
11	79.87	7.54	1.19	22.24	392.21	0.40
12	79.87	3.96	1.19	22.24	392.21	0.19
13	66.00	5.75	0.90	27.50	30.00	0.11
14	79.87	7.54	0.61	22.24	392.21	0.21

Continue Table 1

Factors						Response
Run	*A*	*B*	*C*	*D*	*E*	Absorbance
15	52.13	7.54	0.61	32.76	392.21	0.39
16	79.87	3.96	1.19	32.76	177.79	0.40
17	52.13	3.96	1.19	22.24	392.21	0.38
18	52.13	7.54	1.19	32.76	177.79	0.12
19	79.87	7.54	0.61	32.76	177.79	0.21
20	52.13	7.54	0.61	22.24	392.21	0.42
21	79.87	3.96	1.19	22.24	177.79	0.36
22	52.13	7.54	0.61	22.24	177.79	0.25
23	79.87	7.54	0.61	32.76	392.21	0.39
24	66.00	5.75	0.90	27.50	285.00	0.18
25	66.00	5.75	0.90	27.50	285.00	0.18
26	52.13	3.96	1.19	32.76	392.21	0.41
27	79.87	3.96	0.61	32.76	392.21	0.43
28	52.13	3.96	1.19	32.76	177.79	0.23
29	52.13	7.54	1.19	22.24	177.79	0.10
30	66.00	5.75	0.90	27.50	285.00	0.18
31	66.00	5.75	0.90	27.50	285.00	0.18
32	52.13	3.96	0.61	22.24	177.79	0.20
33	33.00	5.75	0.90	27.50	285.00	0.16
34	52.13	7.54	1.19	32.76	392.21	0.15
35	52.13	7.54	1.19	22.24	392.21	0.13
36	52.13	7.54	0.61	32.76	177.79	0.29
37	79.87	7.54	1.19	32.76	392.21	0.43
38	66.00	1.50	0.90	27.50	285.00	0.07
39	66.00	5.75	0.90	27.50	285.00	0.18
40	66.00	5.75	1.60	27.50	285.00	0.18
41	79.87	7.54	0.61	22.24	177.79	0.27
42	52.13	3.96	0.61	32.76	392.21	0.31
43	66.00	5.75	0.90	27.50	285.00	0.18
44	79.87	3.96	1.19	32.76	392.21	0.22
45	52.13	3.96	0.61	22.24	392.21	0.31
46	66.00	5.75	0.90	27.50	285.00	0.18
47	66.00	10.00	0.90	27.50	285.00	0.42
48	66.00	5.75	0.90	27.50	540.00	0.29
49	79.87	7.54	1.19	32.76	177.79	0.25
50	52.13	3.96	1.19	22.24	177.79	0.19

Analysis of the real sample. The content of two ampoules was mixed and diluted to 50 mL with water. An accurate amount equivalent to 0.5 mg of the drug was further diluted with the same solvent in a 10 mL volumetric flask. The procedure was continued as described under the general procedure.

Human serum and urine were used as biological samples for the determination of vitamin B_{12}. They were spiked with vitamin B_{12}, and the solid phase extraction technique with a C_{18} cartridge (Supelco Inc., 10 mL) was used for the purification and preconcentration of vitamin B_{12} from the samples [31]. The extracted vitamin B_{12} was determined by the developed method.

Results and discussion. Orange G is a basic dye of a mono-azo group that can be oxidized by oxidizing agents such as bromate in acidic media to produce a colorless oxidized form. It was used as an indicator for the catalytic determination of different species such as vanadate [32]. The absorption spectra of the inhibited and uninhibited reaction mixture at different time intervals are shown in Fig. 1. As can be seen, the change in absorbance was decreased in the presence of vitamin B_{12} at trace levels. Therefore, the sensitive proposed reaction system can be used for the trace amount determination of vitamin B_{12}.

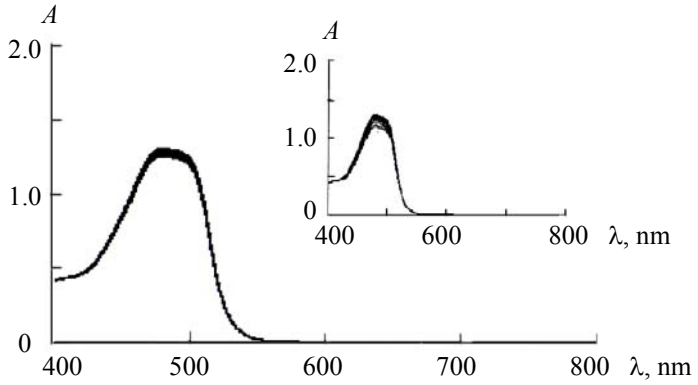

Fig. 1. Spectrum of the sample; conditions: orange G, 6.6×10^{-5} mol/L; sulfuric acid 1.2 mol/L, vitamin B_{12}, 10.0 μg/mL; bromate, 5.0×10^{-3} mol/L; 25°C and 30–360 s; the inset shows a blank spectrum that was recorded in the absence of vitamin B_{12}.

Optimization of the reaction variables. In order to establish the experimental conditions under which the inhibitory effect of vitamin B_{12} and, therefore, the sensitivity in its determination are at a maximum, the dependences of the reaction rate on the reagent concentration, temperature, and time were studied. The change in absorbance after a fixed time as a measure of the initial rate was used to plot the graph for each variable. The optimum conditions were taken from the graphs for the subsequent study of the variables. The reagent concentration optimization was carried out on the uninhibited and inhibited reactions for a constant time of 360 s in the presence of 10.0 μg/mL of vitamin B_{12}.

The experimental results of the study of the orange G concentration effect in the range from 33.0 to 99.0 μmol/L indicate that the difference in absorbance increases with the concentration of orange G up to 66.0 μmol/L (Fig. 2a), which may be attributed to orange G aggregation. Therefore, 66.0 μmol/L of orange G was selected as the optimum value.

The effect of the sulfuric acid concentration on the uninhibited and inhibited reactions was studied in the concentration range from 0.2 to 1.6 mol/L. As shown in Fig. 2b, the reaction rate increases with increase in the concentration of sulfuric acid up to 1.2 mol/L. At higher concentrations, the reaction rate decreased. This decrease at higher acidic conditions may be attributed to the protonation of orange G, which might stop oxidation or make oxidation difficult. Thus, 1.2 mol/L of sulfuric acid was used for further study.

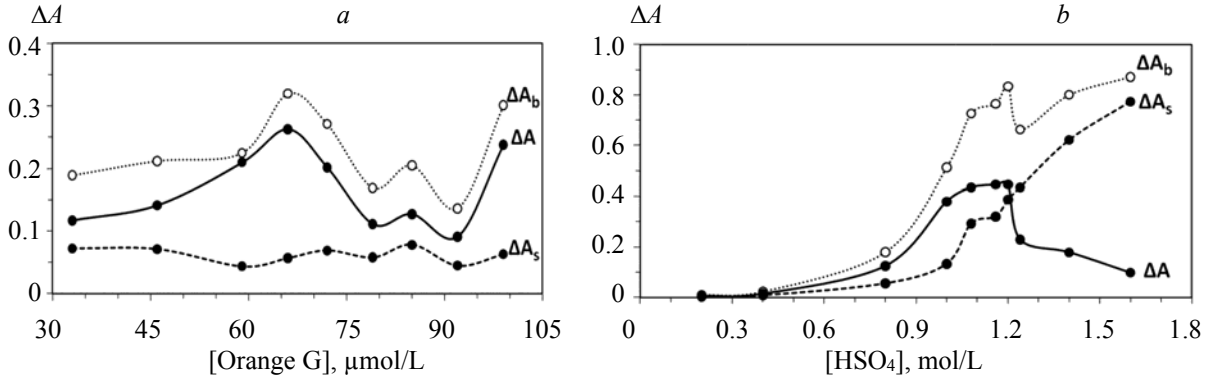

Fig. 2. Effect of the orange G (a) and sulfuric acid concentration (b) on the rate of the uninhibited (ΔA_b) and inhibited (ΔA_s) reactions and the response (ΔA); conditions: vitamin B_{12}, 10.0 μg/mL; bromate, 5.0×10^{-3} mol/L; 25°C and 360 s; a – orange G, 33.0–99.0 μmol/L; sulfuric acid 1.2 mol/L, b – orange G, 6.6×10^{-5} mol/L; sulfuric acid 0.2–1.6 mol/L.

The dependence of the oxidation reaction rate on the bromate concentration was studied in the concentration range from 1.5 to 10.0 mmol/L. As shown in Fig. 3a, under the optimum concentrations of sulfuric acid and orange G, the reaction rate increased up to 4.5 mmol/L of bromate. Decreasing the orange G concentrations led to reducing the net reaction rate. Therefore, the optimum value of 4.5 mmol/L of bromate was selected for following the procedure.

Under the optimum reagents concentration, the temperature effect on the rate of reaction was studied in the range 15–40°C. The maximum sensitivity was obtained at 30°C and selected as optimum

The optimum time was found by measuring the change in the absorbance during 30–540 s. The reaction rate was increased up to 360 s, and for longer times it was reduced (Fig. 3b). Therefore, 360 s was selected as optimum for further study.

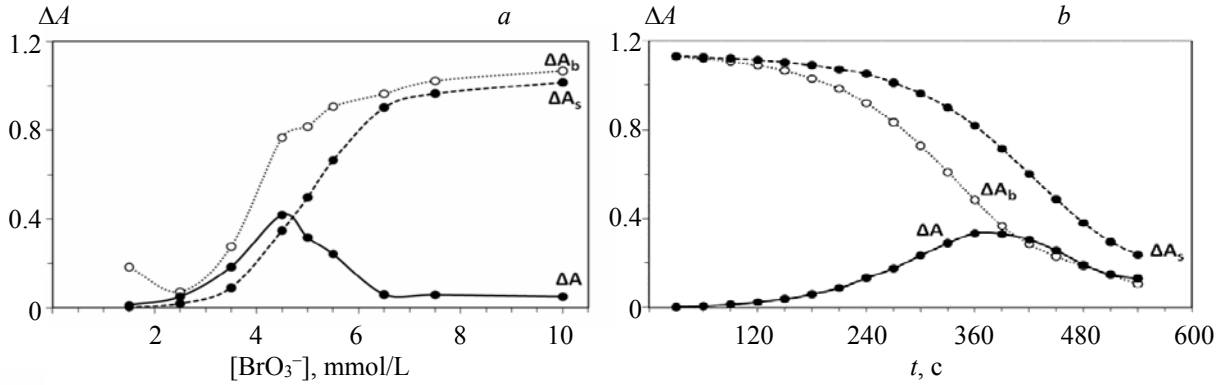

Fig. 3. Effect of the bromate concentration (a) and time (b) on the rate of the uninhibited (ΔA_b) and inhibited (ΔA_s) reactions and the response (ΔA); conditions: orange G, 6.6×10^{-5} mol/L; sulfuric acid 1.2 mol/L, vitamin B_{12}, 10.0 μg/mL; 30 °C and 360 s; bromate, 1.6×10^{-3}–6.5×10^{-3} mol/L (a) and 4.5×10^{-3} mol/L (b).

Optimization using the response surface methodology. The experimental design and results for experimental trials were determined (Table 1). ANOVAs helped to produce the linear models for response surfaces, which elucidated the fitness, accuracy, and significance of the models in addition to the effects of interaction results and individual variables on the responses (Table 2).

TABLE 2. ANOVA Results of the Established Model for Responses

Source	Sum of squares	*Df*	Mean square	*F*-value	*P*-value
Model	0.1119	5	0.0224	2.49	0.0454 significant
Orange G conc.	0.0261	1	0.0261	2.91	0.0953
Bromate conc.	0.0049	1	0.0049	0.5489	0.4627
Sulfuric acid conc.	0.0028	1	0.0028	0.3163	0.5767
Temperature	0.0034	1	0.0034	0.3732	0.5444
Time	0.0746	1	0.0746	8.30	0.0061
Residual	0.3958	44	0.0090		
Lack of fit	0.3832	37	0.0104	5.75	0.0109 significant
Pure error	0.0126	7	0.0018		
Corr. total	0.5077	49			

The correlation coefficients R^2 and adjusted-R^2 were used to test the fit of the model equation

$$Y = 0.2518 + 0.0246A + 0.0107B - 0.0081C + 0.0088D + 0.0415E. \quad (2)$$

R^2 was 0.9721, indicating the model predicted the response well because R^2 was close to 1. The value of the adjusted-R^2 (0.9470) was also very high, indicating a satisfactory adjustment of the mathematical model to the test data.

Additional analysis by ANOVAs was shown by the 2.49 model *F*-value. It implies that the model is significant for absorbance. Moreover, there is only a 4.45% chance that such a large model *F*-value could occur due to the noise. The adequate precision ratio of 6.01 indicates an adequate signal where it measures the signal to noise ratio; a ratio greater than 4 is desirable. *P*-values less than 0.0500 indicate that the model terms are significant, whereas values greater than 0.1000 are usually considered as nonsignificant. Changes in responses for the orange G concentration (*A*), bromate concentration (*B*), sulfuric acid concentration (*C*), temperature (*D*), and reaction time (*E*) are shown by negative and positive coefficients for the primary effects Eq. (2). The absolute value of the coefficients (Eq. (2)) supported a strong correlation with their effects

size. The central composite design of response surface methodology (CCD/RSM) experiments revealed the sequence for significance as follows: concentration > reaction times > reaction temperatures.

The three-dimensional response surfaces were based on Eq. (1) and helped to explain the interactive and main effects of independent variables (Fig. 4).

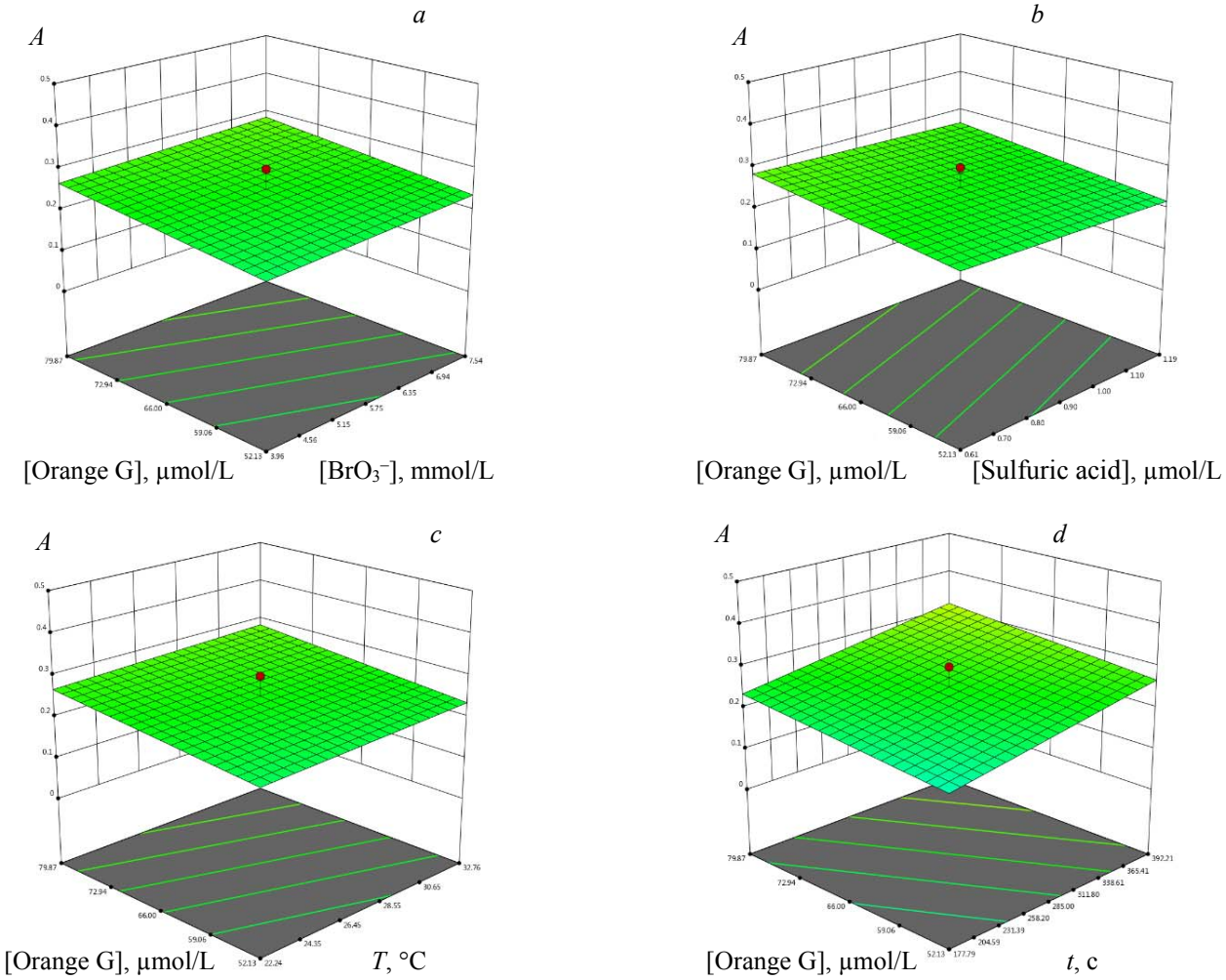

Fig. 4. Mean plot of the tested factors for the absorbance response. a) The effect of the orange G concentration and the bromate concentration on the catalyzed reaction; b) the effect of the orange G concentration and the sulfuric acid concentration on the catalyzed reaction; c) the effect of the orange G concentration and temperature; d) the effect of the orange G concentration and the reaction time on the catalyzed reaction.

Analytical parameters. Under the optimum experimental conditions, the calibration curve was obtained over the range 1.0–200.0 μg/mL, including two linear segments of 1.0–50.0 and 50.0–200.0 μg/mL. Analysis of the data gave the following regression equation: $\Delta A = 0.0039$ [Vitamin B_{12}] + 0.3038 ($R^2 = 0.9978$) for the first and $\Delta A = 0.0007$ [Vitamin B_{12}] + 0.4647 ($R^2 = 0.9986$) for the second linear segment, where ΔA is the difference in absorbance between the blank and the sample, [Vitamin B_{12}] is the Vitamin B_{12} concentration in mg/L, and R^2 is the correlation coefficient, as shown in Fig. 5. The detection limit ($3S_b/m$) was 0.56 μg/mL.

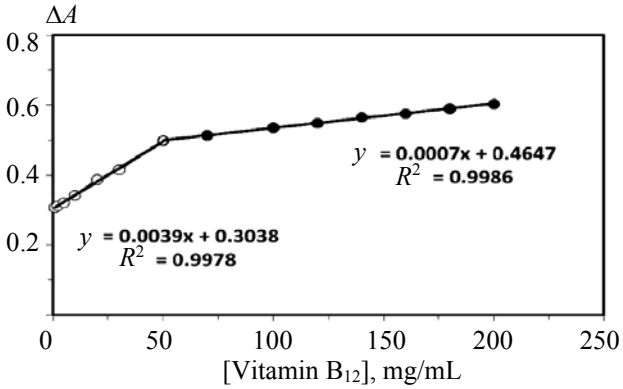

Fig. 5. Calibration plot of the absorbance versus the concentration of vitamin B_{12}.

Interference studies. The interfering effect of foreign species in the determination of 50.0 μg/mL of vitamin B_{12} was investigated. The tolerance limit was defined as the concentration of the added species causing an error (analytical signal) more than ± 5%. The results are given in Table 3. The obtained results show that Fe^{+2}, NO_2^-, and Cl^- have a serious interfering effect.

TABLE 3. Tolerance Limit for Foreign Species on the Determination of 50.0 μg/mL of Vitamin B_{12}

Foreign species	Tolerance limit ($W_{\text{Vitamin B12}}/W_{\text{species}}$)
Na^+, K^+, Ca^{2+}	1000
EDTA	1000
NO_3^-, PO_4^{3-}	1000
Mn^{2+}	950
Mg^{2+}	910
Glucose	850
Saccharose	800
Fructose, ethanol, methanol	750
CN^-	740
Urea	400
Uric acid	300
SCN^-	250
Fe^{+2}	20
Cl^-, NO_2^-	15

Real sample analysis. The accuracy and applicability of the proposed method has been confirmed by the determination of vitamin B_{12} in pharmaceutical and biological samples. Pharmaceutical sample preparation was performed using the mentioned procedure. After sample preparation, they were analyzed using the recommended procedure. The results of the determinations were given in Table 4. The precision (RSD%) varies in the range 0.9–1.2% for vitamin B_{12} ampoules using the recommended procedure, respectively. The reliability of the method has been assessed by a statistical *t*-test. It was found that the experimental values differ from the critical value (3.18, 95% confidence level, and 3° of freedom). Based on the differences between the critical and experimental values, the systematic error of detecting vitamin B_{12} in pharmaceutical specimen via the presented procedure can be neglected. Also, the procedure was used for the determination of vitamin B_{12} in human serum and urine samples. After sample preparation, the samples were spiked with different amounts of vitamin B_{12}, including two linear segments of the calibration curve, and analyzed using the recommended procedure method. The obtained results of three replicate determinations are given in Table 5. The values of RSD% of the spiked serum and urine samples using the recommended procedure vary over the range 1.1–2.0 and 1.0–1.2%, respectively. With respect to the results of the real sample analysis, the developed method is free from the interfering effect of the matrix and suitable for the analysis of vitamin B_{12} in different samples.

TABLE 4. Determination of Vitamin B_{12} in Vitamin B_{12} Ampule in Dosage 500 mg/ampoule (n = 4)

Found, μg/mL[a]	RSD, %	t-test [b]
494.5±6.0	1.21	0.45
484.0±5.0	1.03	1.6
484.0±5.5	1.14	2.9
488.5±4.5	0.92	1.0

[a] After multiplying the diluting factor 10.
[b] Tabulated t-value for three degrees of freedom at P(0.95) is 3.18.

TABLE 5. Determination of Vitamin B_{12} in Human Serum, Urine, and Vitamin B_{12} Ampoule (n = 3)

Sample	Added, μg/mL	Found, μg/mL	RSD, %	Recovery, %
Serum	–	<DL	–	–
	10.0	9.9±0.2	2.0	99.0
	20.0	20.7±0. 3	1. 9	103.5
	50.0	51.1±0.6	1.2	102.2
	100.0	99.2±1.1	1.1	99.2
Urine	–	<DL	–	–
	10.0	10.1±0.1	1.0	101.0
	20.0	19.8±0. 2	1.0	99.0
	50.0	49.8±0.6	1.2	99.6
	100.0	101.6±1.2	1.2	101.6

Conclusions. Based on the response surfaces, a kinetic spectrophotometric method for the rapid determination of trace amounts of vitamin B_{12} was proposed and checked. The presented method possesses prominent advantages, including instrumental simplicity, reduced reagents consumption, improved sensitivity, analytical efficiency, and easy handling.

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