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Response Surface Methodology in Spectrophotometric Determination of Formaldehyde Using Ch romotropic Acid

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Formaldehyde in small quantities is commonly analyzed by spectrophotometric methods. One of the most-commonly used spectrophotometric techniques for this purpose is based on the reaction with chromotropic acid. Because of its simplicity, sensitivity, selectivity and its low cost, it is still widely used. Investigations for replacing the concentrated sulfuric acid with other acids or using more dilute solutions of sulfuric acid have been performed. Herein, spectrophotometric determination of formaldehyde by chromotropic acid in the sulfuric acid medium is explored and modified by response surface methodology. The reaction was monitored by measuring the absorbance of the product at 574 nm. The factors affecting the response, *i.e.* concentration of sulfuric acid and concentration of chromotropic acid, were explored and optimized using response surface methodology. The calibration curve was linear in the range of 0.03-7.00 mg l^{-1} with detection limit of 0.005 mg l^{-1}. The method was found to be sensitive, selective and was applied to determine the formaldehyde in toothpaste, clothing softener and acetic acid samples with satisfactory results.

Keywords: Formaldehyde, Chromotropic acid, Health products, Response surface methodology

INTRODUCTION

The use of formaldehyde covers a wide range of fields. In food chemistry, it is used as a food additive and is also employed as chemical intermediate in the industrial synthesis of a large number of organic compounds. Moreover, formaldehyde is commonly used in the production of plastics and it can be added to some pharmaceutical products as a preservative. Formaldehyde kills viruses, bacteria, fungi and parasites and has found wide use as a disinfectant with a broad efficiency [1]. High level of formaldehyde toxicity [2,3] necessitates the control over the content of this substance in environment, industrial products, medical preparations, and even in some food products [4]. In the past years, the formaldehyde has received a great deal of attention due to their recognized toxic activity associated with eyes and upper respiratory tract [5]. Formaldehyde is both a multi-tonnage industrial product and an essential metabolite of living systems [2]. It is classified as a mutagen and a possible carcinogen [3]. Recent research demonstrated the presence of formaldehyde in fruit, vegetables, meat and biological liquids of humans [2].

Due to the large usage of formaldehyde and its possible exposure-related health effects, much concern has arisen over the sensitivity and accuracy of analytical methodology for this compound. Several analytical methods have been reported for formaldehyde measurement such as Fourier transform infrared spectroscopy (FTIR) [6], differential optical absorption spectroscopy (DOAS) [7], laser induced fluorescence spectroscopy (LIFS) [8], tunable diode laser absorption spectroscopy (TDLAS) [9], spectrofluorimetry [10], gel filtration chromatography [11] and amperometry [12].

Small amounts of formaldehyde are commonly analyzed by spectrophotometric methods [13,14] and, one of these, the chromotropic acid method [15,16] was established as an international reference method. Despite the advent of more

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sophisticated techniques, it is still widely used because it is simple, sensitive, inexpensive and very selective. Investigations for replacing the concentrated sulfuric acid with other acids or using more dilute solutions of sulfuric acid have been performed [17]. Replacing concentrated sulfuric acid by hydrochloric acid or phosphoric acid resulted in the decrease in sensitivity of the chromotropic acid method [17]. However, using concentrated hydrochloric acid in conjunction with hydrogen peroxide compensated some of the loss in sensitivity. Loss in sensitivity was also observed using dilute solutions of the sulfuric acid [18].

In the present work, the reaction between formaldehyde and chromotropic acid was explored by central composite design. In this approach, the effect of factors influencing the formation of the product, *i.e.* concentration of chromotropic acid and concentration of sulfuric acid, can be investigated simultaneously.

EXPERIMENTAL

Instrumentation

Recording of the absorption spectra in the spectral range of 200-600 nm was performed by an Agilent 8453 UV-Vis spectrophotometer with diode array detector, equipped with 1 cm path length quartz cells.

Reagents and Solutions

Formaldehyde (35%, w/w), sulfuric acid (98%, w/w) and chromotropic acid disodium salt (dehydrate) were all purchased from Merck (Darmstadt, Germany). All the solutions were prepared from the analytical reagent grade using doubly distilled water. A formaldehyde stock solution was prepared by diluting appropriate volume of the reagent solution labeled as 35% (w/w) by doubly distilled water. However, the exact concentration of formaldehyde in the reagent solution was determined by iodometric titration, which the corresponding results showed 33.5% (w/w) formaldehyde in solution. Stock solution of chromotropic acid was 5% (w/v) in doubly distilled water.

Calibration Curve

The calibration curve was prepared as follows. Firstly, appropriate volumes of formaldehyde standard solution was transferred into 10 ml volumetric flasks, followed by addition of 0.14 ml of 5% CA solution and 5.2 ml concentrated sulfuric acid (98%). After completing the mixture to the mark by doubly distilled water and shaking well, the absorbance values were recorded at 574 nm against the reagent blank. Calibration graph was prepared by plotting absorbance against formaldehyde concentration. For any analyzed sample, the linear least square equation of the calibration graph was used to convert absorbance into formaldehyde concentration.

Procedure for the Real Samples

For determination of formaldehyde in toothpaste and clothing softener, 1.0 g of each sample was dissolved in doubly distilled water and filtered through a Whatman filter paper. The filtrate was placed in a 100 ml volumetric flask and completed to the mark with doubly distilled water. For determination of formaldehyde in acetic acid, no preparation was carried out and the sample was used directly. Into six 10 ml volumetric flasks, 0.14 ml chromotropic acid solution (5%, w/v) and 5.20 ml concentrated sulfuric acid (98%) were added and the volumes were completed to the mark with commercial acetic acid as sample. To prepare the spiked samples, the same number of flasks was chosen, and in addition to above quantities, 1.00 mg l^{-1} of formaldehyde was added. After recording the spectra of the prepared samples against reagent blank, the absorbance values at 574 nm were used to calculate the concentrations based on the calibration results.

RESULTS AND DISCUSSION

Standardization of the Formaldehyde Reagent Solution

A volume equivalent to 3.0 ml of freshly prepared formaldehyde reagent solution with nominal concentration of 35% was transferred into a conical flask. After adding 5.0 ml of a standard iodate solution (0.005 M), 2.5 ml of sodium hydroxide solution and 0.664 g potassium iodide (KI), the solution was allowed to stand for 5 min. Then, the solution was acidified with 0.55 ml of sulfuric acid and titrated for excess of iodine by standard sodium thiosulfate solution. When the color of the solution became pale straw, 1 ml of starch solution was added. After addition of starch

solution, the color was immediately changed to deep blue-black. The titration was continued until the color changed from deep blue-black to colorless. Similarly, the blank titration was performed. The difference between titration values of blank and sample was used for calculation of formaldehyde contents in the stock solution. Calculations based on the results of titration revealed that the purity of the reagent formaldehyde solution is 33.5%.

Reaction and Absorption Spectrum

Determination of the trace amounts of formaldehyde would be based on the reaction between formaldehyde and chromotropic acid. The reaction requires a strong acidic medium such as concentrated sulfuric acid (98%). The reaction produces a purple compound. This reaction is consonant with Beer-Lambert's law and the product can be measured by the spectrophotometer. The reaction mechanism can be seen elsewhere [19-21].

The absorption spectra of the reagent blank solution containing chromotropic acid and concentrated sulfuric acid and the reaction product with formaldehyde is shown in Fig. 1. As seen in Fig. 1, the reagent blank has two main absorption bands at the region 350-700 nm. One of them is located at about 360 nm and another at 480 nm. The main absorption peak of the product is at 574 nm. This band is free from the interference of the reagent blank.

Experimental Design and Optimization of the Factors

Using design of experiment (DoE), the maximum amount of information of the system is extracted in an economical way [22]; such as information about the interaction between the factors. For this purpose, all factors were changed from one experiment to the next, simultaneously. The reason for performing this type of experiment is that variables can influence each other and the optimal value for one of them may be dependent on the values of the others [23]. Central composite design (CCD) is an efficient technique for optimization of the group response surface methodology. In CCD, the central point for each factor is 0 in coded unites and the design is symmetrical around it. A total of 13 experiments with two

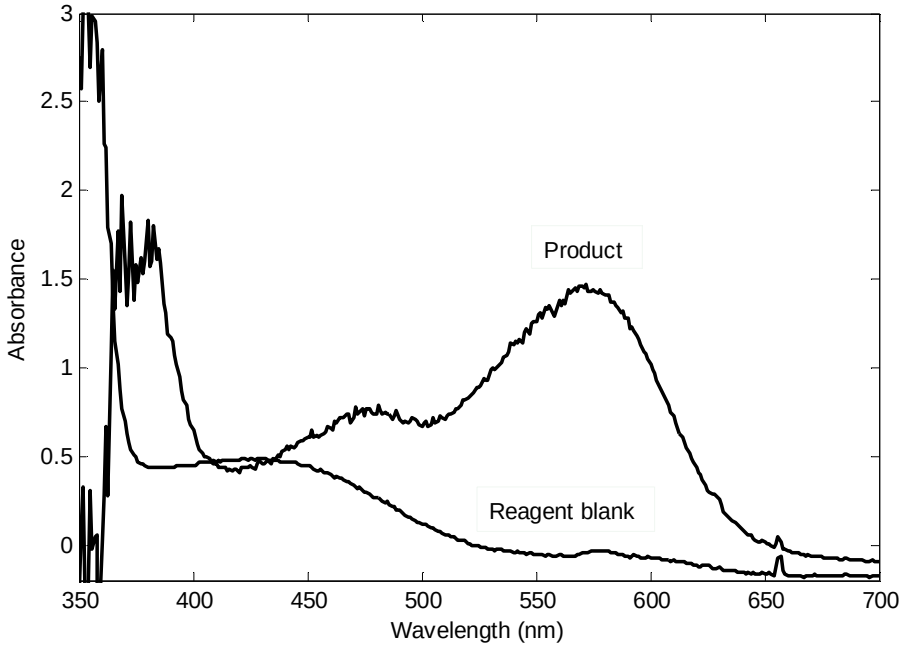

Fig. 1. Absorption spectra of the reagent solution containing chromotropic acid (0.07%, w/v) and concentrated sulfuric acid (51%, w/w) and the product of the reaction between formaldehyde (3.00 mg l^{-1}) and chromotropic acid.

Table 1. Experiments Designed Based on the Central Composite Design with Two Factors

Experiment No.	Volume of chromotropic acid in ml (x_1)	Volume of concentrated sulfuric acid in ml (x_2)	Absorbance
1	0.35	3.5	0.381
2	0.35	3.5	0.250
3	0.56	3.5	0.335
4	0.20	2.0	0.380
5	0.20	5.0	0.508
6	0.35	3.5	0.138
7	0.14	3.5	0.416
8	0.35	3.5	0.378
9	0.50	5.0	0.918
10	0.50	2.0	0.261
11	0.35	5.6	0.125
12	0.35	1.4	0.027
13	0.35	3.5	0.286

Table 2. ANOVA Results of the Experiments in Table 1

Term	Coefficient	t[a]	p[b]
Constant	0.325	8.830	0.000
x_1	-0.046	-1.370	0.220
x_2	0.090	2.670	0.040
x_1x_1	0.049	1.510	0.180
x_2x_2	-0.041	-1.260	0.250
x_1x_2	-0.004	-0.080	0.940
Regression			
R	0.856		0.090
F	3.290		

[a]t statistics. [b]Probability value. [c]F statistics.

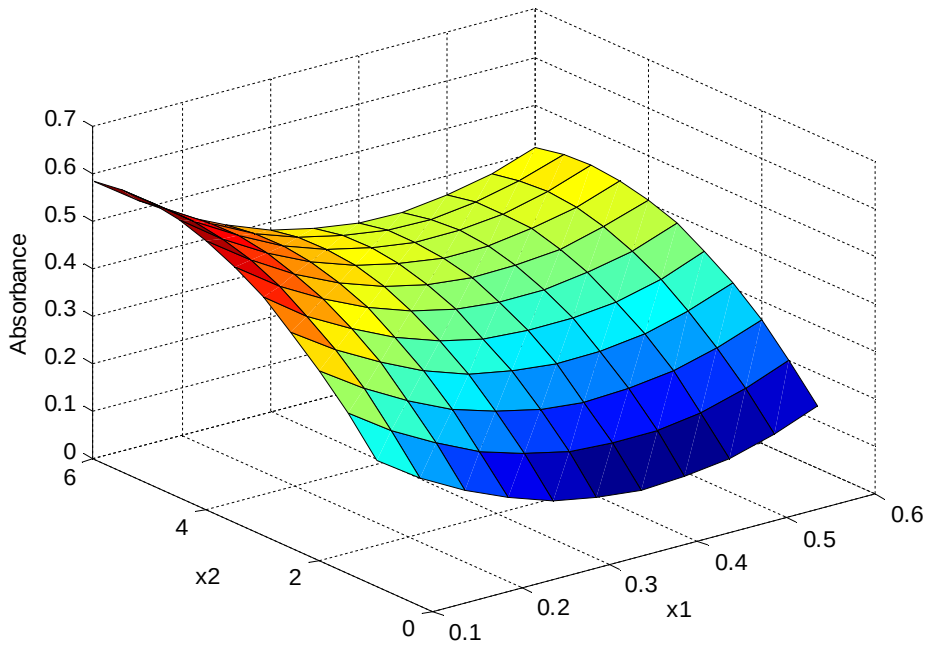

Fig. 2. Variation of the response (absorbance) with the volume of chromotropic acid (x_1) and volume of the concentrated sulfuric acid (x_2).

Table 3. Analytical Data of the Constructed Calibration Curve

Parameters	Results
Linear range (mg l^{-1})	0.0300-7.0000
Limit of detection (LOD) (mg l^{-1})	0.0050
Limit of quantification (LOQ) (mg l^{-1})	0.0162
Slope	0.3990
Intercept	0.0020
Correlation coefficient (r)	0.9810
λ_{max} (nm)	574

factors, *i.e.* volume of chromotropic acid solution (5%, w/v) and volume of the concentrated sulfuric acid, were designed (Table 1). Among 13 experiments, five replicates of the central experiment (0.35 ml of chromotropic acid and 3.5 ml concentrated sulfuric acid) were considered. The response of the experiment was the absorbance of the reaction product of formaldehyde with chromotropic acid (5.0 mg l^{-1}) in each condition.

To set up a model for the variation of response with two factors, analysis of variance (ANOVA) was performed. The model contains linear terms (b_1x_1 and b_2x_2), square terms ($b_{11}x_1x_1$ and $b_{22}x_2x_2$), an interaction term ($b_{12}x_1x_2$) and a constant (b_0). The results of ANOVA including the coefficients of the model (b_0, b_1, b_2, b_{11}, b_{22} and b_{12}) and

Table 4. Results of the Application of the Method for Determination of Formaldehyde in Different Real Samples

Real sample	Spiked ($mg\ l^{-1}$)	Predicted ($mg\ l^{-1}$)	Formaldehyde content	RE (%)	RSD (%)
Acetic acid[a]	0.00	1.17	2.57 $mg\ l^{-1}$		
	1.00	2.22		5.0	5.37
Toothpaste[b]	0.00	1.88	403.2 $mg\ kg^{-1}$		
	0.46	2.33		2.2	2.46
Clothing softener[b]	0.00	0.85	183.2 $mg\ l^{-1}$		
	0.46	1.30		2.2	6.34

[a]Mean of six determinations. [b]Mean of five determinations.

Table 5. Effect of Potential Interferents in the Determination of Formaldehyde

Interferent	Tolerance limit ($mg\ l^{-1}$)
Fe^{3+}	1
Fe^{2+}	50
Mg^{2+}	100
Ca^{2+}	50
NO_3^-	20
NO_2^-	5
CH_3COO^-	5
SO_4^{2-}	100
H_2O_2	1
CH_2O_2	50
C_2H_5OH	200
CH_3COOH	2000<
CH_3CHO	50

Table 6. Comparing the Results of the Proposed Method with other Methods Reported for Determination of Formaldehyde

Remarks	Reagent	Limit of detection ($mg\ l^{-1}$)	Dynamic linear range ($mg\ l^{-1}$)	Ref.
Flow-injection solid phase spectrophotometry	Fluoral P	0.03000	0.050-1.500	[24]
Spectrophotometric	Bromate-eosin Y	0.00988	0.030-0.600	[25]
Cloud point extraction chromatography	2,4-Dinitrophenylhydrazine	0.00070	0.172-0.385	[26]
Resonance fluorescence	Pyronine Y and potassium bromate	0.00380	0.0127-2.280	[27]
Flow-injection spectrophotometric	Pyrogallol red and bromate	0.36000	0.470-40.00	[28]
Fluorescence	Pyronine Y and sodium periodate	0.00002	0.000-0.300	[29]
Flow injection analysis with spectrophotometric	Brilliant green-sulphite	0.02000	0.200-3.000	[30]
Electrochemical	β-Nicotinamide adenine dinucleotide	0.01600	0.100-1.000	[31]
Flow injection spectrophotometry	Phloroglucinol	0.02300	0.025-0.300	[32]
Spectrophotometry	Chromotropic acid	-	0.200-4.000	[33]
Spectrophotometry	Chromotropic acid	-	0.050-2.000	[34]
Spectrophotometry	Chromotropic acid	0.00500	0.030-7.000	This work

statistics values corresponding to the significance of the terms have been collected in Table 2.

Based on the ANOVA table (Table 2), the amount of the chromotropic acid (x_2) is significant at 95% confidence level since corresponding calculated p value is lower than 0.05. Moreover, the negative sign of the coefficient of this factor shows that a lower amount of chromotropic acid is suitable for maximizing the response. Based on the ANOVA table (Table 2), there is no interaction between the concentration of sulfuric acid and chromotropic acid (p value for the term x_1x_2 is higher than 0.05). The statistics of the model (regression) are satisfactory implying that it can be used to prediction purposes.

The results of ANOVA can be used to construct response surface. The response surface shows the variation of the response with the studied factors. The response surface of the current study is shown in Fig. 2. As seen in Fig. 2, higher amounts of the concentrated sulfuric acid increase the response. However, in volumes about 5 ml of the concentrated sulfuric acid, the response tends to level off (see Fig. 2). This indicates that very high concentration of sulfuric is not necessarily needed. In the moderate levels

of chromotropic acid, the observed response is low. However, in the lower and higher amounts of chromotropic acid, response increases. This increase is more pronounced when amount of chromotropic acid is lower. By using response optimizer, the optimal values of the volume of chromotropic acid and concentrated sulfuric acid were obtained as 0.14 and 5.2 ml, respectively.

Analytical Characteristics

The calibration graph was obtained by plotting absorbance versus formaldehyde concentration using the developed method under the optimal conditions. The calibration graph was linear in the concentrations range of 0.03-7.00 mg l^{-1} for formaldehyde. The statistics of the calibration are reported in Table 3. The limit of detection (LOD) and quantitation (LOQ) were calculated according to the ICH guidelines (LOD = 3 × s_b/m and LOQ = 10×s_b/m, where s_b is the standard deviation of the blank signal and m is the slope of the calibration plot). The linearity of the calibration curve was validated by the high value of correlation coefficient of the regression equation. The high values of molar absorptivity and low values of LOD indicate that the proposed method is very sensitive.

Application to Real Samples

In order to validate the method, it was used to determine formaldehyde in acetic acid, toothpaste and a softener. The samples were also spiked with appropriate amounts of formaldehyde. The results are collected in Table 4. As can be seen, the accuracy of the method is good. In acetic acid, 2.567 mg l^{-1} of formaldehyde was found which indicates that the commercial acetic acid used in the laboratory has some impurity of formaldehyde. In toothpaste, formaldehyde is used for sterilization and acts as softener in softener solutions.

Interference Study

The effect of potential interferents in determination of formaldehyde by the developed method was studied. The results were given in Table 5. As can be seen, in most cases, interference occurs in high concentration of the examined interferent. The most serious interferents are Fe^{3+} and H_2O_2. The reactive NO_2^- ion can interfere at relatively low concentrations. However, in the examined real samples, nitrite is not present.

Comparison of the Proposed Method with the Reported Ones

Some of the published methods for the determination of formaldehyde are collected in Table 6. The methods are compared based on the linear range and detection limit. The detection limit of the proposed method is one of the lowest values in Table 6. Compared with the spectrophotometric methods reported in Table 6, the linear range of the proposed method is the widest. In the last entries of Table 6, the results of spectrophotometric methods for determination of formaldehyde based on the chromotropic acid are also included.

CONCLUSIONS

Here, a simple and sensitive spectrophotometric method for the fast determination of formaldehyde in different samples was optimized by response surface methodology. Results of experimental design showed that the concentration of sulfuric acid is statistically an important factor in determination of formaldehyde by chromoptropic acid. Applying the method to the real samples resulted in a satisfactory precision and accuracy. In optimal conditions, the developed procedure had a very low detection limit. The low detection limit proved that the developed procedure was sensitive. The proposed method had a wide dynamic linear range, and required no complicated instruments which are usually needed for health products control.

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