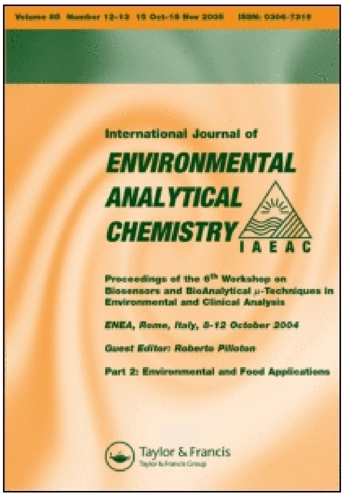

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MONITORING OF ORTHO-PHOSPHATES IN WASTEWATERS BASED ON THE OXIDIZING EFFECT OF MOLYBDENUM ON DIPHENYLAMINE IN A FORMIC ACID SOLUTION ENVIRONMENT

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A new approach towards the spectrophotometric determination of ortho-phosphates is described. The method is based on the reaction of phosphate with the by-products of the oxidation reaction between diphenylamine and molybdenum (VI) in a formic acid solution environment. The final product absorbs at 340 nm allowing for the determination of ortho-phosphates at concentrations as low as $0.44\,\mathrm{mg\,L^{-1}}$ by using a very small sample volume. The calibration curve is linear in the range of 0.5–$3\,\mathrm{mg\,L^{-1}}$ yielding recoveries higher than 96% in various sample matrices. Under the optimum experimental conditions side reactions with other ions that usually have high affinity for molybdenum ions are absent or minimum allowing for the interference free determination of PO_4^{3-}. The only interference rises from the partial decomposition of organic or acid-hydrolysable phosphorus, which is codetermined with ortho-phosphates as a result of the extreme acidic conditions. However, their effects are minor compared to the interferences usually observed with the traditional methods.

Keywords: Ortho-phosphates; Molybdenum; Diphenylamine; Wastewaters; Spectrophotometry

INTRODUCTION

Enrichment of aquatic areas with nutrients often results in the growth of micro or macro algae and algal blooms, which lead to undesirable ecological impacts. These impacts are mostly related to the phenomenon of eutrophication of water bodies. To prevent enrichment of aquatic areas and study their ecological state three key nutrients are monitored on a routine basis, nitrate, ortho-phosphate and ammonium. Among these, phosphorus has received particular attention as large quantities have been traditionally used in commercial and industrial applications [1].

The determination of phosphorous in waters and wastewaters has been described by a variety of methods [2–5]. Some of them are based on the formation of heteropoly complexes with molybdate ($H_2PMo_{12}O_{40}^-$) in an acidic medium [5,6]. In these

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complexes, the central atom is phosphorous and the coordinated groups the molybdate anions. Measurements are then performed spectrophotometrically either directly to the original yellow heteropoly acid complex or after reduction to blue compounds with various reducing agents [7].

However, several disadvantages have been recognized by the use of these traditional methods. Yellow heteropoly acid complexes between molybdate and other species like silicon and arsenate may be formed under the same experimental conditions thus interfering with the determination of phosphorous (as ortho-phosphates). Furthermore, the presence of high amounts of ferric ions or yellowish organic substances which are often present in natural waters and wastewaters may cause positive deviation in the absorbance of the molybdenum-phosphates complex. Reduction of these complexes to less reactive or inactive forms alleviates these interferences [3]. Nevertheless, in these cases, the excess of molybdate strongly absorbs in the same region (ultraviolet) while at least two maxima are observed indicating the presence of two or more chromophore centers as a result of the two valence forms of molybdenum (V and VI) [3]. At the same time, blue heteropoly complexes are sparingly soluble in water and are present in the colloid state [3], which increases the effect of electrolytes and temperature on the color of solution and requires very accurate adherence to the determination procedure. Solvent extraction of the heteropoly complex as an ion-pair with a cationic dye alleviate many of these interferences [8–10] but the use of solvents is generally decreasing in analytical chemistry applications over the past years as a function of the development of other safer and more accurate methods of extraction [1].

In this context, a new method for the determination of ortho-phosphates in wastewater samples is presented. The method takes advantage of the oxidizing effect of molybdenum on diphenylamine in a formic acid environment. The oxidation products of this reaction react with ortho-phosphates to yield a complex absorbing at 340 nm. Under the optimum experimental conditions ions which are present in wastewater and are known to interfere with the traditional molybdenum blue method like arsenate, silicate or vanadate did not posse any adverse effects allowing thus for the interference free determination of ortho-phosphates in various wastewater samples.

EXPERIMENTAL

Reagents. Diphenylamine (99%) was obtained from Fluka Chemika and working solutions of 10 g L^{-1} were prepared in methanol and stored at 4°C. Ammonium heptamolybdate tetra hydrate was purchased from Mallinckrodt (St. Louis, Mo) and working solutions of 100 g L^{-1} were prepared in doubly distilled water and filtered to remove any undissolved material. Potassium dihydrogen ortho-phosphate (BDH Chemicals Ltd, UK) was used for preparing standard PO_4^{-3} solutions of 100 mg L^{-1}. Formic acid (98–100%) was obtained from Riedel de Haen. HACH spectrophotometry reagents (Phosver 3, Cat. No. 2125-99) were used as purchased.

Apparatus. A Shimadzu UV-2100 spectrophotometer with matched quartz cells of 1 cm path length was used for recording normal spectra measurements. A HACH DR/2000 spectrophotometer was used for the application of the reference molybdenum blue method according to the method described in the HACH water analysis handbook

(Method 490) [11] based on the method proposed by Standard Methods for Examination of Water and Wastewater [12].

Samples and Sampling Procedure

Samples of wastewater were obtained from the municipal wastewater treatment plant of Ioannina and consisted of samples from the influent wastewater stream (untreated sewage), after primary sedimentation (settled sewage) and from the effluent of the plant (treated sewage). All samples were filtered through a Whatman No 40 filter to remove any suspended material and subjected to the determination of ortho-phosphates according to the proposed and the reference HACH method.

Procedure

In 4.5 mL of concentrated formic acid 100 mg L^{-1} of diphenylamine (DPA) are added (50 μL from a 10 g L^{-1} stock solution) followed by 1 g L^{-1} Molybdenum (VI) (50 μL from a working solution of 100 g L^{-1}). The mixture is shaken and 300 μL of sample are added to it followed by 100 μL of doubly distilled water to reach a final volume of 5 mL. Measurements are performed spectrophotometrically against reagent blank within 5 min after sample introduction.

RESULTS AND DISCUSSION

Before proceeding with the validation and evaluation of the analytical merits of the proposed method a thorough optimization procedure was undertaken in order to obtain the best reaction yield for PO_4^{-3}. It is well known that experimental designs based on the single-factor-at-a-time method are unable to detect the presence of factor interactions. However, the strong acidic conditions employed in this method as well as the interactions between the variables (oxidation of DPA by Mo(VI), complexation with PO_4^{-3}, etc) suggest possible interactions which may complicate the process of achieving reliable and reproducible results, especially during the analysis of real samples. To overcome this problem several methods are called on such as factorial designs, steepest ascent, simplex methods etc. [13,14]. To determine the optimum conditions for a reaction, optimization designs that can assume nonlinear models, have to be used [15]. In this context, an optimization procedure based on a Central Composite Design (CCD) approach was decided. The CCD is based on a full factorial two-level design which is augmented by the center and star points and can achieve a saving in the number of experimental runs required [16] while reveal the presence of factor interactions [15–17].

Initial preliminary experiments using the classical single-factor-at-a-time approach served to detect the variables and their respective working ranges that have influence on the reaction. Four variables were included in the initial experiments: the amount of reagents (DPA and Mo(VI)), the water-to-formic acid and the reaction time. The response of the product of the reaction as a function of the reaction time showed that the reaction allows for reproducible results after 5–10 min following sample introduction, irrespectively of the concentration of reagents or water volume in solution. This behavior will be discussed again further below.

Based on this information a full second-order CCD was proposed in order to study the reaction variables. Since it is advisable to keep the reagent consumption at the bare minimum and reaction time low, the variable values were narrowed down and each variable was assigned the values given in Table I. The chemometric design required 17 experiments (14 experimental points and three center points) which were conducted at random. The values representing the reaction yields of $3\,\mathrm{mg\,L^{-1}}$ PO_4^{3-} were calculated by subtracting the absorbance of the blank from this of the sample.

The results of the optimization procedure are given in Table II. In order to understand the way in which the reaction variables affect the reaction, the variables must be considered along with nonlinear effects and interaction terms. Hence, the results were subjected to multiple linear regression using a full second-order polynomial model. The outcome of the statistical treatment of data is summarized in Table III. At 95% confidence level ($p < 0.05$) certain variables are statistically significant while for others a weaker significance is observed. The main and quadratic term of water volume was not significant while the lack of significance of the cross-product terms of DPA-V_{H2O} and Mo(VI)-V_{H2O} suggests nonsignificant interactions between these variables at the zone studied.

TABLE I Factor levels of the CCD for the study of the reaction yield

Coded levels	*Diphenylamine* ($\mathrm{mg\,L^{-1}}$)	*Molybdenum* (VI) ($\mathrm{g\,L^{-1}}$)	*Water volume*[a] (μL)
$-\alpha$	50	0.5	50
-1	100	1.0	100
0	150	1.5	200
$+1$	200	2.0	300
$+\alpha$	300	2.5	500

[a] Additional water volume added to the final solution (not including sample volume of 300 μL and volume of Mo solution).

TABLE II The three-factor CCD and the sample absorbance

Exp. no.	*Diphenylamine*	*Molybdenum (VI)*	*Water volume*[a]	*Absorbance (against reagent blank)*	*HCOOH/Water ratio*[b]
1	−1	−1	−1	0.198	5.2
2	+1	−1	−1	0.135	7.3
3	−1	+1	−1	0.138	8.1
4	+1	+1	−1	0.130	9.0
5	−1	−1	+1	0.193	5.7
6	+1	−1	+1	0.133	6.1
7	−1	+1	+1	0.143	8.1
8	+1	+1	+1	0.123	5.7
9	0	0	0	0.126	6.7
10	0	0	0	0.127	6.7
11	−2	0	0	0.167	6.7
12	+2	0	0	0.142	7.3
13	0	−2	0	0.169	6.1
14	0	+2	0	0.144	7.3
15	0	0	−2	0.138	6.1
16	0	0	+2	0.133	9.0
17	0	0	0	0.118	4.3

[a] Total volume including the volume of sample, Mo solution and additional water needed to reach a final volume of 5 mL. Whenever total water volume exceeded 500 μL the volume of formic acid was reduced respectively; [b] Not included in the CCD.

TABLE III Summary of multiple regression and ANOVA results for orthophosphates absorbance signal

Term	*Coefficient*	*Sum of squares*	*df*	*p-value*[a]
Intercept	*0.12267*			*0.0000*
DPA	*– 0.01250*		1	*0.0082*
Mo	*– 0.01100*		1	*0.0144*
DPA · Mo	*0.01200*		1	*0.0391*
DPA · DPA	*0.00898*		1	*0.0263*
Mo · Mo	*0.00955*		1	*0.0207*
Regression		0.007557	5	
Linear			2	
Square			3	
Residual		0.00124	11	
Total		0.008797	16	
R	0.904			
R^2	0.818			
Adjusted R^2	0.806			

[a]The significance of data in italics is 95% ($p < 0.05$).

From the available data, a reduced model was set forth which takes into account only the significant contributions. As illustrated in Table III the main effect (coefficient) of DPA and Mo(VI) have a negative sign, which indicates that the absorbance signal is deteriorated as their concentrations increase. This behavior can be attributed to the enhancement of the oxidation activity of Mo(VI) under the strong acidic conditions, which may result in secondary nonpredicted reactions that can prohibit or interfere with the reaction pathway of phosphates. With the volume of water being the only exception, the rest of the second-order parameters, which describe curvature effects, are statistically significant. The interaction parameters responsible for curvature and twisting effects are nonsignificant with the DPA-Mo(VI) factor being the exception.

The absorbance signal as a function of the two studied variables was investigated by keeping the rest of the variables constant at the medium level ('0' coded level) of the experimental domain. The 3-D response surfaces are depicted in Fig. 1. As can be seen, optimum experimental conditions correspond to the following uncoded values: $[\mathrm{DPA}] = 50\,\mathrm{mg\,L^{-1}}$, $[\mathrm{Mo(VI)}] = 0.8\,\mathrm{g\,L^{-1}}$ and $V_{\mathrm{H2O}} = 300\,\mu\mathrm{L}$. The highest signal is obtained when [DPA] and [Mo(VI)] are maintained at low levels (-1 uncoded value and below) and water volume at medium to high levels (1 uncoded values and above). Additionally, no signal deterioration is obtained when V_{H2O} is further increased up to the maximum value of the CCD (+2 uncoded value) (Fig. 1(b)), which practically means that higher sample volume may be used if necessary.

Based on the reaction mechanism of DPA oxidation under strong acidic conditions and the results of the optimization procedure described above, a potential reaction pattern was proposed. As can be seen in Fig. 2, the oxidation of DPA first proceeds by an irreversible process to the colorless diarylbenzidine, which then is reversibly oxidized in two successive single-electron steps to the blue–violet product diquinonediimine [18]. This blue–violet oxidized form is generally unstable and slowly deteriorated by an irreversible process [18]. It is suspected that Mo(VI) enhance the oxidation of DPA towards diquinonediimine which probably reacts with $\mathrm{PO_4^{-3}}$ or with the heteropolyacid

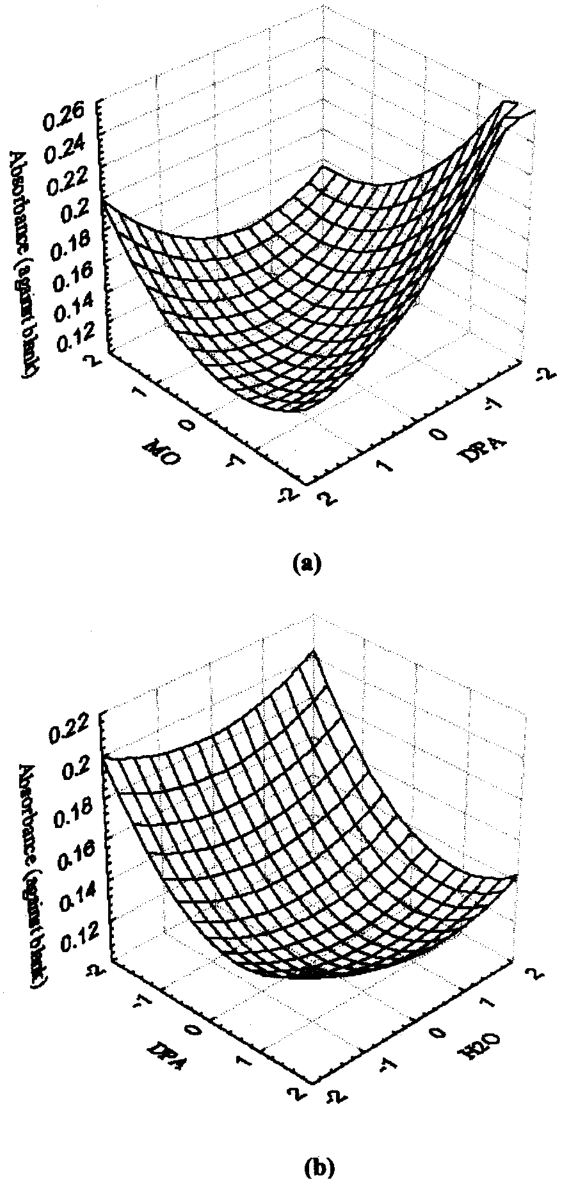

FIGURE 1 Response surfaces of the multivariate optimization procedure for the determination of orthophosphates: spectrophotometric signal (against blank) as a function of: (a) diphenylamine and molybdenum (VI) concentration; (b) diphenylamine and water volume.

($H_2PMo_{12}O_{40}^{-}$), which is very reactive, with the available *N* groups. This hypothesis was further supported by two observations. In the first place, the color of the mixture (formic acid, DPA and Mo(VI)) turns to blue–violet, which denotes the formation of diquinonediimine, almost immediately after the introduction of Mo(VI). Furthermore, the product of the reaction between DPA–Mo(VI) and phosphates declines progressively as the time of reaction increases above 5–10 min. This was evidenced

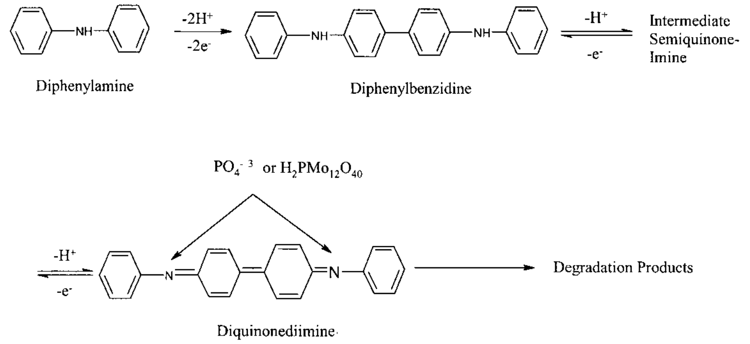

FIGURE 2 Proposed reaction pattern of ortho-phosphates complexation in the molybdenum(VI)–diphenylamine–formic acid mixture.

with the aid of properly designed experiments. Different solutions consisting of various amounts of DPA (10, 30 and 70 mg L^{-1}) and Mo(VI) (0.1, 0.3 and 0.7 g L^{-1}) were prepared and a reaction time profile was generated for these three solutions ranging from 0 to 30 min. It was observed that irrespectively of the amount of reagents in solution the signal was deteriorating after 5 min and almost significantly diminished after 10 min following sample introduction. This fact was attributed to the instability of the diquinonediimine and to the respective phosphates complex. Moreover, if the addition of PO_4^{-3} was made 20 min after the initiation of the oxidation reaction no absorbance signal was observed, revealing thus that the interaction with PO_4^{-3} or with the $H_2PMo_{12}O_{40}^-$ takes place with intermediate products and not with the final degradation products.

Figures of Merit

Under the optimized experimental conditions linear calibration curves in the range of 0.5–3 mg L^{-1} were constructed according to the linear expression: $A_{PO4} = 0.0505$ (± 0.00113) $C_{PO4} - 0.0084$ (± 0.0022), $R^2 = 0.9980$. The detection limit was as low as 0.15 mg L^{-1} with a quantification limit of 0.44 mg L^{-1}. The sensitivity of the method was 0.15 mg L^{-1} with relative standard deviation of 4.2% ($C_{PO4} = 1$ mg L^{-1}, $n = 3$).

Effect of Foreign Ions

The effect of a wide variety of anions and cations usually present in wastewaters was investigated by spiking appropriate amounts of the relative ions to a reference phosphates solution of 3 mg L^{-1}. The anions investigated (NO_3^-, NO_2^-, Cl^-, ClO^-, F^-, HCO_3^-, Br^-, I^-, SO_4^{-2}) did not posse any adverse effects on the analytical signal of the target species at the concentration levels studied (25 times higher than PO_4^{-3}). Of the various cations examined (Ca^{+2}, Mg^{+2}, Cd^{+2}, Pb^{+2}, Cr^{+3}, Fe^{+2}, Fe^{+3}, Ni^{+2}, Co^{+2}) no adverse effects were observed at concentrations level investigated (up to 100 mg L^{-1}). Of special concern, was the reactivity of metallic anions such as CrO_4^{-2} and VO_3^- but mostly SiO^{-3}, AsO_4^{-3}, with molybdate at acidic conditions to form heteropoly acid complexes. Under the specified experimental conditions though no adverse effects were observed even at equivalent concentrations of ortho-phosphates with these metallic species. Finally, the effect of humic and fulvic acids on the absorbance signal was found to be practically trivial.

TABLE IV(a) Determination of ortho-phosphates in wastewater with the proposed and a reference method. All concentrations are in mg L^{-1}

Sample point[a]	*Number of sample*	*Proposed method*	*Reference method*	*Relative error* (%)
Untreated sewage	1	16.2	15.5	+4.5
	2	30.0	28.8	+4.2
	3	48.8	47.5	+2.7
Settled sewage	1	8.8	8.6	+2.3
	1	15.5	15.0	+3.3
	3	28.7	28.4	+1.0
Treated Sewage	1	10.0	10.2	−1.9
	2	14.6	14.7	−0.7
	3	20.2	20.6	−1.9

[a] Spot samples. Do not reflect the real performance of the facilities.

TABLE IV(b) Recoveries from fortified samples (estimated according to the IUPAC recommendations). All concentrations are in mg L^{-1}

Sample	*Measured*	*Spiked*	*Found*	*Recovery* (%)
Untreated sewage (Sample no. 2)	30.0	5.0	34.7	96
Settled sewage (Sample no. 2)	15.5	5.0	20.5	100
Treated sewage (Sample no. 1)	10.0	5.0	15.1	102

Determination of Phosphates in Real Samples

The determination of ortho-phosphates was performed with the proposed method and with a reference technique to verify the accuracy of the proposed approach. The results from the analysis of different wastewater samples with both methods are gathered in Table IV. As can be seen, the method provided a satisfactory description of PO_4^{-3}concentration. The comparison of the two methods also shows that in the treated wastewater samples the two methods gave similar results. In the untreated wastewater samples though, the results with the proposed method were quite higher. This fact can be attributed to the partial decomposition of organic or acid-hydrolysable phosphorus, which is codetermined with ortho-phosphates as a result of the extreme acidic conditions. However, this interference was not found to produce any significant deviations in most of the cases.

In order to further investigate the efficiency of the proposed method several samples were spiked with appropriate amounts of ortho-phosphates and analyzed according to the described procedure. The results presented in Table IV(b) show that satisfactory recoveries were obtained even from the complex wastewater matrix.

CONCLUSIONS

A new spectrophotometric method for the determination of ortho-phosphates in wastewater has been described. The method is based on the oxidizing effect of molybdenum (VI) on diphenylamine in a formic acid solution environment. Under the optimum experimental conditions the determination of PO_4^{-3} is feasible at concentrations as low as 0.44 mg L^{-1} without interferences thus alleviating the need for laborious and time consuming steps of interference elimination through other reactions or by extraction procedures. The method was successfully applied for the determination of PO_4^{-3} in real samples yielding recoveries in the range of 96–102%. The only interferences possibly arises from the presence of high amounts of organically complexed or acid-hydrolysable phosphorous but their effect is too small to posse any significant adverse effects on the determination of ortho-phosphates with the proposed procedure, even in too complex matrices (e.g. wastewater).

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