


Usability of simplified UV–Vis spectrophotometric methods for the determination of nitrate in the presence of organic matter and chloride as interfering factors

Marcela Fernandes Silva ^{a,*}, Luana Bermejo de Lima^b, Carolina de Camargo^a and Claudia Telles Benatti^a

^a Civil Engineering Department, Universidade Estadual de Maringá, Maringá-PR, Brazil

^b Chemistry Department, Universidade Estadual de Maringá, Maringá-PR, Brazil

*Corresponding author. E-mail: mfsilva2@uem.br

 MFS, 0000-0001-7433-8387

ABSTRACT

The presence of nitrate in groundwater and surface water in concentrations above levels suitable for human consumption is an increasingly common problem. This is mainly due to human activity, such as the excessive use of fertilizers and the inadequate deactivation of pits. Water analysis laboratories very often carry out analyses to determine nitrate to control the presence of this anion in water intended for human consumption. One of the most commonly used methods is spectrophotometry, both with commercially available kits and by direct measurement of specific wavelengths. However, in these methods, the presence of organic matter and chloride can influence the results obtained. Thus, in this study, the applicability of the simplification (without acidification) of two methods for the determination of nitrate in water by ultraviolet–visible absorbance was verified in the presence of the above-mentioned interfering factors, and it was found that the absorbance method at 220 nm corrected for the absorbance at 275 nm, in its simplified form without acidification, can be used with concentrations of organic matter of 20 mg L⁻¹ for high nitrate concentrations (10 mg L⁻¹ NO₃-N).

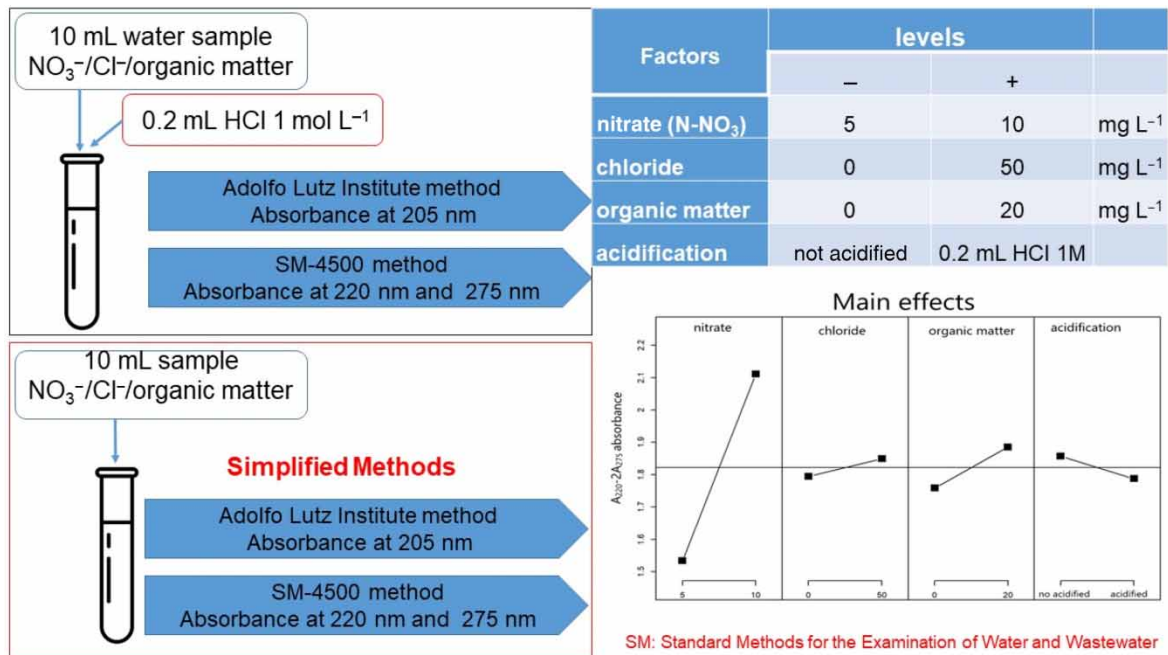
Key words: acidification, chloride, nitrate, organic matter, spectrophotometry

HIGHLIGHTS

- SM-4500 without acidification is suitable for nitrate determination in the presence of chloride and organic matter.
- The presence of organic matter up to 20 mg L⁻¹ did not interfere with the analysis of nitrate by the simplified method.
- The SM-4500 method should not be used at nitrate concentrations up to 10 and 20 mg L⁻¹ of organic matter.
- Avoiding the use of HCl is in line with the fifth principle of green chemistry.

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GRAPHICAL ABSTRACT



INTRODUCTION

Ultraviolet-visible (UV-Vis) spectrophotometry plays a crucial role in the characterization of water due to its effectiveness in the analysis of inorganic and organic compounds. It works by measuring the absorption or transmission of electromagnetic radiation in the UV-Vis regions of the electromagnetic spectrum. This technique is widely used in water analysis and environmental applications and provides a cost-effective and versatile method for assessing water quality. UV-Vis spectrophotometers enable rapid measurements, are simple, inexpensive, and require minimal sample preparation, making them valuable for drinking water quality monitoring and process control (Thomas *et al.* 2017; Shi *et al.* 2022).

A recurring problem in the analysis of individual species using UV spectrometry is interference due to absorption by other components in the analyzed systems, and the resulting increase in background absorption as well as the possible exceeding of measurement limits. Natural water consists of a complex matrix of components, of which mainly the inorganic components absorb in the UV wavelength range from 195 to 280 nm. (Collos *et al.* 1999; Johnson & Coletti 2002; Birkmann *et al.* 2018), while organic compounds absorb in the wavelength range from 220 to 280 nm, with absorption at 254 nm being typical for measurements of aromatic compounds (Korshin *et al.* 2009; Albrektienè *et al.* 2012). This results in a UV multicomponent spectrum, which is determined by superimposing the absorbances of the individual species. The direct measurement of nitrate by absorption of UV light is used in several methods, such as the Standard Methods for the Examination of Water and Wastewater 4500 $\text{NO}_3\text{-B}$ method (APHA/AWWA/WEF 2017) (measurement at 220 nm corrected by measurement at 275 nm), and the method described by the Adolfo Lutz Institute (Instituto Adolfo Lutz 2008) (measurement at 205 nm), both of which recommend prior acidification of the samples with HCl. The first method uses the intense absorption of nitrate in the 220 nm range. However, UV light is also strongly absorbed by dissolved organic substances in the same region of the spectrum (APHA/AWWA/WEF 2017), and certain saline constituents (such as hydroxides and carbonates) can also cause interference (Gross *et al.* 1999). Therefore, this method proposes to perform the measurement at two wavelengths for water with dissolved organic matter. In this method, the absorbance is measured at 220 nm, and a second reading at 275 nm, where the nitrate does not absorb UV light, is used as a correction for the absorbance of the organic matter at the shorter wavelength. In the second method, the absorbance is measured directly at 205 nm. Although Edwards *et al.* (2001) report that this method can be used without interference from the presence of organic matter at concentrations up to 20 mg L^{-1} , Zhang *et al.* (2021) report that a humic acid concentration of 5 mg L^{-1} absorbance at

205 nm is negligible. According to Souto *et al.* (2006), interference from chloride ions was observed at concentrations above 50 mg L⁻¹ when nitrate was determined at 205 nm.

Edwards *et al.* (2001) reported that, based on the observed characteristics of the studies of both methods, it would be logical to choose a wavelength with greater sensitivity (205 nm) for concentrations up to 4 mg L⁻¹ NO₃-N and less sensitive wavelengths (220 nm) for concentrations above that. These results were confirmed by previous studies (Silva *et al.* 2023), where linearity of absorbance at 205 nm was observed at concentrations up to 3 mg L⁻¹ NO₃-N. Different methods have been developed to analyze the quantification of nitrate (exclusively or together with other parameters) by reading the absorbance at different wavelengths (multivariate analytical models) or the second derivative in UV spectrophotometry, but they are suitable for specific samples, such as wastewater or seawater, or for the determination of different compounds together (Karlsson *et al.* 1995; Dahlén *et al.* 2000; Causse *et al.* 2017; Meyer *et al.* 2018).

The variability of the natural water composition in terms of pH and alkalinity, characteristics that indicate the need for acidification of samples according to Examination of Water and Wastewater (2017) and Gross *et al.* (1999), suggest that for low alkalinity water (with low concentrations of hydroxides and carbonates), acidification may not be necessary for spectrophotometric methods, which has been confirmed in previous studies, allowing the analysis to be performed in less time and with less risk, considering that the determination of nitrate concentration is increasing in many water analysis laboratories. Avoiding the use of excipients in the analytical procedures is also in line with the fifth principle of green chemistry, a growing and necessary ideology of developing green methods in analytical chemistry (Anastas & Eghbali 2010).

Therefore, the aim of the present work is to evaluate the applicability of the investigated methods for samples containing organics and chloride ions in the determination of nitrate concentration by UV spectrophotometry, as well as the need for prior acidification of samples for analysis, as recommended in the methods evaluated here, using a 2⁴-fold design.

MATERIALS AND PREPARATION

Materials

- Ultrapure water (AP) (Table 1);
- Standard nitrate NO₃-N solution (1,000 ± 6 mg L⁻¹, QuimLab-SpecSol);
- Standard chloride Cl⁻ solution (1,004 ± 6 mg L⁻¹, QuimLab-SpecSol);
- Standard organic matter solution (COD) (potassium hydrogen phthalate) (1,000 ± 6 mg L⁻¹, QuimLab-SpecSol).

Table 1 | Ultrapure water characteristics

Parameter	Analysis method	Value
pH	SM 4500-H ⁺ B ^a	8.01
Conductivity (μS cm ⁻¹)	SM 2510 B ^a	1.30
Fe ³⁺ (mg L ⁻¹)	Merck Spectroquant 14761 ^b	<0.03
Mn ²⁺ (mg L ⁻¹)	DIN 38406-2 ^c	<0.06
SiO ₂ (mg L ⁻¹)	SM 4500-SiO ₂ D and E ^a	<0.09
Na ⁺ (mg L ⁻¹)	SM 3500-Na ⁺ B ^a	<0.01
K ⁺ (mg L ⁻¹)	SM 3500-K ⁺ B ^a	<0.01
NO ₃ -N (mg L ⁻¹)	DIN 38405-9 ^c	<0.14
Cl ⁻ (mg L ⁻¹)	SM 4500-Cl ⁻ E ^a	<2.42
Cl ₂ -free residual chlorine (mg L ⁻¹)	SM 4500-Cl ₂ G ^a	<0.11
Total hardness (mg CaCO ₃ L ⁻¹)	SM 2340 C ^a	3.55
Organic matter (mg L ⁻¹)	SM 4500-O D ^a	<0.1

^aStandard Methods for the Examination of Water and Wastewater (APHA) (AWWA), 23^a ed., 2017.

^bMerck Spectroquant kit.

^cDeutsches Institut für Normung E.V. (DIN).

Preparation of the test solution

Using a mixture of ultrapure water and standard solutions of $\text{NO}_3\text{-N}$, Cl^- and organics, intermediate solutions were prepared with concentrations based on the limits set by the Brazilian Ministry of Health Decree No. 888 (2021) which amended Annex XX of the Brazilian Ministry of Health Consolidation Decree No. 5 (2017). These concentrations were also based on known levels of nitrate, chloride, and organics found in real groundwater and surface water samples from the Maringá-PR region, as well as on the acidification of the samples. The concentration of $\text{NO}_3\text{-N}$ varied between 5 and 10 mg L^{-1} , and the interference of chloride was evaluated with a concentration of Cl^- of 50 mg L^{-1} and of organic matter at 20 mg L^{-1} (Mantovani *et al.* 2018; Rezende *et al.* 2015, 2019; Brasil. Ministério da Saúde 2021). Acidification was tested with 1 M HCl (Dahlén *et al.* 2000; Oliveira 2007).

METHODS

For both methods evaluated, the absorbance measurements were made in triplicate, and the values reported refer to the mean of these values.

*Method 1: Spectrophotometric $\text{NO}_3\text{-N}$ determination method at 205 nm (A_{205}) from Instituto Adolfo Lutz (Scorsafava *et al.* 2018) (IAL method)*

In a test tube, 10 mL of the sample (for non-acidified assays) or 10 mL of the sample and 0.2 mL HCl 1 mol L^{-1} solution were homogenized (for acidified assays). Measurements were performed at 205 nm in a Merck Prove 600 UV-Vis spectrophotometer, using a quartz bucket with an optical path length of 10 mm.

Method 2: Standard Methods $\text{NO}_3\text{-N}$ spectrophotometric determination at 220 and 275 nm (APHA/AWWA/WEF 2017) (SM-4500 method)

In a test tube, 10 mL of the sample (for non-acidified tests) or 10 mL of the sample and 0.2 mL HCl 1 mol L^{-1} solution were homogenized (for acidified tests). Measurements were performed at 220 and 275 nm (designated A_{220} and A_{275} , respectively) in a Merck Prove 600 UV-Vis spectrophotometer, using a quartz bucket with an optical path length of 10 mm.

The nitrate absorbance (A_{NO_3}) was determined according to Equation (1):

$$A_{\text{NO}_3} = A_{220} - (2A_{275}) \quad (1)$$

If the correction value ($2A_{275}$) is more than 10% of A_{220} , the method should not be used.

Factorial planning

To evaluate the importance of variables in relation to absorption by $\text{NO}_3\text{-N}$, a factorial design 2^4 was performed according to Table 2. The factorial planning was performed without experiments with central points because one of the factors has no quantitative levels, which makes analysis at the central point impossible. The factorial planning matrix with the respective response values for A_{205} (for the IAL method) and A_{NO_3} (for the SM-4500 method) is shown in Table 3. To avoid statistical bias in the results, the tests were performed randomly.

Table 2 | Factors and levels values in factorial planning

Factors	Levels		
	-	+	
Nitrate	5	10	mg L^{-1}
Chloride	0	50	mg L^{-1}
Organic matter	0	20	mg L^{-1}
Acidification	Not acidified	0.2 mL HCl 1 M	

After preparing the solutions, the absorbance values of the samples were determined using the IAL method and the SM-4500 method.

R software was utilized to analyze the factorial design data using the FrF2 package of functions. The effects of the factors and their interactions as well as the obtained results were evaluated using regression models for each response variable, using confidence intervals at a 95% level.

Table 3 | Values of the factorial planning matrix for Methods 1 and 2

Run	Factorial 2 ⁴			
	Nitrate (mg L ⁻¹)	Chloride (mg L ⁻¹)	Organic matter (mg L ⁻¹)	Acidification
1	5	0	0	Not acidified
2	10	0	0	Not acidified
3	5	50	0	Not acidified
4	10	50	0	Not acidified
5	5	0	20	Not acidified
6	10	0	20	Not acidified
7	5	50	20	Not acidified
8	10	50	20	Not acidified
9	5	0	0	0.2 mL HCl 1 M
10	10	0	0	0.2 mL HCl 1 M
11	5	50	0	0.2 mL HCl 1 M
12	10	50	0	0.2 mL HCl 1 M
13	5	0	20	0.2 mL HCl 1 M
14	10	0	20	0.2 mL HCl 1 M
15	5	50	20	0.2 mL HCl 1 M
16	10	50	20	0.2 mL HCl 1 M

The β_0 coefficient obtained for each model is referred to as the axis coefficient, interpreted as the mean of y when all control variables are zero. The T -statistic presented for each of the coefficients (β_1 to β_n) aims to test the statistical significance of the effect of each factor on the response variable. The significance of the T -statistic is indicated by the p -value (MONTGOMERY 2005). For this design, the Breusch–Pagan tests indicate that the residuals of both regressions are homogeneous and satisfy the assumption of non-heteroscedasticity, and the Durbin–Watson tests indicate that the errors generated by the residuals of both regressions are independent and there is no effect of autocorrelation. The coefficient of determination (R^2) is a measure of how well the model explains the variability of the data.

RESULTS AND DISCUSSION

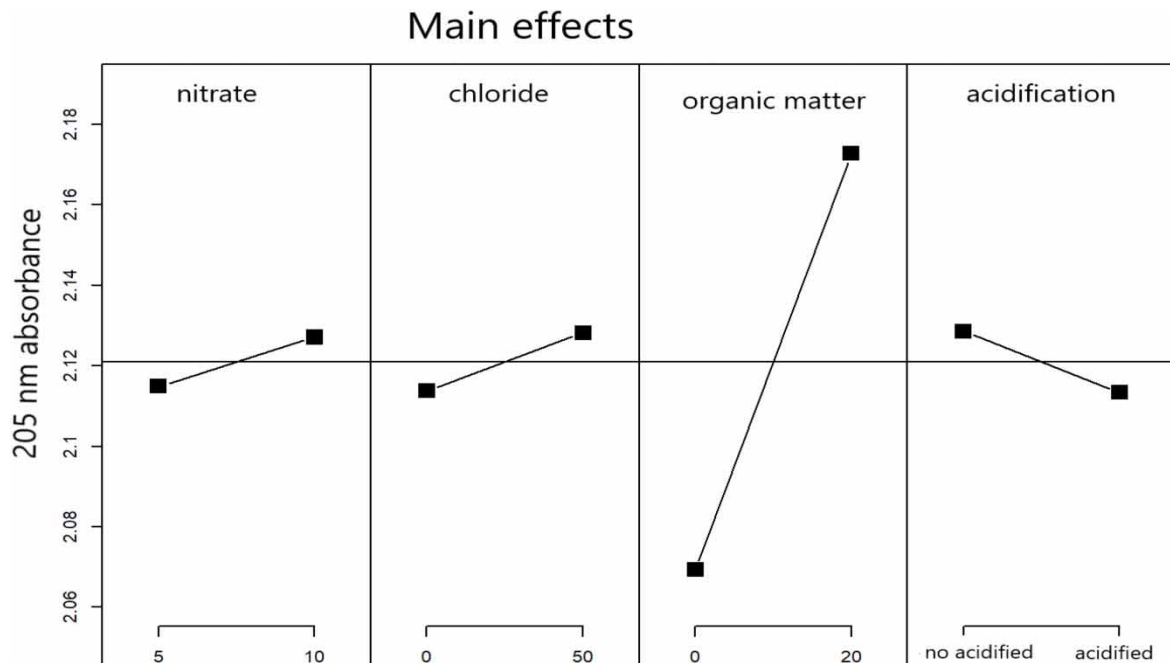
The results for absorbance at 205 nm (A_{205}) (IAL method) and $A_{NO_3} = A_{220} - (2A_{275})$ (SM-4500 method) are presented in Table 4. The IAL method has no specific requirements for applicability, except for the possibility of deviations from Beer's law at concentrations above 6 mg L⁻¹ (Scorsafava *et al.* 2018). In addition, the SM-4500 method is applicable when the correction value ($2A_{275}$) is less than 10% of A_{220} . Otherwise, the method should not be used (APHA/AWWA/WEF 2017). Hence, its applicability was confirmed through the examination of the studied samples. It is worth noting that the method SM-4500 was found to be unsuitable for acidified samples containing 20 mg L⁻¹ of organic matter, regardless of low (5 mg L⁻¹) or high (10 mg L⁻¹) nitrate content (essays 13–16), as well as for non-acidified samples with any tested concentration of organic matter but with the lowest nitrate concentration (5 mg L⁻¹) (essays 5 and 7). Based on these findings, it can be concluded that the SM-4500 method can be used to samples containing up to 20 mg L⁻¹ organic matter when the nitrate concentration is high (10 mg L⁻¹) without the need to acidify the samples with HCl, indicating that no acidification step is required.

Taking into account the concentration range of the IAL method and the corrections needed for the SM-4500 method, both approaches were included in all experiments. This comprehensive approach enabled factorial planning analyses to provide insight into the significant factors influencing absorption. The results of the marginal means for the absorbance at 205 nm (for the IAL method) are shown in Figure 1, while the results for the absorbance at 220 nm corrected for the absorbance at 275 nm (for the SM-4500 method) are shown in Figure 2.

For the absorbance in the IAL method (Figure 1), it can be seen that the organic matter was the most influential factor (highest mean difference between factors). The other factors had little or no significant influence on this

Table 4 | Results obtained (nitrate-related absorbance) for factorial design using Methods 1 and 2

Essay	A_{205}	$A_{NO_3} = A_{220} - (2A_{275})$	SM-4500 method applicability
1	2.054	1.415	Applicable
2	2.088	2.113	Applicable
3	2.080	1.436	Applicable
4	2.088	2.146	Applicable
5	2.145	1.659	Not applicable
6	2.176	2.136	Applicable
7	2.222	1.811	Not applicable
8	2.176	2.140	Applicable
9	2.038	1.337	Applicable
10	2.088	2.111	Applicable
11	2.038	1.404	Applicable
12	2.080	2.108	Applicable
13	2.155	1.533	Not applicable
14	2.166	2.059	Not applicable
15	2.187	1.676	Not applicable
16	2.155	2.073	Not applicable

**Figure 1** | Marginal means diagram for the main effects of absorbance at 205 nm and the IAL method.

method. The importance of organic matter for this method is justified because this method is suitable for water with low organic matter content (Mazon *et al.* 2005), since according to Edwards *et al.* (2001), both organic matter (between 2 and 12 mg L⁻¹ of polymaleic acid) and nitrate (between 0.4 and 4 mg L⁻¹ NO₃-N) strongly absorb wavelengths of this range. The fact that nitrate concentrations of 5 or 10 mg L⁻¹ of NO₃-N have no effect on absorbance indicates that this method is not a valid alternative for the determination of nitrate in the concentration range studied, since absorbance is expected to vary as a function of analyte concentration. This method was previously considered inadequate due to nonlinearity in the range of 1 to 7 mg L⁻¹ NO₃-N (Mazon *et al.* 2005).

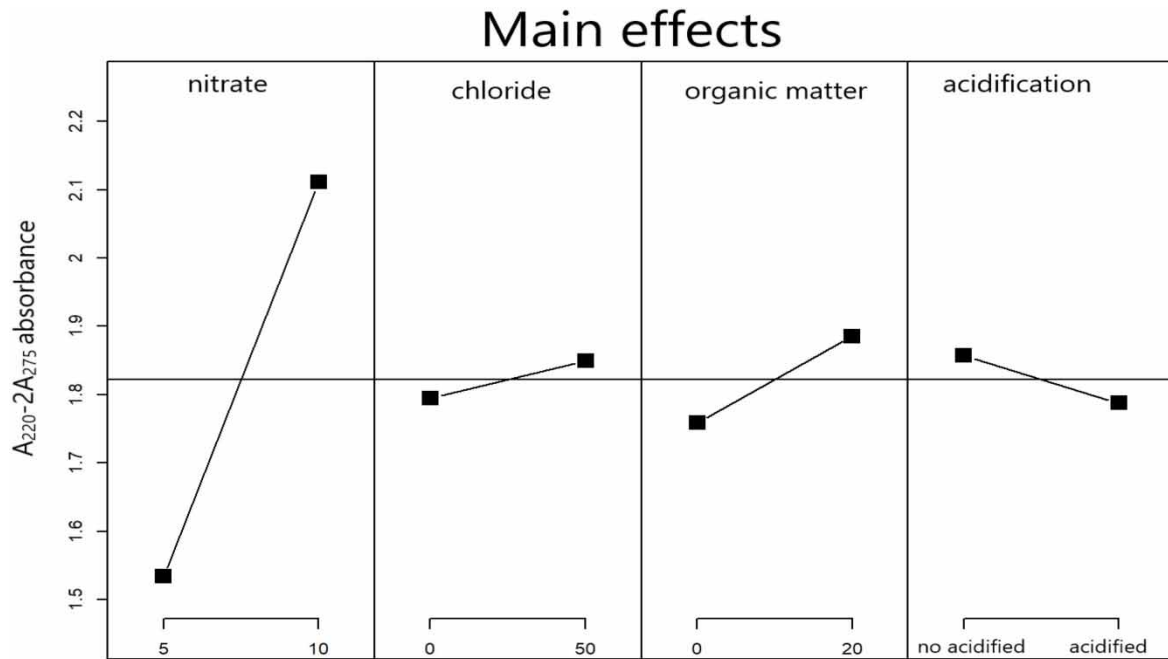


Figure 2 | Diagram of marginal mean values for the main effects of absorbance at 220 nm corrected by absorbance at 275 nm using the SM-4500 method.

In the results of the SM-4500 method (Figure 2), it can be seen that nitrate was the most influential factor, followed by organic matter. The influence of nitrate is to be expected since the 220 nm absorption method is designed to quantify this ion. Chloride and acidification of the medium, on the other hand, apparently had little or no effect in this case. The potential to omit acidification from the procedure is once again emphasized, thereby simplifying the analysis.

To determine whether the influence of the factors observed in the limit plots was statistically significant in relation to the results obtained (A_{205} and A_{NO_3}), factorial regression models capable of describing the relationship between the response and the terms of the model obtained were constructed.

For the IAL method (absorbance at 205 nm), as already expected from the analysis of the marginal mean plots, it was found, in terms of model coefficients, that only the organic matter showed a statistically significant effect, with the coefficient $\beta_{\text{Organic matter}} = 0.05175$, $t(5) = 15.66$, $p < 0.0001$. This means that a change in organic matter concentration from 0 to 20 mg L⁻¹ leads to an increase in method absorbance of 0.05175 on average, holding the effects of the other factors constant. However, as discussed in connection with the marginal mean plots, the non-significance of nitrate in this case (at concentrations of 5 and 10 mg L⁻¹) indicates that this method is unsuitable for quantifying nitrate. Regarding the interactions, the steeper the lines go up or down and across, the more influential is the interaction of the tested factors. Figure 3 shows that the interaction between the factors nitrate and chloride and nitrate and organic matter has statistical significance at the 95% confidence level. Both interactions resulted in a decrease in method absorbance, taking into account the constant effects of the other factors, with an average decrease in absorbance of 0.0096 for an interaction between nitrate concentration and chloride concentration, and an average decrease in absorbance of 0.0106 for an interaction between nitrate concentration and organic matter concentration. From the latter two values, it appears that the interactive effect of the factors with the greatest impact was that between the nitrate concentration and the organic matter concentration. These data suggest that the non-significance of nitrate is due to the lack of linearity of absorbance at 205 nm with respect to nitrate concentration, but possibly such interactions at lower concentrations, where the behavior of absorbance at 205 nm is linear with respect to nitrate concentration, could actually affect the quantification of this ion.

For the SM-4500 method, as also expected from the analysis of the plots, the factorial model showed statistical significance for the data, $F(5, 10) = 189.5$, $p < 0.0001$, $R^2 = 0.9974$, which means that the variation of the factor levels in the model explained about 99.74% of the variation of the absorbance values for the SM-4500 method. The model estimated an overall mean absorbance value of 1.822, which was statistically significant at the 95% confidence level, $\beta_0 = 1.822$, $t(5) = 256.87$, $p < 0.001$. Regarding the coefficients of the model, it is now

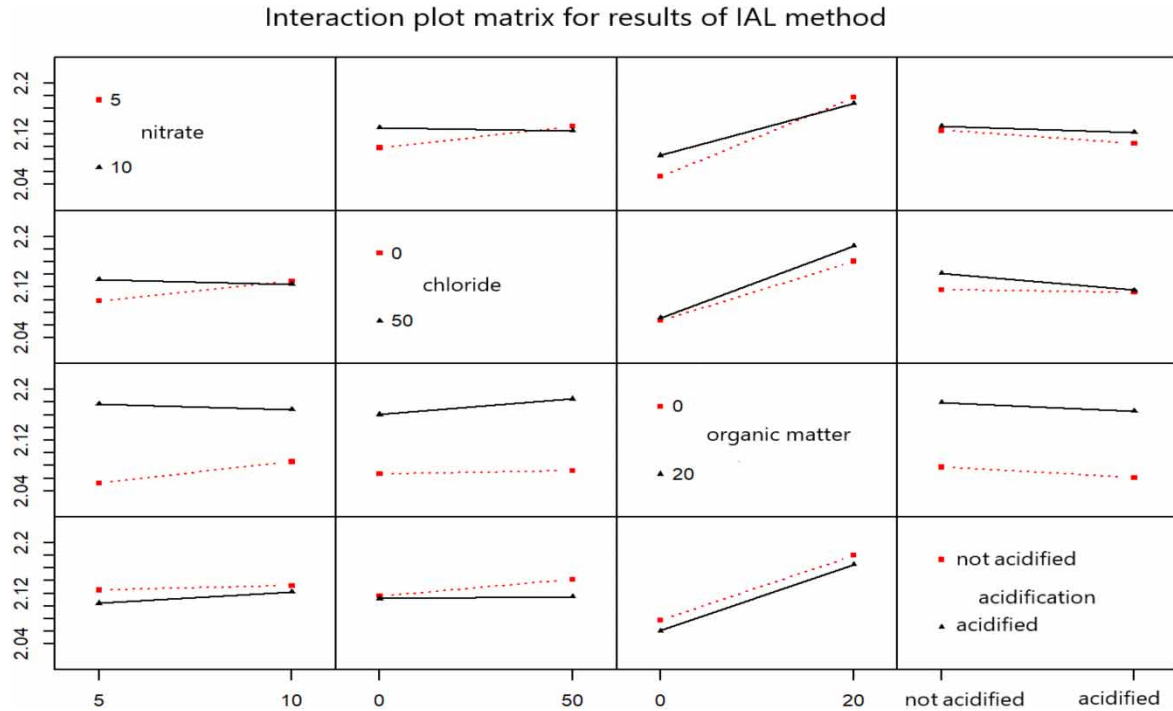


Figure 3 | Interaction diagrams of the factors for the absorption method at 205 nm.

found that all factors have a statistically significant effect: $\beta_{\text{nitrate}} = 0.2884375$, $t(5) = 40.66$, $p < 0.0001$; $\beta_{\text{chloride}} = 0.0269375$, $t(5) = 3.80$, $p = 0.0127$; $\beta_{\text{organic matter}} = 0.0635625$, $t(5) = 8.96$, $p = 0.0003$; and $\beta_{\text{acidification}} = -0.0346875$, $t(5) = -4.89$, $p = 0.0045$. This means that changes in the concentrations of nitrate, chloride, and organic matter lead to an increase in absorbance, while acidification of the medium leads to a decrease, indicating that the acidification step leads to an underestimated quantification of nitrate. For each case, the value of the coefficients indicates how much the absorption increases or decreases on average. As for the interactions (Figure 4), the interactions between nitrate and chloride and between nitrate and organic matter were the only

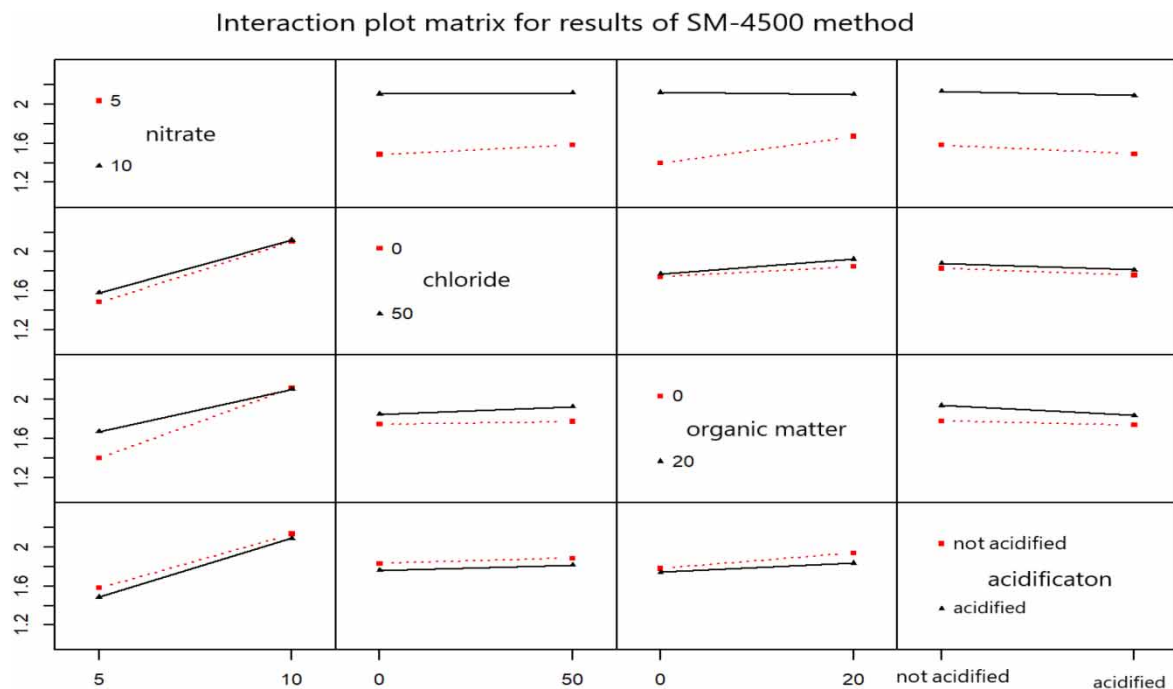


Figure 4 | Interaction diagrams of the factors for the SM-4500 method.

ones that showed statistical significance at the 95% confidence level. Both of these interactions, holding the effects of the others constant, also resulted in a decrease in method absorbance for an interaction between changes in nitrate concentration and chloride concentration, the average decrease in absorbance was 0.0209375, and for an interaction between changes in nitrate concentration and organic matter concentration, the average decrease in absorbance was 0.0723125. From these last two figures, it can be seen that the interactive effect of the factors with the greatest impact was again that between the nitrate concentration and the concentration of organic matter, which indicates the need for correction with the absorbance at 275 nm.

CONCLUSIONS

In the factorial design, it was found that in the IAL method, the nitrate concentration (of 5 and 10 mg L⁻¹ NO₃-N) has no significant effect on the absorption. This indicates that this method does not obey Beer's law in these concentration ranges, so it should not be used for its intended purpose regardless of the presence or absence of confounding factors. In terms of factorial design, the SM-4500 method had as significant factors the nitrate concentration, the chloride concentration, the organic matter concentration, and the acidification of the sample. However, one should consider the applicability of this method (the double absorbance for organic material should not exceed 10% of the absorbance for nitrate), which indicates that this method in its standardized form should not be used in standard methods (with acidification) in the presence of organic material at a concentration of 20 mg L⁻¹ for nitrate concentrations up to 10 mg L⁻¹ NO₃-N. Considering the results obtained in this study and the trend toward high nitrate concentrations increasingly observed in water analysis laboratories, the SM-4500 method in its simplified form (without acidification) can be considered suitable for nitrate determination, even in the presence of chloride (up to 50 mg L⁻¹) and organic matter (up to 20 mg L⁻¹) as interfering factors, with a confidence level of 95%, and environmentally friendly.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

- Albrektienė, R., Rimeika, M., Zalieckienė, E., Šaulys, V. & Zagorskis, A. 2012 **Determination of organic matter by UV absorption in the ground water**. *J. Environ. Eng. Landscape Manage.* **20**, 163–167. <https://doi.org/10.3846/16486897.2012.674039>.
- Anastas, P. & Eghbali, N. 2010 **Green chemistry: Principles and practice**. *Chem. Soc. Rev.* **39**, 301–312. <https://doi.org/10.1039/B918763B>.
- APHA/AWWA/WEF 2017 **Standard Methods for the Examination of Water and Wastewater**. American Public Health Association, American Water Works Association, Water Environment Federation, Denver, CO.
- Birkmann, J., Pasel, C., Luckas, M. & Bathen, D. 2018 **UV spectroscopic properties of principal inorganic ionic species in natural waters**. *Water Pract. Technol.* **13**, 879–892. <https://doi.org/10.2166/wpt.2018.097>.
- Brasil. Ministério da Saúde 2021 Portaria GM/MS N° 888, de 4 de maio de 2021. Altera o Anexo XX da Portaria de Consolidação GM/MS n° 5, de 28 de setembro de 2017, para dispor sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potab.
- Causse, J., Thomas, O., Jung, A.-V. & Thomas, M.-F. 2017 **Direct DOC and nitrate determination in water using dual pathlength and second derivative UV spectrophotometry**. *Water Res.* **108**, 313–319.
- Collos, Y., Mornet, F., Sciandra, A., Waser, N., Larson, A. & Harrison, P. J. 1999 **An optical method for the rapid measurement of micromolar concentrations of nitrate in marine phytoplankton cultures**. *J. Appl. Phycol.* **11**, 179–184. <https://doi.org/10.1023/A:1008046023487>.
- Dahlén, J., Karlsson, S., Bäckström, M., Hagberg, J. & Pettersson, H. 2000 **Determination of nitrate and other water quality parameters in groundwater from UV/Vis spectra employing partial least squares regression**. *Chemosphere* **40**, 71–77. [https://doi.org/10.1016/S0045-6535\(99\)00242-8](https://doi.org/10.1016/S0045-6535(99)00242-8).
- Edwards, A. C., Hooda, P. S. & Cook, Y. 2001 **Determination of nitrate in water containing dissolved organic carbon by ultraviolet spectroscopy**. *Int. J. Environ. Anal. Chem.* **80**, 49–59. <https://doi.org/10.1080/03067310108044385>.
- Gross, A., Boyd, C. E. & Seo, J. 1999 **Evaluation of the ultraviolet spectrophotometric method for the measurement of total nitrogen in water**. *J. World Aquac. Soc.* **30**, 388–393. <https://doi.org/10.1111/j.1749-7345.1999.tb00690.x>.
- Instituto Adolfo Lutz 2008 **Métodos físico-químicos para análise de alimentos**, 1st digital edn. (Zenebon, O., Sadocco Pascuet, N. & Tiglea, P., eds). Instituto Adolfo Lutz, São Paulo.

- Johnson, K. S. & Coletti, L. J. 2002 *In situ* ultraviolet spectrophotometry for high resolution and long-term monitoring of nitrate, bromide and bisulfide in the ocean. *Deep Sea Res. Part Oceanogr. Res. Pap.* **49**, 1291–1305. [https://doi.org/10.1016/S0967-0637\(02\)00020-1](https://doi.org/10.1016/S0967-0637(02)00020-1).
- Karlsson, M., Karlberg, B. & Olsson, R. J. O. 1995 Determination of nitrate in municipal waste water by UV spectroscopy. *Anal. Chim. Acta* **312**, 107–113. [https://doi.org/10.1016/0003-2670\(95\)00179-4](https://doi.org/10.1016/0003-2670(95)00179-4).
- Korshin, G., Chow, C. W. K., Fabris, R. & Drikas, M. 2009 Absorbance spectroscopy-based examination of effects of coagulation on the reactivity of fractions of natural organic matter with varying apparent molecular weights. *Water Res.* **43**, 1541–1548. <https://doi.org/10.1016/j.watres.2008.12.041>.
- Mantovani, D., Oliveira, A. T. A. B., De Bezerra, C., Cusioli, L. F., Rezende, D., Bergamasco, R. & Soares, P. F. 2018 Monitoramento da qualidade da água com classificação hidroquímica da bacia do Pirapó na cidade de Maringá-PR. *J. Exact Sci.* **18**, 5–12.
- Mazon, E. M. d. A., Oliveira, A. C. G. d., Brígido, B. M. & Freitas, V. P. d. S. 2005 Estudo comparativo de métodos para determinação de nitrato em águas para consumo humano. *Rev. Inst. Adolfo Lutz Impresso* **64**, 110–116.
- Meyer, D., Prien, R. D., Rautmann, L., Pallentin, M., Waniek, J. J. & Schulz-Bull, D. E. 2018 *In situ* determination of nitrate and hydrogen sulfide in the Baltic Sea using an ultraviolet spectrophotometer. *Front. Mar. Sci.* **5**, 431.
- MONTGOMERY, D. C. 2005 *Design and Analysis of Experiments*. John Wiley & Sons, New York.
- Oliveira, F. M. d. 2007 Aplicação e testes do método APHA-4500-NO₃-C para determinação de nitratos em águas por leitura em UV com segunda-derivada. *Rev. Anal.* **27**, 80–88.
- Rezende, D., Valim Junior, N. C., Nishi, L., Mantovani, D., Vieira, A. M. S. & Bergamasco, R. 2015 Avaliação de nitrato nas águas de fontes subterrâneas da bacia hidrográfica do Ribeirão Borba Gato, Maringá-PR. *Blucher Chem. Eng. Proc.* **2**, 1954–1959.
- Rezende, D., Nishi, L., Coldebella, P. F., Mantovani, D., Soares, P. F., Junior, N. C. V., Vieira, A. M. S. & Bergamasco, R. 2019 Evaluation of the groundwater quality and hydrogeochemistry characterization using multivariate statistics methods: Case study of a hydrographic basin in Brazil. *Desalin. Water Treat.* **161**, 203–215.
- Scorsafava, M., Souza, A. & Alves, G. 2018 Método espectrofotométrico na região UV a 205 nm para determinação de íon nitrato em água: Validação. *Rev. Inst. Adolfo Lutz* **76**, 1/10.
- Shi, Z., Chow, C. W. K., Fabris, R., Liu, J. & Jin, B. 2022 Applications of online UV-Vis spectrophotometer for drinking water quality monitoring and process control: A review. *Sensors* **22**, 2987. <https://doi.org/10.3390/s22082987>.
- Silva, M. F., Silva, C. F. d. S., Zavarize, D. G. & Benatti, C. T. 2023 Verificação da adequação de diferentes métodos espectrofotométricos para determinação de nitrato em água. *DELOS Desarro. LOCAL Sosten.* **16**, 2867–2882. <https://doi.org/10.55905/rdelosv16.n47-022>.
- Souto, M. A. M., Okada, M. M., Okada, I. A. & Dovidauskas, S. 2006 A determinação de nitrato em águas por espectrofotometria UV: Usos e precauções. *Rev. Inst. Adolfo Lutz Impresso* **65**, 66–70.
- Thomas, O., Causse, J., Jung, A.-V. & Thomas, M.-F. 2017 Chapter 7 – Natural water. In: *UV-Visible Spectrophotometry of Water and Wastewater (Second Edition)* (Thomas, O. & Burgess, C., eds). Elsevier, pp. 225–259. <https://doi.org/10.1016/B978-0-444-63897-7.00007-X>.
- Zhang, H., Wu, Q., Li, Y. & Xiong, S. 2021 Simultaneous detection of nitrate and nitrite based on UV absorption spectroscopy and machine learning. *Spectroscopy* **36**, 38–44.

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