DERIVATIVE SPECTROPHOTOMETRY FOR NO₃⁻ DETERMINATION IN SANITARY SEWAGES

Tayane Cristiele Rodrigues Mesquita[1] (iD), Pamela Lais Cabral Silva[2] (iD), Lucas Rafael Lommez Vaz[3] (iD) & Alisson Carraro Borges[4] (iD)

1 - Agricultural Engineer, PhD student of the Graduate Program in Agricultural Engineering - Agricultural Engineering Department, Federal University of Viçosa. Email: tayanemesquita@yahoo.com.br

2 - Environmental Engineer, MSc student of the Graduate Program in Agricultural Engineering - Agricultural Engineering Department, Federal University of Viçosa. Email: pamela_lais@hotmail.com

3 - Agricultural Engineer, PhD student of the Graduate Program in Agricultural Engineering -Agricultural Engineering Department, Federal University of Viçosa. Email: lucasrlv@yahoo.com.br

4 - Professor of the Graduate Program in Agricultural Engineering - Agricultural Engineering Department, Federal University of Viçosa. Email: borges@ufv.br

Keywords:	ABSTRACT

ABSTRACT

The objective of this study was to observe the feasibility of using the UV spectrophotometric method of the second derivative in the determination of nitrogen in the form of nitrate in sanitary sewers, since this method is indicated for water with low content of organic matter. Therefore, the results obtained in that method were compared with the salicylate methodology. The Mann-Whitney test showed a significant difference ($\alpha = 5\%$) between the results obtained in both methods. However, when compared, the difference between them was less than 0.25 mg L^{-1} in half of the observations. Despite this significant difference, the use of the second derivative method for sanitary sewage samples showed satisfactory performance.

ESPECTROFOTOMETRIA DERIVATIVA PARA DETERMINAÇÃO DE NO₃⁻ EM ESGOTOS SANITÁRIOS

RESUMO

O objetivo deste estudo foi verificar a viabilidade de uso do método espectrofotométrico UV da segunda derivada na determinação de nitrogênio na forma de nitrato em esgotos sanitários, posto que tal método é indicado para água com baixo teor de matéria orgânica. Para isso, compararam-se os resultados obtidos no referido método com a metodologia do salicilato. O teste de Mann-Whitney apontou diferença significativa ($\alpha = 5\%$) entre os resultados obtidos nos dois métodos. Ainda assim, quando comparados, a diferença entre eles foi inferior a 0,25 mg L^{-1} em metade das observações. Apesar dessa diferença significativa, o uso do método da segunda derivada para amostras de esgotos sanitários apresentou desempenho satisfatório.

INTRODUCTION

Nitrogen is an element found in the nature in several forms and oxidation states. The presence of nitrogen in the form of nitrate in water bodies can cause several adverse effects on the ecosystem and public health (ALIMOHAMMADI *et al.*, 2018; TEMKIN *et al.*, 2019; BLAISDELL *et al.*, 2019). Consequently, its determination and monitoring in water bodies and effluents is essential. The methods most commonly used in environmental samples include spectrophotometric techniques, electrochemical detection, chromatography and capillary electrophoresis (SINGH *et al.*, 2019).

The method based on derivative spectrophotometry (APHA, 2017) is simple to be executed, in which the calculation of the second derivative of the spectrum is established in order to eliminate interferences, such as organic matter, present in samples that can absorb ultraviolet radiation (UV). Thus, this method is recommended for water with a low organic matter content, therefore, it is not suitable for wastewater. However, Hoather and Rackham (1959) and Ferree and Shannon (2001) observed satisfactory results in the quantification of NO_3^- in household sewage, instigating why this method is not considered for wastewater.

Therefore, the objective of this study was to verify the feasibility of using the second derivative method to determine nitrate in sanitary effluents.

MATERIAL AND METHODS

To evaluate the viability of the second derivative (DM) method in determining $N-NO_3^-$ in sanitary effluents, a comparison was made with the salicylate (SM) method, described by Yang *et al.* (1998) and widely used.

The raw sewage samples were collected from a sewage collection network in the city of Viçosa, Minas Gerais State (MG), immediately acidified (H_2SO_4) and filtered through membrane filters (0.45 μm). After, they were divided into 20 portions and randomly assigned to the two methods being evaluated.

Calibration curves were constructed for both methods. For SM, it was used the following concentrations: 0.5; 1.0; 1.5; 2.0; 2.5 and 5.0 mg

L^{-1} $N-NO_3^-$, and the absorbance readings were taken in a quartz cuvette (10 mm) in a HACH spectrophotometer (DR6000) for $\lambda = 410\ nm$. In relation to DM, a scanning was performed every 5 nm in the 200-250 nm range for the concentrations 0.5; 1.0; 1.5; 2.0 and 2.5 mg L^{-1}, according to the linearity method (APHA, 2017).
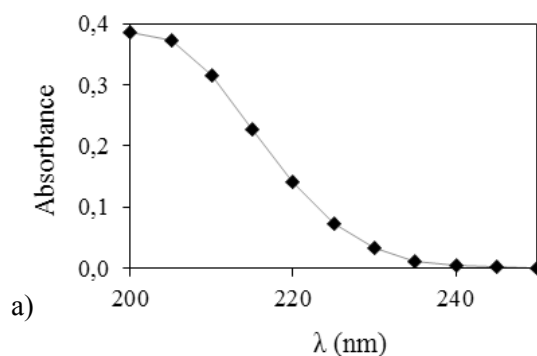
The second derivative of the spectra generated by the reading of standard solutions and samples was calculated. The absorbance data at each evaluated wavelength were plotted and then the least squares method was used to obtain the regression equations, fitted in a second-degree polynomial model. From the models obtained previously, values of the second derivatives were determined, which were then plotted according to the concentrations of $N-NO_3^-$, in which a linear equation was fitted.

Likewise, the concentrations of $N-NO_3^-$ in the sewage samples were obtained using the values of the second derivative and the equation of the analytical curve of the standard solutions (concentration *versus* 2nd derivative).

Finally, ANOVA's normality and homoscedasticity assumptions (Shapiro-Wilk and Cochran) were tested, whereas the statistical difference between the methods was performed using the Mann-Whitney (U) non-parametric test in the statistical program R 3.5.3®, for a 5% significance level.

RESULTS AND DISCUSSION

From the SM calibration curve ($R^2 = 0.99$), the $N-NO_3^-$ concentrations were obtained in the evaluated sewage samples. Absorbance values between 0.305 and 0.319 and concentration between 3.59 to 3.77 mg L^{-1} ($\bar{X}$=3.68 mg L^{-1}) were observed, showing a high precision (relative standard deviation <2%).

a)

b)
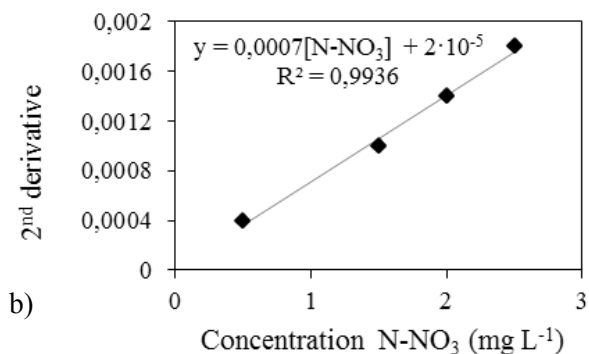
Figure 1. a) Spectrum obtained from the scanning of the standard 0.5 mg L^{-1} N-NO$_3^-$ for 200-250-nm λ b) model fitted to the concentration data of N-NO$_3^-$ standard solutions and second derivative value of the spectrum generated for 200-250-nm λ.

Figure 1a shows the spectrum obtained for the 0.5 mg L^{-1} N-NO$_3^-$ standard solution. From the spectra generated for the evaluated standard solutions and the adjustment of the values of the second order model, the values of the second derivative were calculated (Table 1). The models showed a good fit to the data (R^2> 0.97). Through

the concentration values and second derivative, the linear model was obtained (Figure 1b). This model presented a determination coefficient greater than 0.99, indicating linearity of the method in the evaluated concentration range.

From the model indicated in Figure 1b, as well as from the achievement of spectra generated for λ from 200 to 250 nm, N-NO$_3^-$ concentrations were obtained in the sewage samples, as observed in shown in Table 2.

The models fitted for the spectra of the sewage samples did not show differences in the coefficients of the regression quadratic term, which yielded values for the second derivative and equal concentrations for all samples. As a result, a residual variance equal to zero was obtained, which resulted in the lack of homogeneity of variances of the experimental residues between the data obtained for both methods, through the Cochran test (p <0.0001).

Therefore, a non-parametric alternative was used to check the difference between the methods. The Mann-Whitney test showed a significant difference (α = 5%) between the results obtained in both methods (p <0.0001). However, it should be

Table 1. Models fitted to the spectra generated for the standard solutions and values calculated for the second derivative

Concentration (mg L^{-1})	Equation	R^2	2nd derivative
0.5	y = 0.0002x^2 – 0.0928x + 11.532	0.9720	0.0004
1.5	y = 0.0005x^2 – 0.2620x + 32.483	0.9729	0.0010
2.0	y = 0.0007x^2 – 0.3448x + 42.800	0.9724	0.0014
2.5	y = 0.0009x^2 – 0.4300x + 53.375	0.9719	0.0018

Table 2. Models fitted to the spectra generated for the sewage samples, values calculated for the second derivative of the curve and N-NO$_3^-$ concentrations

Sample	Equation	2nd derivative	Concentration (mg L^{-1})
1	y = 0.0006x^2 – 0.3085x + 40.891	0.0012	3.37
2	y = 0.0006x^2 – 0.3106x + 41.073	0.0012	3.37
3	y = 0.0006x^2 – 0.3128x + 41.319	0.0012	3.37
4	y = 0.0006x^2 – 0.3170x + 41.785	0.0012	3.37
5	y = 0.0006x^2 – 0.3134x + 41.350	0.0012	3.37
6	y = 0.0006x^2 – 0.3190x + 41.954	0.0012	3.37
7	y = 0.0006x^2 – 0.3206x + 42.133	0.0012	3.37
8	y = 0.0006x^2 – 0.3236x + 42.485	0.0012	3.37
9	y = 0.0006x^2 – 0.3292x + 43.092	0.0012	3.37
10	y = 0.0006x^2 – 0.3238x + 42.511	0.0012	3.37

observed that this difference between the methods may come from the fact that all samples analyzed in the DM method had equal concentrations, which was associated with the absence of differences in the coefficients of the regression quadratic term. Even so, the comparison between SM and DM showed that the largest difference between them was 0.40 mg L^{-1}, although this contrast was less than 0.25 mg L^{-1} ($\bar{X}$=0.3 mg L^{-1}) in half of the observations. It is important to highlight that the comparison between DM and ion chromatography for drinking water showed a maximum difference of 1.6 mg L^{-1} in concentration between the methods, mostly less than or equal to 0.5 mg L^{-1}. Yet, this method was recommended for the APHA manual (2017). Therefore, despite the significant difference between the methods, the DM for sanitary sewers presented a performance comparable to the DM for water with a low organic matter content.

The SM presented concentrations, approximately 8.3% higher than the DM, which indicates its tendency to underestimate N-NO$_3^-$ concentrations. Such fact may be associated with the matrix effect resulting from the interference of organic compounds present in the sewage sample that can have a suppressive action on N-NO$_3^-$. Oliveira (2007), in an experiment that evaluated the application of DM in pond water did not verify the interference of compounds present in the matrix in the N-NO$_3^-$ determination. However, the matrix evaluated by the aforementioned author presents less complexity than that verified in sanitary sewers.

SM is based on colorimetry principles and, although it provides results considered accurate due to the removal of interfering compounds, it is a laborious method. On the other hand, the DM is highly practical and does not require the use of reagents for analysis in comparison to the other available methods (APHA, 2017). Such characteristics result in the insertion of the DM in the green chemistry scenario that aims at processes that use and generate fewer chemical residues (SILVA *et al.,* 2005).

CONCLUSIONS

- The second derivative ultraviolet method

(DM) for N-NO$_3^-$ quantification showed a simple performance when compared to the salicylate method (SM). The DM application assessment in sewage samples indicated an underestimation of approximately 8.3% when compared to the SM, which may be related to the matrix effect caused by the presence of organic interferences in the sanitary sewage. Thus, the methods showed significant differences between them, where variations in concentrations of up to 0.4 mg L^{-1} N-NO$_3^-$, were obtained, which are, however, lower than those observed for other methods recommended in APHA (2017). Thus, it can be concluded that for the conditions assessed in this work the use of DM for domestic sewage samples was shown to be viable.

REFERENCES

ALIMOHAMMADI, M.; LATIFI, N.; NABIZADEH, R.; YAGHMAEIAN, K.; MAHVI, A. H.; YOUSEFI, M.; HEIDARINEJAD, Z. Determination of nitrate concentration and its risk assessment in bottled water in Iran. **Data in Brief**, v. 19, p. 2133-2138, 2018.

APHA. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 23 ed, Washington: APHA, 2017.

BLAISDELL, J.; TURYK, M. E.; ALMBERG, K. S.; JONES, R.M.; STAYNER, L.T. Prenatal exposure to nitrate in drinking water and the risk of congenital anomalies. **Environmental research,** v.176, p. e108553, 2019.

FERREE, M.; SHANNON, R. Evaluation of a second derivative UV/Visible spectroscopy technique for nitrate and total nitrogen analysis of wastewater samples. **Water research**, v.35, n.1, p. 327-32, 2001.

HOATHER, R. C.; RACKHAM, R. F. Oxidised nitrogen in waters and sewage effluents observed by ultra-violet spectrophotometry. **The Analyst**, v. 84, p. 548-551, 1959.

OLIVEIRA, F. M. Aplicação e testes do método Apha-4500-NO3-C para determinação de nitratos em águas por leitura em UV com segunda-derivada. **Revista Analytica**, v.27, p. 80-88, 2007.

SILVA, F. M.; LACERDA, P. S. B.; JONES JUNIOR, J. Desenvolvimento sustentável e química verde. **Química Nova**, v. 28, n. 1, p. 103-110, 2005.

SINGH, P., BEG, Y. R., NISHAD, G. R. A review on spectroscopic methods for determination of nitrite and nitrate in environmental samples. **Talanta**, v. 191, n. 1, p. 364-381, 2019.

TEMKIN, A.; EVANS, S.; MANIDIS, T.; CAMPBELL, C.; NAIDENKO, O. V. Exposure-based assessment and economic valuation of adverse birth outcomes and cancer risk due to nitrate in United States drinking water. **Environmental research**, v.176, p. e108518, 2019.

YANG, J. E.; SKOGLEY, E. O.; SCHAFF, B. E.; KIM, J. J. A simple spectrophotometric determination of nitrate in water, resin and soil extracts. **Soil Science Society of American Journal,** v. 62, n. 4, p. 1108-1115, 1998.