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Development of Green-Assessed and Highly Sensitive Spectrophotometric Methods for Ultra-Low-Level Nitrite Determination Using Rhodanine and 7-Hydroxycoumarin in Environmental Samples

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Abstract

Rapid, sensitive, and environmentally sustainable spectrophotometric methods for the determination of nitrite (NO_2^-) in environmental specimens are proposed. The presented procedures are grounded in the diazotization of sulphathiazole (STZ), followed by coupling with rhodanine (RDN) or 7-hydroxycoumarin (7-HC) in an alkaline medium, and the results were studied. This reaction gave an intense soluble red color at 504 nm and a pale red color at 525 nm for RDN and 7-HC, respectively. The conditions producing the maximum performance and other important analytical criteria in relation to the proposed procedures were investigated to enhance their sensitivity. Beer's law was abided by for NO_2^- over the concentration ranges of 0.08–2.0 μg mL^{-1} and 0.04–2.4 μg mL^{-1} using RDN and 7-HC, respectively. The lower limit of detection (LLOD), lower limit of quantification (LLOQ), molar absorptivity (ε), and Sandell's sensitivity were calculated as follows: 0.0303 μg mL^{-1}, 0.0918 μg mL^{-1}, 4.20×10^4 L mol^{-1} cm^{-1}, and 1.63×10^{-6} μg cm^{-2} (in the case of RDN); and 0.0387 μg mL^{-1}, 0.1172 μg mL^{-1}, 6.90×10^4 L mol^{-1} cm^{-1}, and 1.00×10^{-6} μg cm^{-2} (in case of 7-HC). Furthermore, the ecological implications were assessed using three green assessment methodologies: Analytical Eco-Scale (ESA), Analytical GREEnness metric (AGREE), and Green Analytical Procedure Index (GAPI). Thus, our proposed procedures are fully validated and implemented in order to carry out NO_2^- quantification in the selected ecological samples (water and soil samples).

Keywords: sodium nitrite; rhodanine; 7-hydroxycoumarin; sulfathiazole; spectrophotometry; greenness assessment

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1. Introduction

Nitrogen-containing species continue to receive considerable scientific attention due to their dual role as vital nutrients required for the sustenance of living organisms and as potential environmental pollutants capable of exerting adverse ecological and health effects [1,2].

Nitrite (NO_2^-) is a reactive nitrogen-containing variety that has garnered growing attention across diverse areas because of its potential noxious and beneficial effects. In

forensic chemistry, NO_2^- is one of the principal inorganic products generated during primer detonation and gunpowder combustion [3]. Industrially, NO_2^- is an authorized additive in meat products, serving as an antimicrobial, flavor enhancer, and colorant [4]. Medically, at specific concentrations, the dietary intake of NO_2^- has a curative effect since it is considered as a replacement pathway for nitric oxide (NO) production, which is a potent cardiovascular relaxant [5]. NO_2^- itself does not directly cause cancer, but it can react with intermediate species in the nitrogen cycle or during food manufacturing to form carcinogens [6]. NO_2^- can oxidize iron in hemoglobin within red blood cells, resulting in methemoglobin production, which cannot transport oxygen. This can lead to methemoglobinemia, also known as "blue baby syndrome," a condition especially detrimental for infants [7]. Furthermore, it has the potential to bind with secondary amines and amides in the digestive tract, resulting in carcinogenic N-nitroso compound formation [8,9]. The produced N-nitroso compounds were found to be responsible for inducing congenital malformations in central nervous system abnormalities [10], abortions [11], gastric and esophageal cancer [12], and spontaneous intrauterine growth restriction [13]. Trace levels of NO_2^- can also exist in various excipients during the manufacturing and storage of pharmaceuticals, such as preservatives, dyes, and stabilizers, and may form nitrosamines when reacting with secondary amines under acidic conditions [14], which requires strict control by regulatory agencies according to the International Council for Harmonisation (ICH) [15].

NO_2^- is an essential nitrogen nutrient for plants and can serve as a key water pollutant and an indicator of water quality [16]. Environmentally, NO_2^- is one of the main contaminants of water resources due to the over-application of nitrogenous fertilizers, leading to NO_2^- seepage into adjacent water sources (including groundwater, surface water, and treated drinking water) and soil [17]. Owing to its high water solubility and low retention in soils, NO_2^- tends to relocate readily through groundwater. Increased NO_2^- concentrations are considered a hazardous pollutant and are extremely toxic to aquatic systems, and they can lead to deleterious changes in biodiversity, triggering ecological system disruptions. Excessive NO_2^- concentrations in aquatic systems prompt nutrient overloading, introduce stimulated algal blooms, diminish oxygen amounts, increase aquatic system temperatures, and finally, trigger disruptions in various life forms in the affected aquatic systems [18,19]. NO_2^- enters the human body mainly through contaminated drinking water and nitrite-preserved foods, especially cured meats. It can also be formed endogenously through a reduction in dietary nitrate (NO_3^-) by bacteria in the mouth and gastrointestinal tract. Minor exposure may occur via inhalation or dermal contact in occupational or environmental settings [20,21].

NO_2^- concentrations in drinking water should not exceed 6.5×10^{-5} M (3.0 μg mL^{-1}) and 2.17×10^{-5} M (1.0 μg mL^{-1}), as per the norms of the World Health Organization (WHO) [20] and U.S. Environmental Protection Agency (U.S. EPA) [22], respectively. The acceptable daily intake for NO_2^- is 0.06 and 0.07 mg Kg^{-1} body weight, as stated by the EU Scientific Committee for Food (SCF) [23] and Food and Agriculture Organization (FAO)/World Health Organization (WHO) [24], respectively.

Due to heightened concern regarding the quality of different water sources (such as tap water, bottled water, groundwater, rivers, wastewater, and industrial discharges), NO_2^- concentrations, either individually or correlated with other constituents, can provide a pollution index in water, making the accurate monitoring of NO_2^- and its concentration determination in water extremely important. This helps in evaluating pollution sources, assessing treatment efficiency, and ensuring compliance with international water quality standards. According to the literature, several analytical approaches have been utilized for NO_2^- quantification in various disciplines. Such analytical techniques, including chro-

matography [25–27], electrophoresis [28,29], chemiluminescence [30,31], electrochemical methods [32–34], fluorescence spectrometry [35,36], flow-injection analysis (FIA) [37–41], and spectrophotometry [42–60], have been utilized in the determination of NO_2^- in various applications.

The approved method for NO_2^- analysis relies on sulfanilic acid, which is first diazotized, and the resulting diazonium salt is subsequently coupled with N-(1-naphthyl)ethylenediamine hydrochloride, a process that results in the formation of carcinogenic compounds [61].

Sulfa drugs showed promising results when utilized as an electrophilic reactant for eco-toxicant spectrophotometric analyses [46,47,56]. Herein, we use sulfathiazole (STZ) as an electrophilic reagent for NO_2^- determination.

Both rhodanine (RDN) and 7-hydroxycoumarin (7-HC) were successfully utilized as effective coupling agents in diazotization reactions, forming brightly colored products that exhibit strong absorbance in the visible region, enabling spectrophotometric determination of various analytes [62,63].

Despite the rise of modern sensitive analytical methods, spectrophotometric analysis is still a cornerstone in various analytical laboratories, from environmental monitoring and water quality testing to pharmaceutical purity control and biochemical assays. Its high sensitivity and selectivity, versatility, practicality, simplicity, cost-effectiveness, high accuracy and precision, and speed make it an indispensable tool for both routine analyses and research applications [64,65].

The main objective of the current work was therefore to establish eco-friendly, specific, sensitive, cost-effective, and reliable analytical procedures that are potentially applied in nitrite (NO_2^-) spectrophotometric quantification in different ecological specimens. The recommended procedures are based on the diazotization of STZ, which is then coupled with RDN or 7-HC. The attributes of the developed method (e.g., LLOD, LLOQ, ε, etc.) were determined and compared with other spectrophotometric methods. Furthermore, the greenness assessment related to the proposed procedures was investigated and proved using three green evaluation tools: the Analytical Eco-Scale (ESA), the Analytical GREEnness metric (AGREE), and the Green Analytical Procedure Index (GAPI).

2. Experimental Procedure

2.1. Apparatus

All absorption measurements were conducted at room temperature using a Labomed double-beam UV-visible programmable spectrophotometer supplied with 1 cm quartz cuvettes and operated by a PC running the spectrophotometric software UV Win V.5.0.4. A software program was utilized for absorbance measurements. All pH measurements were made using Hanna Instruments pH Meter HI 2210, USA, fitted with a combined glass electrode, and the total accuracy was 0.01 pH units.

2.2. Materials

All utilized chemicals were of analytical grade and comprised high-purity reagents, and they were employed without any purification steps. All chemicals utilized in this study, including sodium nitrite ($NaNO_2$), RDN, 7-HC, STZ, hydrochloric acid (Conc. HCl), nitric acid (Conc. HNO_3), potassium dichromate ($K_2Cr_2O_7$), sodium hydroxide (NaOH), chloroform ($CHCl_3$), potassium permanganate ($KMnO_4$), ethylenediaminetetraacetic acid (EDTA), and sodium carbonate (Na_2CO_3), were procured from Sigma-Aldrich and BDH. Throughout the entire experimental work, all solutions were produced and diluted utilizing bi-distilled water. All glass tools were exhaustively immersed in an acidified solution of $K_2Cr_2O_7$ and then flushed using Conc. HNO_3; finally, they were washed repeatedly utilizing bi-distilled water.

2.3. Reagent and Standard Solution Preparation

Standard solution of 1000 μg mL^{-1} $NaNO_2$ (Merck, Rahway, NJ, USA) was formulated as mentioned previously [37]. Then, 0.150 g of $NaNO_2$ was dried for 4 h at 110 °C, dissolved in bi-distilled water, and shaken well, and the volume was brought to the mark using the same solvent and kept in a refrigerator. Some precautions were considered to ensure the stability of the prepared $NaNO_2$ solution. A small quantity of NaOH was added to inhibit the degradation of NO_2^- into nitrous acid (HNO_2) [37]. A minimal amount of $CHCl_3$ was added to prevent bacterial proliferation, and it was standardized according to $KMnO_4$ [66,67]. $NaNO_2$ stock solutions were serially diluted using bi-distilled H_2O to obtain daily working solutions.

Since STZ is completely soluble in dilute acids [68], the STZ stock solution (9.79×10^{-4} M) was prepared by solubilizing 0.125 g of STZ in 0.6 M HCl, and the total volume (500 mL) was completed using the same solvent.

Stock solutions of RDN (3×10^{-3} M) and 7-HC (5×10^{-3} M) were prepared by dissolving the required quantity in a specific volume of methyl alcohol and sodium hydroxide, respectively; then, the total volume was completed up to 250 mL with the same solvent.

To study the foreign ions' influence on the effectiveness of the outlined method, a set of potential interfering ions solutions was prepared at suitable concentrations, and they were used whenever required.

2.4. Recommended Procedure

2.4.1. Spectrophotometric Determination of NO_2^- Using RDN

Using RDN, in a 10 mL volumetric flask, a solution containing a mixture of an aliquot of 0.08–2.0 μg mL^{-1} of $NaNO_2$ and 0.4 mL of STZ 250 μg mL^{-1} was maintained for 2 min at ambient temperature to ensure that diazotization was achieved. Afterwards, 0.5 mL of RDN 3×10^{-3} mol/L and 1.5 mL of the NaOH-KCl buffer (pH ≈ 11.2) were subsequently added to the resulting diazonium salt, and bi-distilled H_2O was employed to bring the final volume up to the mark. The resulting final solution was well mixed and permitted to settle for 5 min to ensure the complete progression of the coupling reaction. The final red-colored product was transferred into a quartz cuvette to measure its absorbance at λ_{max} = 504 nm versus a blank solution.

2.4.2. Spectrophotometric Determination of NO_2^- Using 7-HC

Using 7-HC, a specific volume of the 7-HC stock solution was added in a 10 mL volumetric flask filled with 0.4 mL of STZ 250 μg mL^{-1}. The final $NaNO_2$ concentration will lie in the range of 0.04–2.4 μg mL^{-1}. This mixture was thoroughly shaken and left for 2 min. at room temperature to end the diazotization step. Afterwards, we added 3.2 mL of 7-HC (5×10^{-3} M) and 2.8 mL of 1.0 M NaOH and then adjusted the solution to the final volume with bi-distilled H_2O. Using the corresponding blank reagent as a reference, the optical response of the produced azo dye was recorded at λ_{max} = 525 nm.

It should be noted that the maximum absorbance of the produced azo compound is recorded immediately without the need to wait after mixing all components. The mechanism of the suggested reaction is depicted in Figure 1.

Spectral changes during the reaction course were recorded (at 504 nm and 525 nm in case of RDN and 7-HC, respectively) against a blank solution, which was prepared identically, except that NO_2^- was not added. The Beer law calibration curve was graphically represented by charting absorbance in relation to the computed NO_2^- concentration.

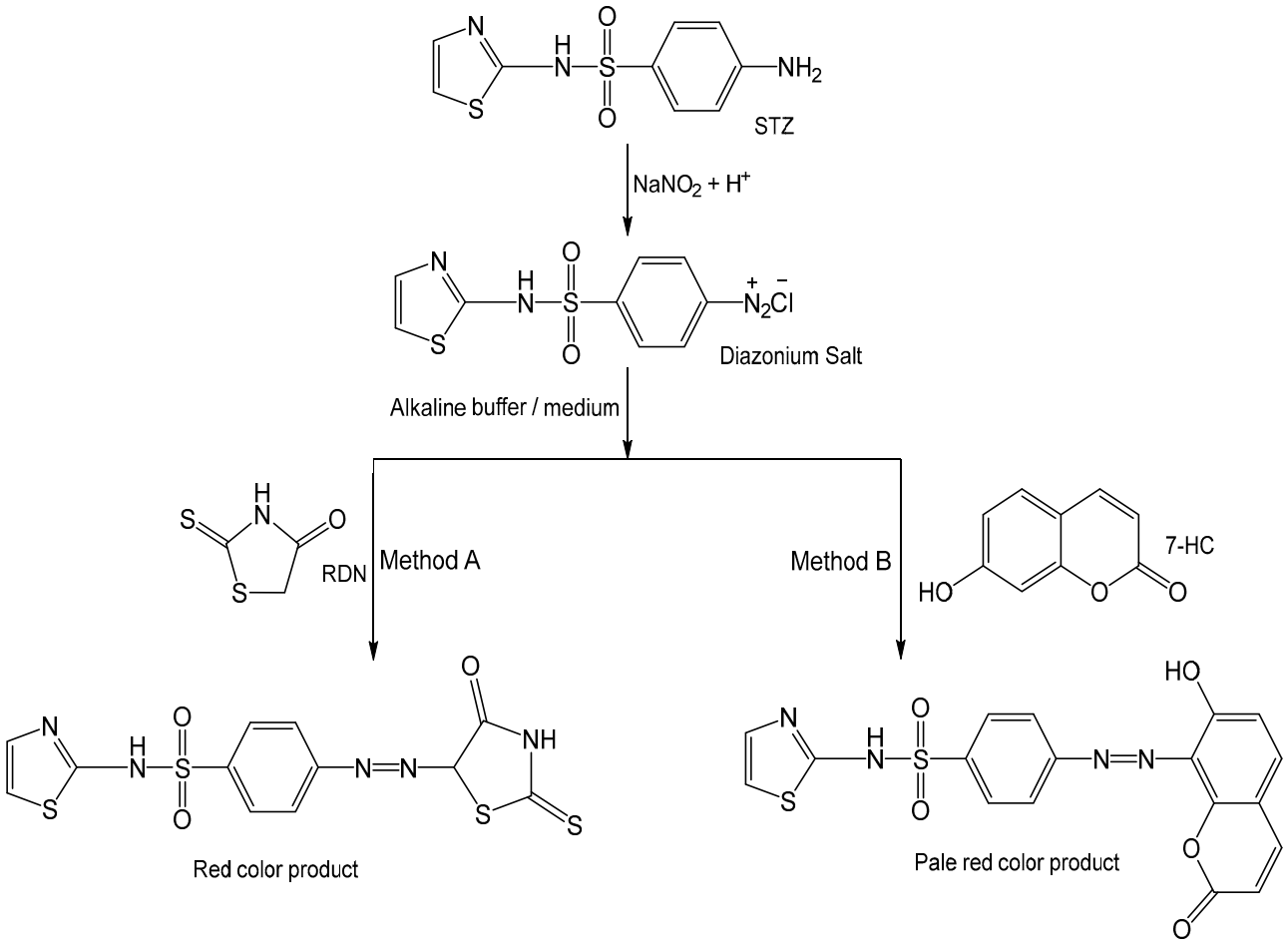


Figure 1. Reaction scheme for the colored product of STZ using RDN (Method A) and 7-HC (Method B).

2.4.3. Application of the Developed Methods for NO_2^- Determination

The presented methods were suggested for NO_2^- determination in different environmental samples: tap water, wastewater, and soil samples. NO_2^- was determined directly in the made-up solutions utilizing the standard addition method, where absorbance was measured and recorded (at 504 nm and 525 nm in the case of RDN and 7-HC, respectively) against the blank solution, which was prepared under identical conditions but with NO_2^- excluded.

Spectrophotometric Determination of NO_2^- in Water Samples

Aliquots of water samples (either tap or wastewater) were exposed to a mixture of 0.5 mL of 1.0 M NaOH and 0.5 mL of 0.2 M EDTA and mixed thoroughly. The resulting precipitate was discarded by centrifugation. EDTA was added for the purpose of masking several metal ions, such as Co^{+2}, Cu^{+2}, and Fe^{+3}, which may interfere with the proposed procedure [46]. A specified volume of the produced centrifugate was transferred to a series of 10 mL volumetric flasks. The used chemicals were added to each volumetric flask, as described in the proposed procedures (either using RDN of 7-HC). The constituents of each flask were mixed thoroughly and left to settle for 5 min., and then, the total volume was completed to the mark using bi-distilled water.

Spectrophotometric Determination of NO_2^- in Soil Samples

Approximately 1.0 g of soil sample was weighed and introduced into a 50 mL beaker and extracted three times with 5.0 mL portions of the 1.0% Na_2CO_3 solution. The extract was filtered through Whatman 41 filter paper, and suitable aliquots of the sample solution

were analyzed. In a series of 10 mL volumetric flasks, the above prescribed procedures (either using RDN or 7-HC) for NO_2^- determination were carried out in the presence of a specified volume of soil filtrate, accompanied by successively increased volumes of the standard NO_2^- solution. The ingredients of the individual flask were vigorously shaken and subsequently left to rest for 5 min. before being topped up to the mark with bi-distilled H_2O.

3. Results and Discussion

3.1. Spectrophotometric Determination of NO_2^- Using RDN

This method entails the diazotization step in an acidified medium between NO_2^- and STZ, followed by coupling with RDN in an alkaline medium, yielding the red-colored azo dye depicted in the scheme in Figure 1.

Figure 2A (spectrum a) shows the absorption spectra of the formed STZ-RDN azo compound, which consists of 10 μg mL^{-1} M STZ, 1.5×10^{-4} M of RDN, and a NaOH-KCl buffer (pH ≈ 11.2) in the presence of 0.4 μg mL^{-1} of NO_2^-. This spectrum was recorded against the blank solution, which has the same composition except for the absence of NO_2^- (Figure 2A (spectrum b)). The formed azo compound demonstrated maximum absorbance at 504 nm, while no absorbance was noticed in the blank solution at all.

An acidic medium is necessary for diazotization; if absent, NO_2^- cannot efficiently convert into NO^+, and the overall diazotization would not proceed [69]. Better results were attained when the HCl medium was used for diazonium cation formation since it cleanly generates HNO_2 from $NaNO_2$ and provides a non-oxidizing, weakly coordinating counter-ion (Cl^-) that yields soluble, relatively stable diazonium chlorides [70]. The current study examined HCl concentrations between 0.2 and 1.2 M to identify a suitable concentration for maximal diazotization. Accordingly, STZ was solubilized in 0.6 M HCl to ensure the highest color intensity.

The effect of pH on the formation of the colored azo STZ-RDN compound was investigated over the pH range of 2–13, which was adjusted with NaOH or HCl. This study was carried out using STZ (10 μg mL^{-1}), 0.4 μg mL^{-1} of $NaNO_2$, and 1.5×10^{-4} M of RDN. This study revealed that the maximum absorbance of sensitive azo-colored compounds was recorded in a basic medium with a pH range of 10.8–11.8. Accordingly, a pH value of approximately 11.2 was selected as the optimum condition for the spectrophotometric determination of NO_2^- using RDN as the coupling reagent, as illustrated in Figure 2B.

To select the best buffer type in the current procedure, different buffers like NaOH (1.0 M), the universal buffer, and NaOH-KCl were investigated. The maximum and best absorbance was obtained using the NaOH-KCl buffer. The optimum volume of the NaOH-KCl buffer (pH ≈ 11.2) for coupling reactions between RDN and diazotized STZ was studied using different NaOH-KCl buffer volumes, ranging from 0.4 to 3.2 mL. This study revealed that constant and maximum absorbance was observed in the range between 1.2 and 1.8 mL. Based on this optimization, 1.5 mL of the NaOH-KCl buffer solution (pH ≈ 11.2) was employed in all subsequent experiments; the influence of the buffer volume is presented in Figure 2C.

The impact of RDN concentrations on the formation of the red azo color was investigated using 10 μg mL^{-1} of STZ and 0.4 μg mL^{-1} of $NaNO_2$ in the presence of 1.5 mL of the NaOH-KCl buffer (pH ≈ 11.2). The RDN concentration ranged between 0.24×10^{-4} M and 2.6×10^{-4} M. Figure 2D shows the obtained concentration results. The optimum RDN concentration in the final concentration ranged between 0.96×10^{-4} M and 1.92×10^{-4} M. Consequently, the addition of 0.5 mL of 3×10^{-3} M RDN, corresponding to a final concentration of 1.5×10^{-4} M in a total volume of 10 mL, was found to be sufficient for the recommended procedure.

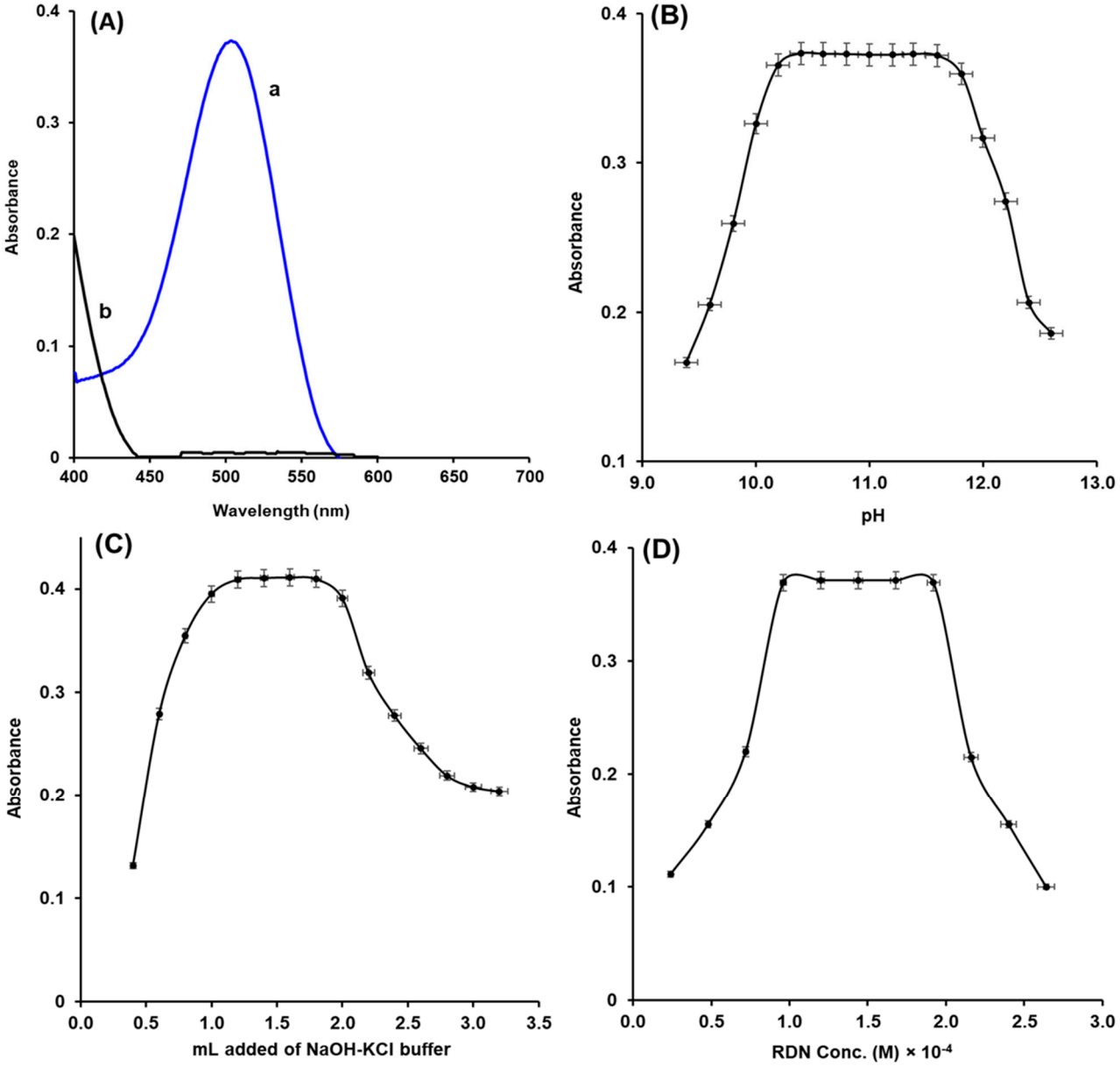


Figure 2. (**A**) Spectral curve for the product of diazonium reaction of 10 μg mL^{-1} STZ (λ_{max} = 504 nm) using RDN: (a) in the presence of 0.4 μg mL^{-1} NO_2^-; (b) in the absence of NO_2^-. Influence of (**B**) pH, (**C**) NaOH-KCl buffer volume, and (**D**) RDN concentration on the formation efficiency of the produced azo compound.

3.2. Spectrophotometric Determination of NO_2^- Using 7-HC

This technique produces a pale red azo dye by diazotizing NO_2^- and STZ in an acidic medium and then coupling the formed diazonium with 7-HC in an alkaline medium, as seen in the scheme in Figure 1.

Figure 3A (curves a and b) shows the absorption spectra of diazotized STZ-7-HC and its blank solution (which has the same constituents except the presence of NO_2^-), respectively. An obvious spectrum with maximum absorbance recorded at 525 nm was recorded for a solution composed of 10 μg mL^{-1} STZ, 0.4 μg mL^{-1} of NO_2^-, and 1.6×10^{-3} M of 7-HC in the presence of a specified volume of 1.0 M NaOH. In the overall scanned wavelength range, there was no noticeable absorbance from the blank solution.

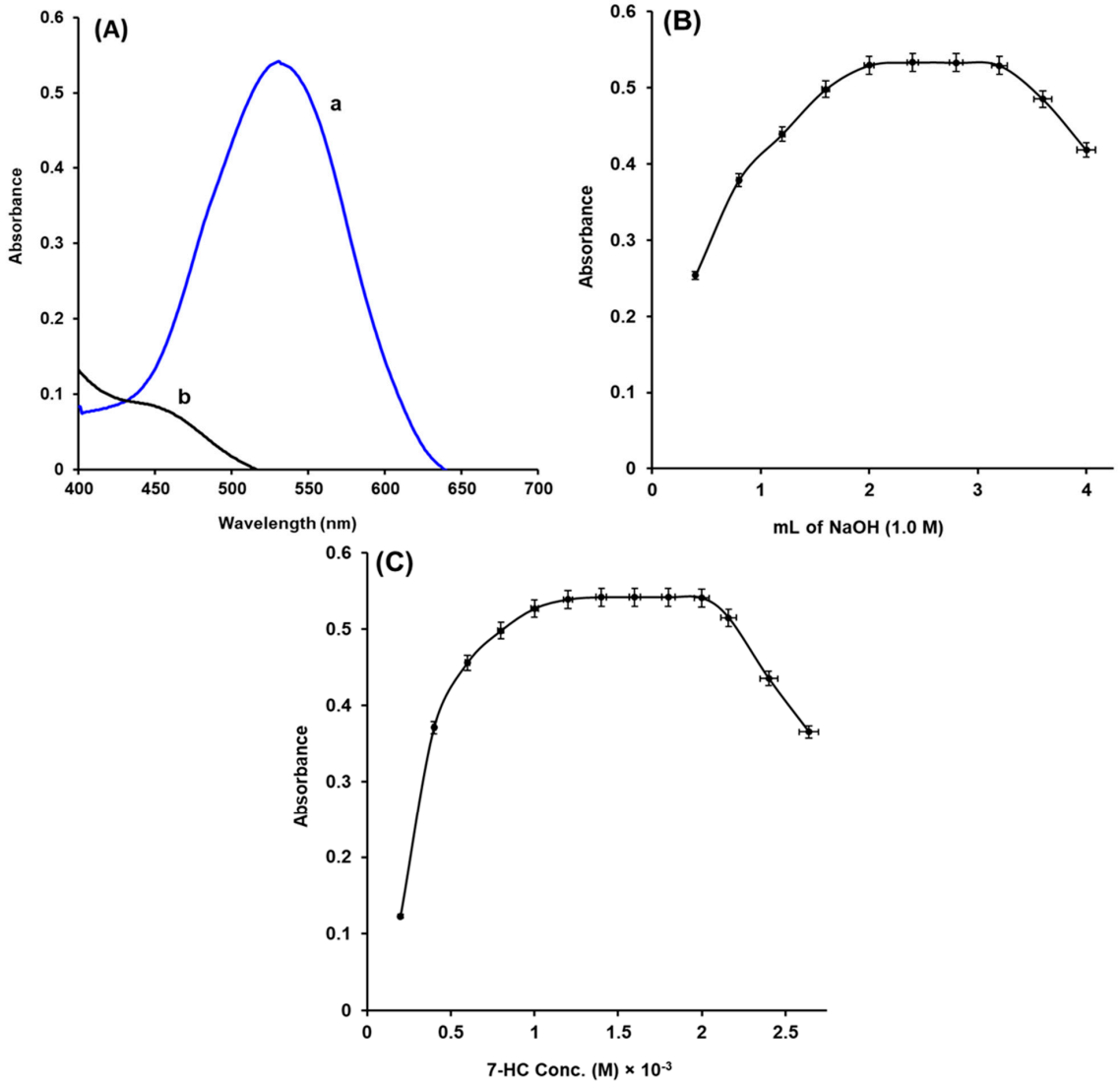


Figure 3. (**A**) Spectral curve for the product of diazonium reaction of 10 μg mL^{-1} STZ (λ_{max} = 525 nm) using 7-HC: (a) in presence of 0.4 μg mL^{-1} NO_2^-; (b) in absence of NO_2^-. Influence of (**B**) mL added of NaOH (1.0 M) and (**C**) 7-HC concentration on the formation efficiency of the produced azo-compound.

Figure 3B shows the basicity impact on the formation of the azo product due to the coupling reaction of diazotized STZ with 7-HC. This study was carried out using 10 μg mL^{-1} STZ (dissolved in 0.6 M HCl), 0.4 μg mL^{-1} of $NaNO_2$, and 1.6 × 10^{-3} M of 7-HC and gradually increasing volumes of 1.0 M NaOH. This effect was studied over a NaOH concentration range of 0.04–0.4 M. The absorbance for the azo product of STZ and 7-HC was found to be maximum and constant at λ_{max} = 525 nm over the NaOH concentration range from 0.20 to 0.32 M. Therefore, 2.5 mL of 1.0 M NaOH in the final volume of 10-mL (0.25 M) was utilized in the recommended procedure for the quantitative determination of NO_2^-.

The effectivity of 7-HC concentrations on the proposed procedure for NO_2^- determination was studied using several 7-HC concentrations starting from 1.2 × 10^{-3} M and ending with 2.0 × 10^{-3} M, which were sequentially added to a series of 10 mL volumetric flasks, where each individual flask contained 10 μg mL^{-1} of STZ and 0.4 μg mL^{-1} of $NaNO_2$, with the subsequent addition of 0.25 M of NaOH. From Figure 3C, which shows the obtained results for the 7-HC concentration study, we can conclude that 3.2 mL of 5 × 10^{-3} M 7-HC (1.6 × 10^{-3} M) was selected for the determination of NO_2^- in the proposed procedure.

3.3. Effect of Time

In the presence of 0.4 μg mL^{-1} of NO_2^- and constant concentrations of all used coupling reagents (RDN or 7-HC), the effect of time on the formed azo dye was evaluated at different time intervals. The reaction proceeded instantaneously, and the resulting color, which appeared directly after completely the mixing of all constituents at room temperature, and the recorded absorbance of the formed azo dye was found to be approximately constant for more than three hours, and no changes were observed. The time-dependent behavior of the produced azo dye is depicted in Figure 4.

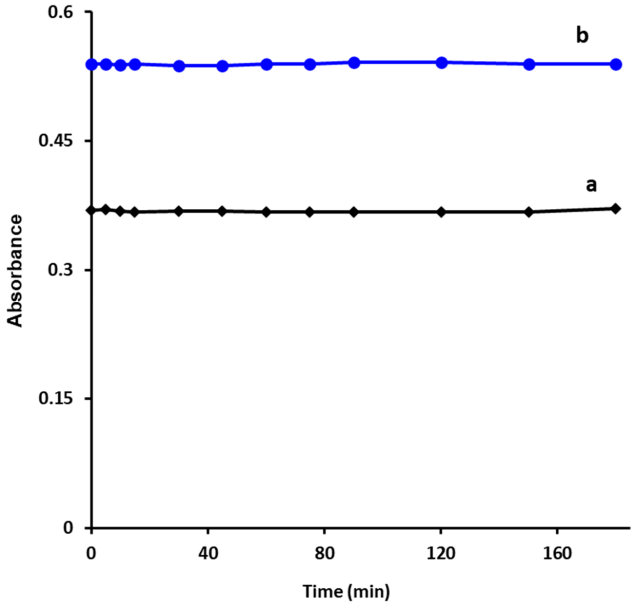


Figure 4. Effect of time on the formed azo dye product using (a) RDN and (b) 7-HC in the presence of 0.4 μg mL^{-1} of NO_2^- at room temperature.

3.4. Analytical Data

The recorded absorption reached its maximum in the absorption spectrum of the formed azo dye at λ_{max} = 504 nm (Figure 5A in the case of RDN) and λ_{max} = 525 nm (Figure 6A in the case of 7-HC), and the blank solution has insignificant absorption versus bi-distilled water in a 1.0 cm quartz cuvette. The calibration graph obtained by the recommended procedure for the determination of NO_2^- using RDN and 7-HC is represented in Figures 5B and 6B, respectively. The calibration graphs indicate that the recommended procedure conformed to Beer's law within the NO_2^- concentration range of 0.08–2.0 and 0.04–2.4 μg mL^{-1} for RDN and 7-HC, respectively. The most characteristic statistical data for the two proposed methods are given in Table 1. The lower limit of detection (LLOD = 3.3 σ/b) and lower limit of quantification (LLOQ = 10 σ/b) were obtained, where σ is the standard deviation of the intercept, and b is the slope of the calibration curve (n = 5) [71]. In compliance with Beer's law, the shown calibration equations in Figures 5B and 6B are expressed without intercepts since they are negligible and statistically insignificant. The obtained values of molar absorptivity (ε), Sandell's sensitivity, correlation coefficients, and other characteristic statistical data shown in Table 1 indicated the good linearity of the constructed calibration graphs and the high precision of the determination of NO_2^- using the proposed methods.

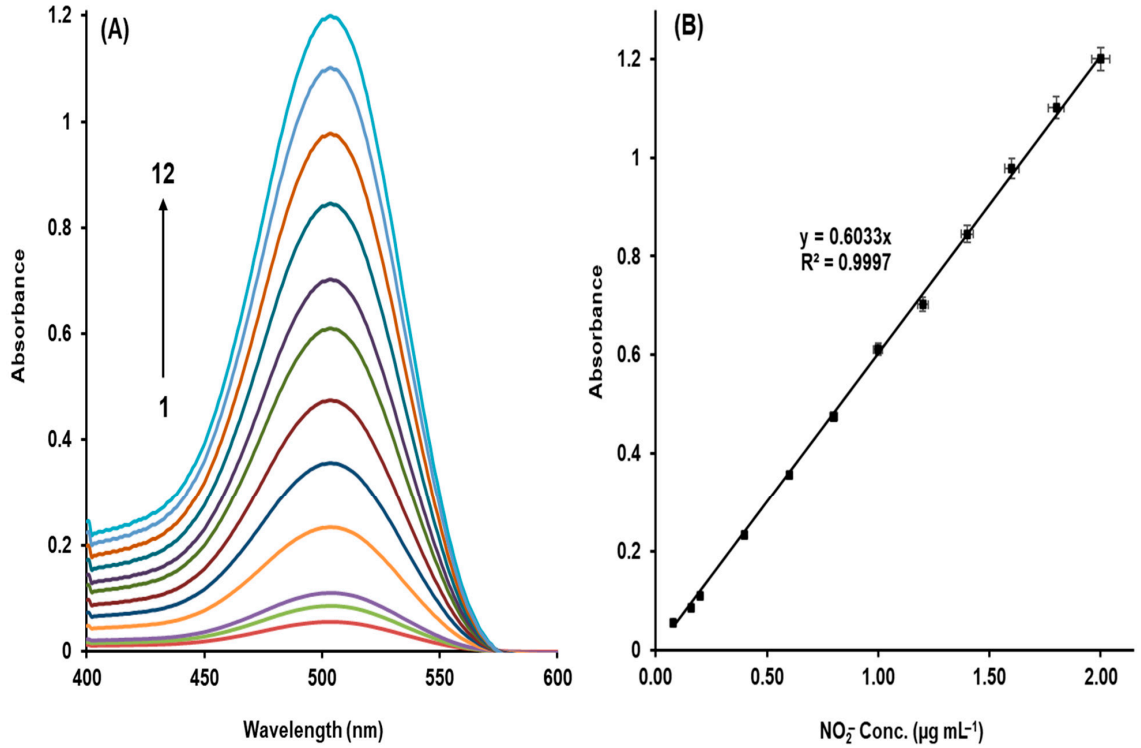


Figure 5. (**A**) Absorption spectra of diazotized STZ-RDN pale red product at λ_{max} = 504 nm in the presence of different NO_2^- concentrations, varying from 0.08 to 2.0 µg mL^{-1}. (**B**) Calibration graph for the formed STZ-RDN azo compound over the concentration range of 0.08–2.0 µg mL^{-1} (0.08, 0.16, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 µg mL^{-1}) for the NO_2^- ion.

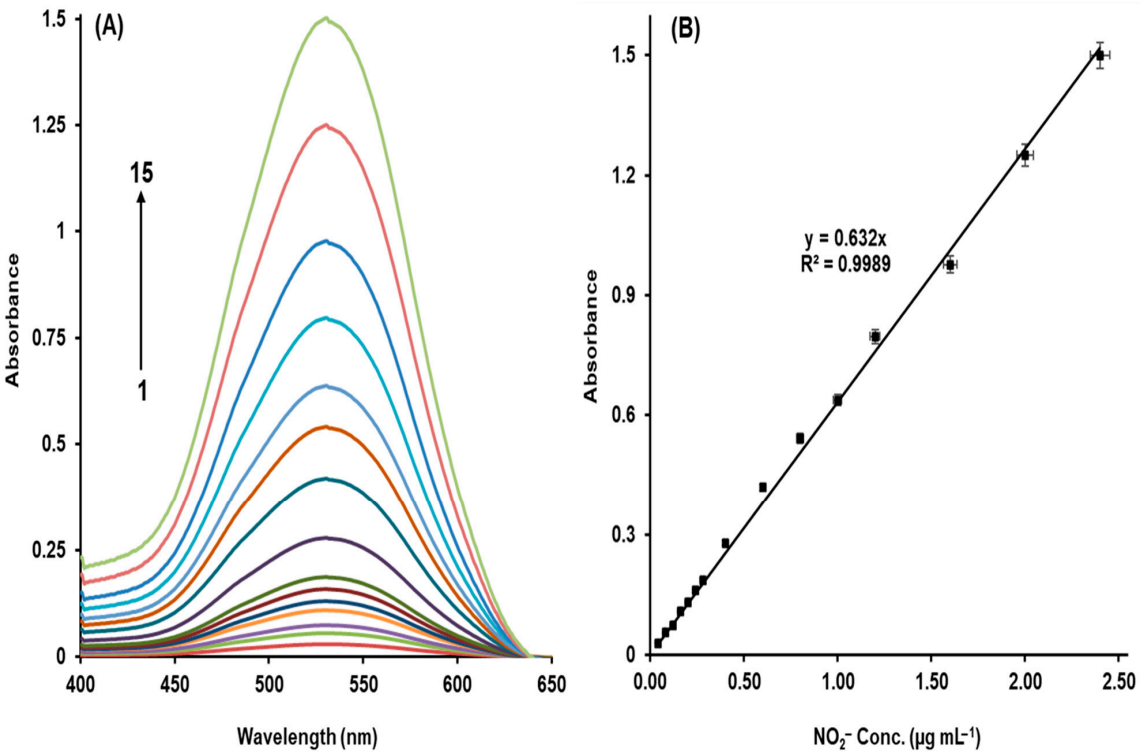


Figure 6. (**A**) Absorption spectra of diazotized STZ-7-HC red product at λ_{max} = 525 nm in the presence of different NO_2^- concentrations, varying from 0.04 to 2.4 µg mL^{-1}. (**B**) Calibration graph for the formed STZ-7-HC azo compound over the concentration range of 0.04 to 2.4 µg mL^{-1} (0.04, 0.08, 0.12, 0.16, 0.2, 0.24, 0.28, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0, 2.4 µg mL^{-1}) for the NO_2^- ion.

Table 1. The characteristic statistical data for the determination of NO_2^- with RDN and 7-HCN.

Parameters	Characteristic	
	Method A: RDN	**Method B: 7-HCN**
Color	Pale red	Red
λ_{max} (nm)	504	525
Beer's law range (μg mL^{-1})	0.08–2.0	0.04–2.4
Molar absorptivity (ε; L mol^{-1} cm^{-1}) × 10^4	4.20	6.90
Sandell's sensitivity (μg cm^{-2}) × 10^{-6}	1.63	1.00
Detection limit (LLOD; μg mL^{-1})	0.0303	0.0387
Quantification limit (LLOQ; μg mL^{-1})	0.0918	0.1172
Correction coefficient (R^2)	0.9993	0.9983
Slope	0.6033	0.6206
Standard deviation of slope (S_b) × 10^{-3}	4.9	7.0
Standard deviation of intercept ($S_{y/x}$) × 10^{-3}	5.6	7.3
Standard deviation	0.011	0.0197
Relative standard deviation (RSD)	2.009	2.1896
Intra-day precision (n = 5), recoveries (%) for studied NO_2^- concentrations *	95.12–99.32	95.75–98.82
Inter-day precision, 5 days, (n = 25) recoveries (%) for studied NO_2^- concentrations *	94.58–99.65	95.23–99.45
Intra-day precision (n = 5), RSD (%) for studied NO_2^- concentrations *	2.45	2.75
Inter-day precision, 5 days, (n = 25) RSD (%) for studied NO_2^- concentrations *	4.25	4.65

* Studied NO_2^- concentrations are 0.5, 1.0, and 1.5 μg mL^{-1}.

Since the calculated LLOD falls below the maximum contamination threshold for NO_2^- in drinking water defined by the WHO (6.5×10^{-5} M (3.0 μg mL^{-1})) [20] and U.S. EPA (2.17×10^{-5} M (1.0 μg mL^{-1})) [22], our suggested spectrophotometric procedures have a considered and real practical application value in order to carry out NO_2^- quantification in fresh and drinking water sources.

3.5. Interference Study

Either using RDN or 7-HC as chromogenic reagents for the determination of NO_2^-, under the achieved optimal circumstances, the selectivity of the recommended methods was studied by the determination of 0.4 μg mL^{-1} NO_2^- in coexistence with various ions that may exist with NO_2^- in the same sample. The permissible limit was defined based on the concentration of ions causing interference. The experimental findings confirmed the absence of significant interference from the examined anions and cations. Accordingly, the proposed spectrophotometric techniques demonstrate high selectivity toward NO_2^- quantification. Table 2 shows the results for such additives.

Table 2. Effect of interfering ions on the determination of 0.4 μg mL^{-1} NO_2^- ions using RDN or 7-HC as chromogenic reagents.

Foreign Ion	Tolerance Limit (μg mL^{-1})
Na^+, K^{+1}, Ca^{+2}, Pb^{+2}, Mg^{+2}, Al^{+3}, Ti^{+4}, EDTA	10,000
SO_4^{-2}, F^-, Br^-, Cl^-, CO_3^{-2}, PO_4^{-3}, NO_3^-	10,000
Bi^{+3}, Ba^{+2}	1000
Ni^{+2}, Co^{+2}, Mn^{+2}, Cr^{+3}	500
Sr^{+2}, Zn^{+2}, Fe^{3+}, Cu^{2+}	100

3.6. Application of the Proposed Methods

To investigate the appropriateness of the recommended procedures for NO_2^- determination in real samples, it was applied to tap water, wastewater, and soil samples. The

collected analysis results are tabulated in Table 3, which shows that the proposed method is accurate, and the results revealed very good recovery and excellent accommodation compared to the results obtained by the reference method [72].

Table 3. Application of the proposed methods for the determination of NO_2^- in tap water, wastewater, and soil.

Proposed Methods	Sample	NO_2^- Added (μg mL^{-1})	Proposed Method		Standard Method		*t*-Test	*F*-Test
			NO_2^- Found * (μg mL^{-1})	Relative Error (%)	NO_2^- Found * (μg mL^{-1})	Relative Error (%)		
Method A: RDN	Tap water	0.2	0.202	1.00	0.203	1.50	0.419	1.53
		0.4	0.403	0.75	0.402	0.50	1.23	3.55
		0.6	0.605	0.83	0.604	0.66	1.40	4.11
	Waste-water	0.3	0.303	1.00	0.302	0.66	0.85	1.63
		0.5	0.504	0.80	0.505	1.00	1.60	4.12
		0.7	0.705	0.71	0.704	0.57	1.25	2.43
	Soil	1.0	0.982	−1.80	1.01	1.00	0.894	4.24
		1.2	1.22	1.60	1.23	2.14	1.40	2.45
		1.4	1.42	1.40	1.44	2.85	2.44	1.23
Method B: 7-HC	Tap water	0.2	0.221	0.25	0.198	−0.90	2.236	1.00
		0.4	0.407	1.80	0.418	4.50	2.00	1.20
		0.6	0.596	0.66	0.606	1.00	1.40	2.70
	Waste-water	0.3	0.303	1.00	0.302	0.66	0.85	1.63
		0.5	0.504	0.80	0.505	1.00	1.60	4.00
		0.7	0.705	0.71	0.704	0.57	1.25	2.41
	Soil	0.4	0.403	1.50	0.402	0.50	1.63	4.00
		0.6	0.596	0.66	0.606	1.00	1.40	2.70
		0.8	1.810	0.12	0.826	3.25	2.236	2.25

* Average of five determinations.

3.7. Comparison with Previously Reported Methods

To confirm the effectiveness and suitability of our proposed methods, we made a comparison between analytical information obtained from our proposed methods and other spectrophotometric methods previously introduced for NO_2^- determination. The information for this comparison is shown in Table 4. The tabulated data proved that our results are much better than most spectrophotometric methods or even very close to some of them. The listed spectrophotometric methods often possess some drawbacks, including reaction time, pH dependence, and the use of large volumes of amines, and sometimes, they are carcinogenic and toxic. Moreover, tabulated comparison data show that some references report lower linearity ranges and LLOD values than those obtained in our study, but these references have their own limitations [37–40,44,54,55]. Conversely, our proposed methods are simple, rapid, and low-cost, requiring minimal sample preparation while offering a satisfactory linearity range and high sensitivity, as they do not require special instruments, cumbersome extraction steps, or even expensive chemicals.

Table 4. Comparison of the proposed spectrophotometric methods and other spectrophotometric methods utilized for NO_2^- determination.

Reagent	Linearity Range *	LOD *	LOQ *	ε *	λ_{max}	Remarks	Refs.
STZ ** + RDN **	0.08–2.00	0.0303	0.0918	4.20	504	Simple, rapid, non-extractive, highly sensitive, and stable	This work
STZ ** + 7-HC **	0.04–2.40	0.0387	0.1172	6.90	525		This work
AHNDMS **	0.1–1.6	0.0069	0.021	2.60	560	Susceptibility to interference from some metal ions (particularly Fe^{3+})	[37]

Table 4. *Cont.*

Reagent	Linearity Range *	LOD *	LOQ *	ε *	λ_{max}	Remarks	Refs.
Barbituric acid	0.00–3.22	0.0166	0.05478	1.53	310	Time-consuming; 30 min before NaOH addition	[38]
Safranin O + PG **	0.002–0.23	0.0005	NA	0.40	610	Require CPE ** using mixed micelle of a nonionic surfactant	[39]
Cefixime + 1-naphthyl amine	0.02–15.00	0.0043	0.014	0.41	360	Time-consuming (max. absorption was reached at 30 min. after mixing)	[40]
PNA ** + EAA **	0.05–6.00	0.05	0.15	1.59	507	Less sensitive	[41]
SAA ** + EAA **	0.2–3.0	0.067	0.20	1.22	356	Less sensitive	[41]
STZ ** + NEDA **	0.054–0.816	0.018	0.054	4.61	546	Interference with higher thiosulfate and sulfite concentrations	[42]
MTL ** + PG **	2.50–30.00	1.60	5.28	0.538	385	Less sensitive	[43]
SCL ** + PG **	2.50–30.00	2.00	6.60	0.331	385	Less sensitive	[43]
TNL ** + PG **	2.50–30.00	1.80	5.94	0.366	385	Less sensitive	[43]
3-nitroaniline + 1-naphthylamine	0.01–1.70	0.0005	0.00165	3.12	515	Utilizing strongly acidic medium and multi-reagent diazotization coupling system	[44]
Sodium-3-mercapto-1-propanesulfonate	3.45–5519.2	2.07	5.52	0.129	547	Less sensitive	[45]
DLLME ** + preconcentration then Griess color	0.1–7.0	0.05	0.10	0.86	410	Less sensitive	[46]
Sulfanilic acid + methyl anthranilate	0.2–8.00	0.93	2.82	1.03	493	Less sensitive	[47]
PNA ** + ethoxy ethylenemaleic ester	0.5–16.00	0.07	0.21	5.04	439	Less sensitive	[48]
PNA ** + ethylcyanoacetate	0.2–18.00	0.05	0.15	1.21	459	Less sensitive	[48]
Sulfanilic acid + resorcinol	0.2–2.6	0.003	0.009	3.22	385	Less sensitive	[49]
Redox reaction with iodide ions in an acidic condition	0.0625–4.00	0.025	0.085	NA	362	Time consuming	[50]
SAA ** + PNZ **	0.13–1.0	0.08	0.264	3.48	540	Less sensitive	[51]
SMX ** + CPN **	0.19–1.0	0.09	0.297	2.57	530	Less sensitive	[51]
SAA 3 + TPN **	0.3–1.6	0.12	0.396	1.60	530	Less sensitive	[51]
MMCBAT ** + N,N-dimethyl aniline	0.05–2.00	0.012	0.0396	2.03	482	Less sensitive	[52]
PNA ** + FRU **	0.02–0.6	0.485	1.470	0.146	680	Less sensitive	[53]
PNA ** + MPAT **	0.4–2.0	0.559	1.695	0.331	395	Less sensitive	[53]
SAA ** + Orcinol	0.005–1.80	0.003	0.009	4.36	427	Very close or lower **(ε)** values	[54]
NO_2^- catalytic effect on oxidative degradation of [BMIM]MR ** by $KBrO_3$	0.006–0.287	0.001	0.003	NA	518	Expensive and require a complex procedure due to using IL ** and modified MR **; interference with higher concentration of regular interferent	[55]

* Linearity range (μg mL^{-1}); limit of detection (μg mL^{-1}); limit of quantification (μg mL^{-1}); molar absorptivity (ε) × 10^4 (L mol^{-1} cm^{-1}). ** 7-HC: 7-Hydroxycoumarin; AHNDMS: 4-amino-5-hydroxy naphthalene-2,7-disulphonic acid mono sodium salt; CPE: cloud point extraction; CPN: 2-chlorophenoxazine; DLLME: dispersive liquid-liquid microextraction; EAA: ethyl acetoacetate; FRU: frusemide; MMCBAT: 4-(1-methyl-1-mesitylcylobutane-3-yl)-2-aminothiazole; MPAT: 5-methyl-4-{[(1*E*)-phenyl methylene]amino}-2,4-dihydro-3H-1,2,4-triazole-3-thione; MTL: metronidazole; NEDA: N-(1-naphthyl)ethylenediamine dihydrochloride; PG: pyrogallol; PNA: *p*-nitroaniline; PNZ: phenoxazine; RDN: rhodanine; SAA: sulfanilamide; SCL: secnidazole; SMX: sulfamethoxazole; STZ: sulfathiazole; TNL: tinidazole; TPN: 2-Trifluoromethylphenoxazine; [BMIM]MR: 1-butyl-3-methylimidazolium-modified methyl red; $KBrO_3$: potassium bromate; IL: ionic liquids; MR: methyl red.

3.8. Greenness Assessment

Recently, there has been a growing global trend to develop new assessment methods, which in turn has become an essential requirement in contemporary research [73,74]. The core objective of these assessment methods is to prove that the proposed analytical method is eco-friendly, since it is executed using fewer hazardous chemicals, safer procedures, and still maintains remarkable analytical performance. To appraise the greenness of the analytical approaches, several evaluation metrics have been devised, including the Analytical Eco-Scale Assessment (ESA) [75], the Analytical GREEnness metric (AGREE) [76], the Green Analytical Procedure Index (GAPI) [77], and the National Environmental Methods Index (NEMI) [78]. Herein, the greenness of our proposed analytical procedures was investigated utilizing ESA, AGREE, and GAPI.

3.8.1. Analytical Eco-Scale Assessment (ESA)

On the analytical Eco-Scale Assessment (ESA), it is postulated that the ideal analytical method would achieve 100 points, and each departure from this ideality is designated as a penalty that is subtracted from a perfect score of 100, lowering the calculated final score for the proposed method. In the ESA system, there are numerous determinants, such as the type and quantity of used chemicals, energy consumption by the utilized electric instruments, waste formation and its processing, and finally, occupational hazards [75]. ESA is not graphically exemplified; instead, it is represented by tabulated numerical values, with a maximum score of 100. If the acquired score is greater than 75, it is regarded as an excellent green analysis; a score in the range of 50–74 is evidence for an acceptable green analysis, and finally, a score below 50 suggests inadequate green analysis. Based on these basic scores, the penalty points are calculated for the proposed spectrophotometric methods for NO_2^- determination either using RDN (Method A) or 7-HC (Method B). As shown in Table 5, the calculated PPs were found to be 87 for the two proposed spectrophotometric methods. Upon this, the proposed analytical methods qualify as outstanding green analytical methods.

Table 5. Calculated PPs score according to the Analytical Eco-Scale (ESA) for the proposed spectrophotometric procedures for the quantification of NO_2^-.

Method A: RDN		**Method B: 7-HC**	
Item	**Calculated PPs**	**Item**	**Calculated PPs**
Chemicals		Chemicals	
Water	0	Water	0
HCl	2	HCl	2
$NaNO_2$	2	$NaNO_2$	2
STZ	1	STZ	1
RDN	1	7-HC	1
NaOH	2	NaOH	2
KCl	0		
Instrument energy		Instrument energy	
UV–Vis spectrophotometer	0	UV–Vis spectrophotometer	0
pH meter		pH meter	
Occupational hazard	3	Occupational hazard	3
Waste	2	Waste	2
ESA score	13	**ESA score**	13

3.8.2. Analytical GREEnness Metric (AGREE)

The Analytical GREEnness metric (AGREE) is an extensive metric framework in which the 12 precepts of green analytical chemistry are deemed to appraise greenness

with respect to the presented analytical method. The ultimate score is displayed in the midpoint of 12 segmented pictograms, with each segment designated with a particular weight and color and associated with the analytical procedure's performance. If the final score approaches 1, the segmented pictogram center acquires a darker green hue, implying a superior environmentally friendly procedure [76]. As indicated by the AGREE metrics, both proposed spectrophotometric procedures for NO_2^- determination received a typical score of 0.61, with a segmented pictogram having a centered green color (Figure 7A). Also, the resulting pictogram for both suggested procedures was identical; the color for the individual segment in the pictogram related to Method A is identical to the color for the corresponding segment in the pictogram related to Method B. This similarity can be attributed to the use of the same chemicals, albeit in different quantities, in both proposed spectrophotometric procedures. Also, the use of RDN in Method A and 7-HC in Method B showed the same color in related segments since both have approximately the same hazardous effect. So, the proposed two spectrophotometric procedures are considered as green methods for NO_2^- quantification.

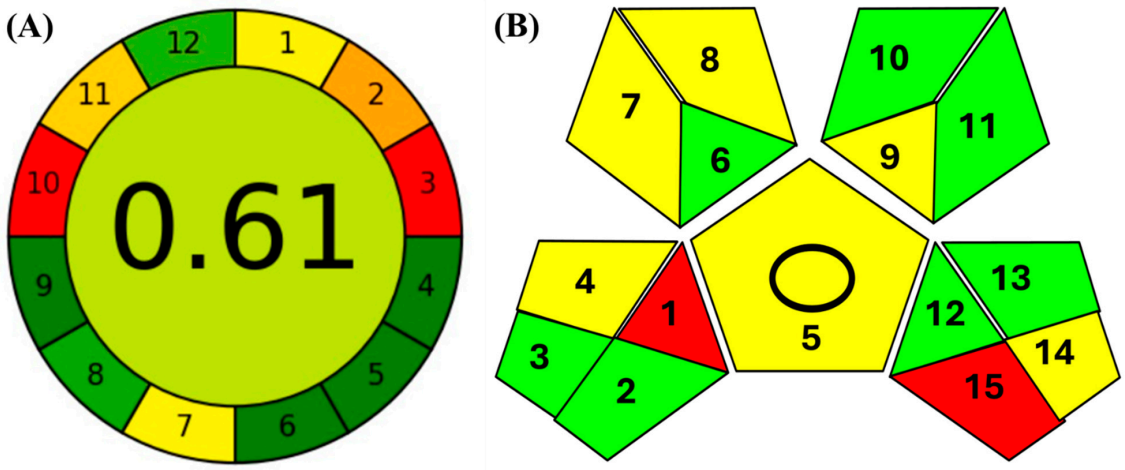


Figure 7. Pictogram of (**A**) AGREE and (**B**) GAPI greenness assessment methods for both spectrophotometric procedures proposed for the quantification of NO_2^-.

3.8.3. Green Analytical Procedure Index (GAPI)

The Green Analytical Procedure Index (GAPI) is a late devised tool that assesses the environmental significance of a proposed method, encompassing every step included in assessed methods, starting from sample gathering to completing the analysis. The obtained outcomes are displayed in five pentagrams, each partitioned into subsections that exemplify diverse facets of the proposed methods: for instance, sample processing stages, amounts and risks of used chemicals, and utilized instrumentation specifications and requirements. Each segment is color-coded: green for low, yellow for medium, and red for high environmental impact [77]. The greenness assessment using GAPI is depicted in Figure 7B. We can assume that the resulting pictogram can be used to express the analytical greenness of both proposed spectrophotometric procedures for NO_2^- determination. The result of the GAPI assessment showed seven green color segments and six yellow color segments, while the last two segments are shown in red color. The first red segment (1) is a result of utilizing offline sampling in research labs, where implementing online or inline approaches is challenging. The second red segment (15) is attributable to the absent wast treatment.

Based on the greenness assessment methods performed in the current article, we can confirm that the strength of our proposed spectrophotometric procedures lies in the use of small quantities of chemicals (since the total volume of the mixture is 10 mL) and the low

energy required for the instruments used (UV-vis spectrophotometer and pH meter), with no heating required. Also, a significant weakness of the proposed method is the lack of a procedure for handling chemical waste from the proposed experiments (even though the waste quantities are less than 10 mL) since the used chemicals have a toxic effect, such as NO_2^-, or a corrosive effect, such as HCl or NaOH.

Finally, the results obtained from greenness assessment using three different methods (ESA, AGREE, and GAPI) proved that the proposed spectrophotometric methods for NO_2^- determination are characterized as moderate-to-good green methods of analysis.

4. Conclusions

In summary, we present two procedures for the spectrophotometric quantification of NO_2^- in environmental specimens without the necessity for pretreatment or extraction. These methods were built upon a diazotization reaction of sulfathiazole, followed by a coupling reaction with RDN or 7-HC. The proposed procedures have significant advantages over several spectrophotometric methods found for NO_2^- determination since they were found to be rapid, sensitive, selective, cost-effective, and environmentally friendly. According to the performed Greenness Assessments (ESA, AGREE, and GAPI), the proposed procedures were characterized as moderate-to-good green methods of analysis. The proposed procedures gave a satisfactory result when executed with respect to the NO_2^- determination of NO_2^- in water and soil specimens.

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