

## Detection of Nitrite in Water Using Minoxidil as a Reagent

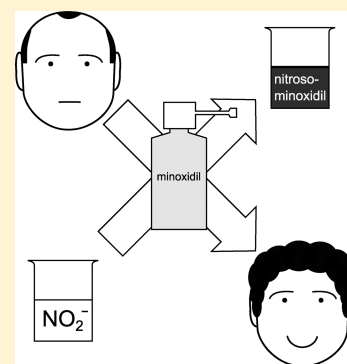
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### Supporting Information

**ABSTRACT:** Water analysis is one of the most important issues in environmental chemistry. The increasing scale of water contamination owing to the presence of nitrate and nitrite in the environment has converted it into one of the most serious public health problems in modern society. Here an easy colorimetric method for determining nitrite in water is reported. The method is based on the reaction of nitrite with minoxidil in acidic media, which gives nitrosominoxidil as a product that shows an absorption band in the  $\lambda = 315\text{--}330$  nm range. Minoxidil was the first drug approved by the U.S. Food and Drug Administration as a treatment against alopecia (hair loss), thus leading minoxidil to be one of the most popular and commonly used drugs that can be purchased freely at low cost on the market in the form of topical solutions. The experiment can be completed over 3.5 h, and it can be extended to include a kinetic mechanistic study of the nitrosation reaction. The nitrite detection range makes the method suitable for environmental, food, and physiological analytical applications. By using a brand-name product, student curiosity and interest is kept high throughout the experiment. Finally, questions are provided in the student handout, requiring the students to engage further in topics associated with the context of this practical work.

**KEYWORDS:** Second-Year Undergraduate, Analytical Chemistry, Environmental Chemistry, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Hands-On Learning/Manipulatives, Physical Chemistry, Water/Water Chemistry, UV-Vis Spectroscopy



Colorimetric determinations are commonly selected as undergraduate chemistry experiments because they offer simplicity and reliable results.<sup>1–3</sup> Using a spectrophotometer is one of the fastest and easiest methods to measure unknown concentrations of a sample substance. The colorimetric experiment presented in this work allows students to detect and quantify nitrite in water. The “real-world” application and use of a brand-name product maintain high student interest throughout the experiment. We believe the work proposed here can be useful as a pedagogical tool to enhance the curiosity, prediction capacity, and creativity of students. At the same time, it helps the students gain a deeper experimental and theoretical kinetic understanding in the laboratory.

Water analysis is one of the most important issues in environmental chemistry, as well as in analytical and general chemistry.<sup>4–7</sup> Two common pollutants are the nitrate and nitrite ions, especially in agricultural areas with high concentrations of nitrate, where some bacteria can reduce it to nitrite.<sup>8</sup> The major sources of nitrite in drinking water are runoff from fertilizer use, leaching from septic tanks, sewage, and the erosion of natural deposits.

The increasing scale of water contamination due to the presence of nitrate in the environment presents a serious public health problem in modern society. Water tests have been strongly recommended for households with infants (blue-baby syndrome), pregnant women, nursing mothers, or elderly people.<sup>9,10</sup> Nitrite can also react with amines present in food to form carcinogenic nitrosamines.<sup>11,12</sup> Both the U.S. Environmental Protection Agency (EPA) and the European Union

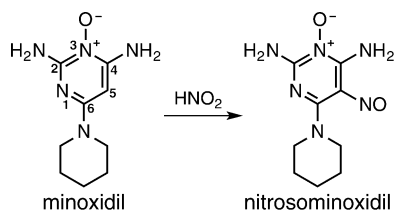
(EU) have set a maximum for the levels of nitrite in drinking water. The EPA maximum contaminant level (MCL) is 1 ppm ( $1.45 \times 10^{-5}$  M).<sup>13</sup> The EU has set the maximum admissible concentration 1 order of magnitude lower at 0.1 ppm ( $1.45 \times 10^{-6}$  M).<sup>14</sup>

Currently, laboratory techniques such as HPLC, potentiometric ion-selective electrodes, and colorimetric assays are used to detect nitrite. The Griess–Ilosvay reagent (a mixture of sulfanilic acid, 1-naphthylamine, and acetic acid) is frequently used to measure the concentration of nitrite in water samples.<sup>15,16</sup> Although this reagent forms a colored compound with nitrite, the product forms after a series of four steps. First, acetic acid converts nitrite into a nitrosating agent. This agent then nitrosates sulfanilic acid, forming a diazonium salt that couples with 1-naphthylamine to afford the colored compound.<sup>17</sup> The fact that this reaction takes many steps and involves a highly unstable diazonium salt has encouraged the search for new colorimetric methods of analysis. An alternative is the direct nitrosation of aromatic rings, which can also be followed by colorimetry.<sup>18</sup> Because nitrosating agents are weak electrophiles, the aromatic rings must be activated by electron-rich substituents.<sup>19</sup>

Minoxidil (6-(1-piperidinyl)-2,4-pyrimidinediamine 3-oxide, CAS 38304-91-5), shown in Scheme 1, was the first drug approved by the U.S. Food and Drug Administration (FDA) as a treatment against alopecia (hair loss).<sup>20</sup> Minoxidil soon

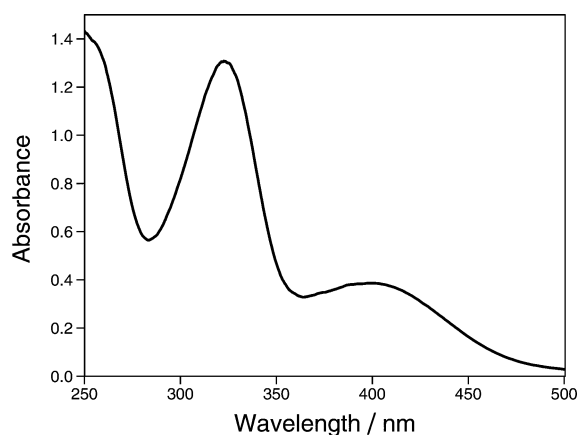
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## Scheme 1. Nitrosation Reaction of Minoxidil



became one of the most popular and commonly used drugs that could be purchased freely at low cost in the form of a topical solution.<sup>21</sup> The most well-known of these on the market is Rogaine. Rogaine is a 2% or 5% solution of minoxidil in ethanol (68–65%), propylene glycol (10%), and water (20%).

The minoxidil nitrosation reaction, shown in Scheme 1, is proposed as a colorimetric experiment for determining nitrite in water. The product has an intense yellow color and shows an absorption band from 315 to 330 nm with  $\lambda_{\text{max}}$  at 325 nm (Figure 1), making it easy to measure the concentration of



**Figure 1.** UV-vis spectrum of nitrosominoxidil: [nitrosominoxidil] =  $1.25 \times 10^{-4}$  M, pH 4.08,  $T = 25.0$  °C.

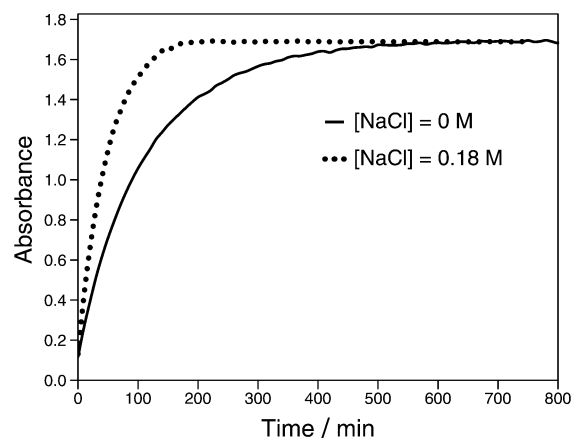
nitrite in water. The molecular structure of minoxidil suggests that this substance undergoes carbon-nitrosation at its C-5 position (Scheme 1).<sup>22</sup> The reaction conditions are optimal at pH 4 because the nitrite decomposition reaction is negligible. With an excess of minoxidil ( $\text{p}K_{\text{a}} 4.6$ ) a buffer is unnecessary. Because halides catalyze the nitrosation reaction (Figure 2), the addition of sodium chloride (NaCl) in a sufficient quantity permits good control over the duration of the lab work, allowing students a suitable amount of lab time to acquire data.

## EXPERIMENTAL DETAILS

### Standard Solutions

The following standard solutions were prepared by the students working in pairs: (a) minoxidil  $3.9 \times 10^{-3}$  M, this solution (25-fold dilution) was prepared from a commercial 2% minoxidil topical solution; (b) hydrochloric acid  $3.4 \times 10^{-2}$  M; and (c) sodium chloride 4.1 M. This basic reagent (BR) was prepared with a mixture of 10 mL, 1 mL, and 1 mL of a, b, and c, respectively. The final concentration of each reagent is given in the figure captions.

When performing this practical work a Shimadzu UV-2401-PC spectrophotometer and a Hanna Instruments pH-211

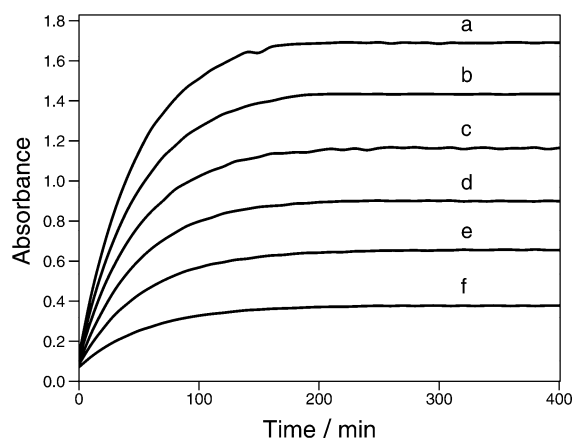


**Figure 2.** Catalytic effect of the  $\text{Cl}^-$  ion on the reaction time of minoxidil nitrosation: [minoxidil] =  $1.74 \times 10^{-3}$  M,  $[\text{NaNO}_2] = 1.42 \times 10^{-4}$  M, pH 4.01,  $T = 25.0$  °C,  $\lambda = 325$  nm.

instrument ( $\pm 0.01$ ) were used. Any other comparable equipment would be equally suitable.

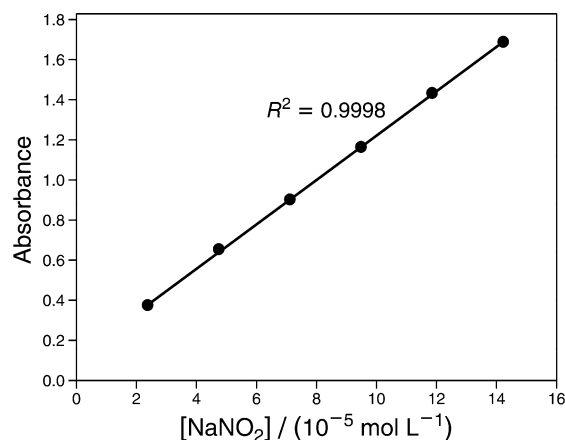
### Calibration Curve

Six different solutions with concentrations ranging from  $5.0 \times 10^{-5}$  to  $3.0 \times 10^{-4}$  M were prepared by the students using a standard  $1.0 \times 10^{-3}$  M  $\text{NaNO}_2$  solution. To create the calibration curve 10 mL from each solution was mixed with 12 mL of BR. The absorption ( $\lambda = 325$  nm) over time was recorded (Figure 3). Each solution was prepared and measured by a pair of students.



**Figure 3.** Typical student kinetic runs for the nitrosation of minoxidil: pH 4.01–4.03,  $T = 25.0$  °C, [minoxidil] =  $1.74 \times 10^{-3}$  M,  $[\text{NaCl}] = 0.18$  M,  $[\text{NaNO}_2] =$  (a)  $1.42 \times 10^{-4}$  M, (b)  $1.18 \times 10^{-4}$  M, (c)  $9.48 \times 10^{-5}$  M, (d)  $7.11 \times 10^{-5}$  M, (e)  $4.74 \times 10^{-5}$  M, and (f)  $2.37 \times 10^{-5}$  M,  $\lambda = 325$  nm.

The absorbances were measured when the plateau was reached, and with the respective concentrations a calibration curve (Figure 4) was obtained that shows an excellent proportionality between absorbance and concentration (Beer–Lambert law;<sup>23</sup> see the Supporting Information). The slope of the curve was determined to be  $11,040 \pm 70$   $\text{L mol}^{-1} \text{cm}^{-1}$ , and the y intercept was  $0.120 \pm 0.007$ . The y intercept is not zero because minoxidil absorbs slightly at  $\lambda_{\text{max}}$ .



**Figure 4.** Student calibration curve for nitrosominoxidil:  $[\text{minoxidil}] = 1.74 \times 10^{-3} \text{ M}$ ,  $[\text{NaCl}] = 0.18 \text{ M}$ ,  $[\text{NaNO}_2] = 2.37 \times 10^{-5} - 1.42 \times 10^{-4} \text{ M}$ , pH 4.01–4.03,  $T = 25.0 \text{ }^\circ\text{C}$ ,  $\lambda = 325 \text{ nm}$ .

### Analysis of Well Water

Samples of water were collected by the authors from a well in the province of Ávila, Spain, filtered and stored in polyethylene plastic bottles with no further pretreatment, and given to the students. For analysis, 10 mL of the sample was mixed with 12 mL of BR. A nitrite concentration of  $2.51 \pm 0.02 \text{ ppm}$  was measured by the instructor. The students' results were in the range 2.47–2.54 ppm. These results are in excellent agreement with that obtained with Zambelli's reagent ( $2.48 \pm 0.06 \text{ ppm}$ ), which is the Spanish Standard Method for the determination of nitrites.<sup>24,25</sup> All data recorded here were obtained at room temperature.

### HAZARDS

Hydrochloric acid causes severe skin burns and eye damage. It emits hydrogen chloride gas, which presents a serious inhalation hazard, and a fume hood should be used. Minoxidil topical solution is flammable because it contains ethanol, and thus, flames and sources of ignition should not be used in the laboratory. Minoxidil and sodium nitrite are harmful if swallowed. Regarding the hazardous characteristics and storage requirements of  $\text{NaNO}_2$ , see ref 26. A motivating article for students can be seen in ref 27. Specific safety considerations are described in the Supporting Information.

### FINAL REMARKS

The experiment can be done within a 3.5 h laboratory period once the solutions have been prepared. The method is simple and accurate. The required materials are inexpensive and commonly available. The assay detection range (0.62–12.42 ppm or  $9 \times 10^{-6}$  to  $1.8 \times 10^{-4} \text{ M}$ ) is suitable for environmental, food, and physiological analytical applications.<sup>28</sup> The experiment may be extended to add a kinetic study of the nitrosation reaction including reaction rates, activation parameters, reaction mechanism, and so forth.<sup>22</sup>

### ASSOCIATED CONTENT

#### Supporting Information

Notes for the instructors; student handout. This material is available via the Internet at <http://pubs.acs.org>.

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#### Notes

The authors declare no competing financial interest.

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