

Influence of ultrasonic waves in the reduction of nitrate to nitrite by hydrazine–Cu(II)

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Abstract

Colorimetric methods are still important for determining nitrate and nitrite. A critical step in the use of these methods to determine nitrate in low concentrations is the reaction time required to totally reduce nitrate to nitrite, i.e., 24 h in the dark. This work involved a study of the influence of ultrasonic irradiation on the nitrate reduction reaction by hydrazine. Our findings indicated that ultrasonic irradiation, associated with copper(II) ion as a catalyst, increased the redox reaction rate, decreasing the reaction time to about 10 min when the power of the ultrasonic irradiation was set in 14.0357 W. The strong influence of the ultrasonic irradiation in the reduction reaction rates can be sustained by an excellent linear correlation ($R^2 = 0.9993$) between the kinetic constants and ultrasonic powers. Nitrate conversion also increased from 68% to 98% at the latter conditions. It thus become clear that high intensity ultrasound is very beneficial for this reduction reaction to proceed in good yield and in short reaction time in comparison to its silent reaction.

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1. Introduction

Nitrate and nitrite are commonly present in the environment. Therefore, determining nitrate in samples taken from the environment is very important, for it involves the quality of drinking water, effluent control, atmospheric aerosol, and acid deposition. Nitrate compounds are highly soluble in water [1], acting as the principal reservoir of combined nitrogen in oceans and exerting a controlling influence on marine life [2–4]. High nitrate levels in rivers lead to increased growth of algae, which, in turn, decreases the level of biologically available oxygen, impairing the ecological balance in rivers and lakes and posing risks to human health. The health hazards include the risk of methemoglobinemia in young infants and of gastric cancer,

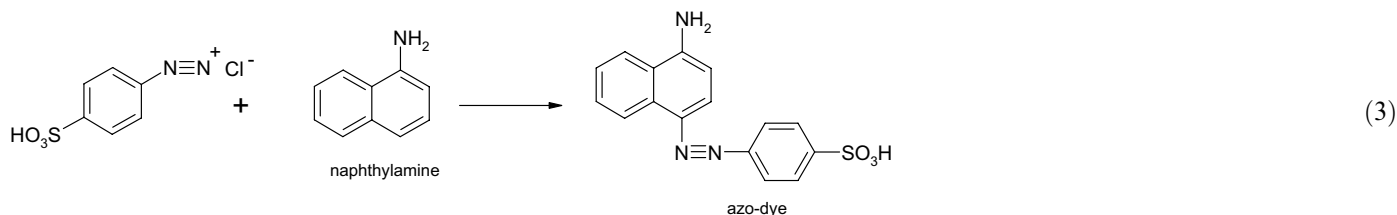
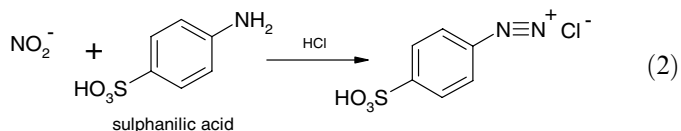
particularly in certain high-risk patient groups. Moreover, sulfate and nitrate are known to be the dominant inorganic species in acidic precipitation, and nitric acid plays a uniquely important role in atmospheric chemistry [5]. Also, nitrate and nitrite are usually added to processed meat products to protect against microorganisms that can cause food poisoning, such as *Clostridium botulinum* [2].

Nitrite can be simply and accurately determined by the highly sensitive colorimetric method. Therefore, nitrate in low concentrations is usually determined by reducing it to nitrite using hydrazine and copper(II) ion catalysts in an alkaline solution, followed by diazotization and a coupling reaction to form an azo dye (reactions Eqs. (1)–(3)), which can be readily measured spectrophotometrically [3–7].



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A critical step in this process is the reduction of nitrate to nitrite. The main problem involved is the reaction time required to completely reduce nitrate, which is 24 h in the dark [3]. Although this method is commonly employed, its use is often impractical. An alternative method is the enzymatic conversion of nitrate to nitrite with nitrate reductase, which is efficient in 5 min [8,9], or the use of energy such as ultrasonic irradiation to hasten the reduction of nitrate to nitrite using a hydrazine–Cu reagent.

Ultrasonic energy using ultrasonic cleaning baths has been employed in analytical chemistry for bottle cleaning and in several different applications [10], such as extraction [11–13], reagent generation [14–19] and to improve mass transport in solution and reaction rates [17,20,21]. Ultrasonic energy has also been used in conjunction with the reduction of the internal pressure of reaction vessels to obtain salts with a high degree of purity [22,23]. Thus, we studied the influence of ultrasonic irradiation on the nitrate reduction reaction using hydrazine, which involved a series of kinetic experiments aimed at shedding light on the influence of the ultrasonic effects on this reaction.

2. Experimental procedure

2.1. Materials

All the solutions were prepared with analytical grade reagents and distilled water further purified in an E-pure system (Barnstead). Fresh solutions of KNO_3 , KNO_2 , and hydrazine–copper reagent (2.0 g l^{-1} of hydrazine hydrate + 1.4 g l^{-1} of CuCO_3) were used. The analytical curves for nitrate and nitrite were made using stock solutions of KNO_3 [$100 \text{ mg N}(\text{NO}_3^-) \text{ l}^{-1}$] and KNO_2 [$100 \text{ mg N}(\text{NO}_2^-) \text{ l}^{-1}$], respectively. The reactions were carried out at pH 9.6 using a sodium phenate buffer.

2.2. Apparatus

The concentrations of nitrite and nitrate were determined using an ion chromatograph (IC) Dionex (Model

4000 (i) equipped with a Rheodyne injector with a $250 \mu\text{l}$ sample loop and a conductivity detector at $30 \mu\text{S}$. The analytical column was an ION PAC AS4A, whose use was followed by a Dionex AMMS-1 fibrous micromembrane

suppressor. A $2.0 \text{ mmol l}^{-1} \text{ Na}_2\text{CO}_3$ + $0.75 \text{ mmol l}^{-1} \text{ NaHCO}_3$ eluant was used at a flow rate of 1.0 ml min^{-1} . Under these conditions, the peak height ratio ($\text{NO}_2^-/\text{NO}_3^-$) for a 0.6 mg N l^{-1} standard solution was 1.1. An Arruda model SX-10 (Brazil) ultrasonic cleaning bath and a probe VibraCell VC-130 (Sonics Instruments, USA) were employed to evaluate the reaction time when exposed to 20 kHz ultrasonic waves.

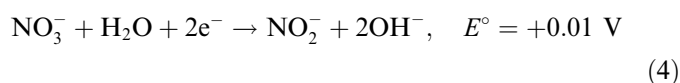
2.3. Procedure

Aliquots of 40 ml of the nitrate solution [$0.6 \text{ mg N}(\text{NO}_3^-) \text{ l}^{-1}$] with 2 ml of sodium phenate buffer (pH 9.6) were reacted with 1 ml of the hydrazine–Cu(II) reagent. The reactions were conducted in the dark under the following conditions: at room temperature and 40°C without ultrasonic irradiation, at room temperature under ultrasonic irradiation using an ultrasonic cleaning bath and at room temperature using an ultrasonic probe set in different powers.

For each condition, aliquots of $250 \mu\text{l}$ were taken at regular intervals over a period of 200 min, injected into the chromatographic system and the signals of nitrate and nitrite measured. The concentrations of nitrate and nitrite were determined based on the corresponding analytical curves. The experiments were repeated three times for each condition and showed very similar results.

3. Results and discussion

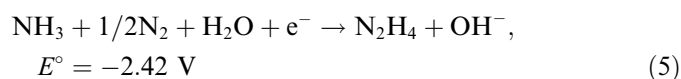
Nitrate ion has a high chemical stability, especially at low levels of concentration. Standard reduction potentials indicate that it should serve as an excellent oxidizing agent, but special conditions such as catalysts are required to make it react with suitable reducing agents. The reduction of nitrate to nitrite is described by the following reaction [24]:



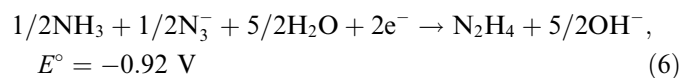
Hydrazine–Cu(II) reducing agent is usually prepared by mixing hydrazine sulfate solution with copper sulfate catalyst and the nitrate reduction is done at pH 9.6 by adding a phenate buffer solution [3]. However, we avoided using sulfate here because sulfate peaks can interfere in chromatographic analyses. But even using hydrazine hydrate and copper carbonate to prepare the hydrazine–Cu(II) reducing agent it was also necessary to add a buffer solution to keep the alkaline medium.

The thermodynamic reducing strength of hydrazine depends on whether it undergoes 1-, 2-, or 4-electron oxidation and whether this is an acid or an alkaline solution. For basic solutions, the reactions and corresponding reduction potentials are as follows [24]:

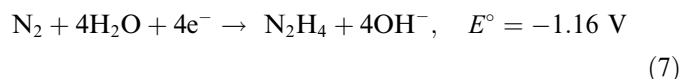
1-Electron change:



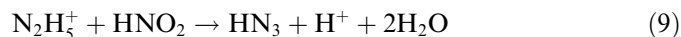
2-Electron change:



4-Electron change:



The oxidizing hydrazine is catalyzed by traces of metal ions such as Cu(II) to form N_2 [24] and the alkaline medium is also suitable for complete oxidation. Thus, it is clear that the most likely reaction to occur involves four electrons. Other metal cations, such as Fe(II), Ni(II), and Pb(II), were evaluated as reduction reaction catalysts in this work, but they failed to improve the nitrate to nitrite conversion rates. No nitrite was formed with Fe(II) and Pb(II), while the Ni(II) catalyst showed a maximum conversion of 40% and even then, not all nitrate consumed appeared to have been converted to nitrite. The copper(II) ion catalyst proved the most effective one in this reduction reaction. However, the reduction must be carried out in alkaline medium since, at pH values below 7, nitrite is rapidly destroyed by hydrazine, according to reactions Eqs. (8)–(10) [3,24].



It was sustained by results of three experiments carried out without adding the phenate buffer solution. The pH was monitored and the values recorded can reach to pH 5, depending on the power of the ultrasonic irradiation. In the acid medium, the nitrite peak disappears, probable destroyed by hydrazine.

Fig. 1 represents the reduction of nitrate to nitrite as a function of time, conducted under different conditions: (1) in the dark at room temperature; (2) in the dark at

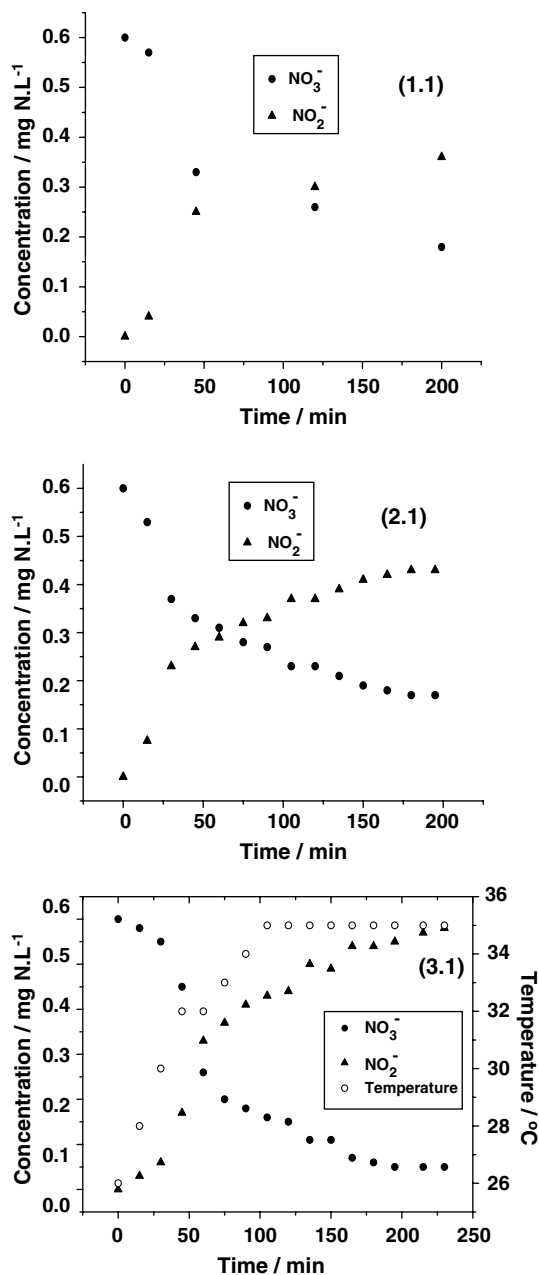


Fig. 1. Reduction of nitrate to nitrite by hydrazine–copper reagent in the dark: (1.1) without ultrasonic irradiation at room temperature; (2.1) without ultrasonic irradiation at 40 °C; (3.1) with ultrasonic irradiation at room temperature.

40 °C; and (3) in the dark under ultrasonic irradiation using an ultrasonic cleaning bath at room temperature. When the reactions were conducted without ultrasonic irradiation, silent conditions, the reduction reaction showed a nitrate conversion of 68% in 200 min. In contrast, when the reactions occurred under ultrasonic irradiation, the nitrate conversion was 92% after 200 min at room temperature. The temperature was monitored during the experiments conducted under ultrasonic irradiation started at room temperature. The highest temperature recorded was 35 °C. Because of this, an experiment was carried out at 40 °C in silence. The nitrate conversion under these

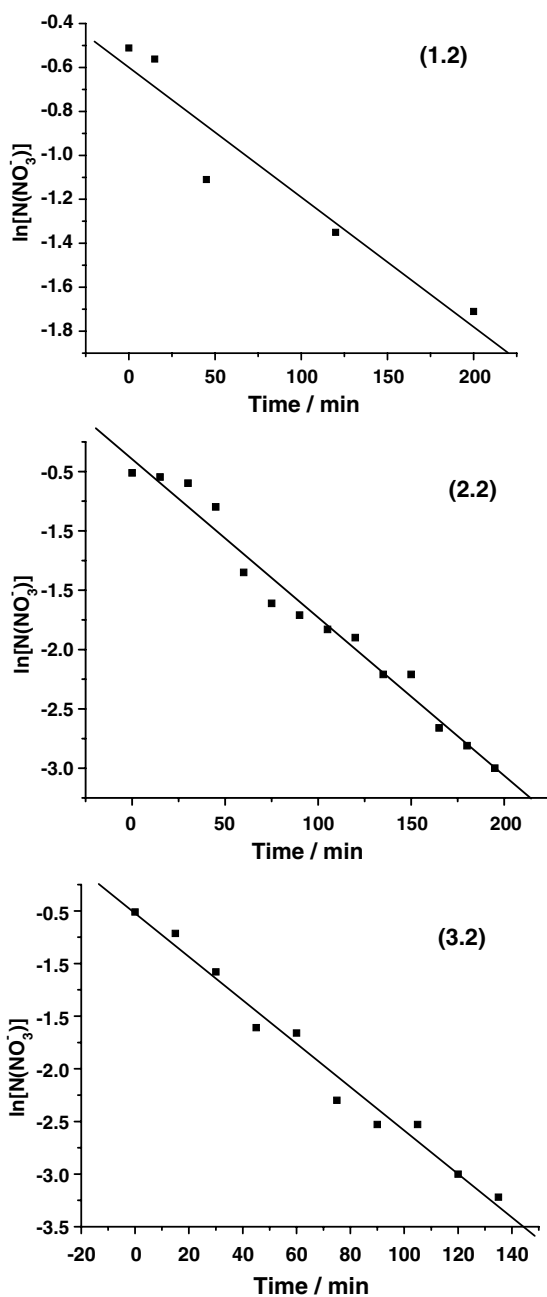


Fig. 2. Time dependence of $\ln[N(\text{NO}_3^-)]$ for reducing nitrate in the dark: (1.2) without ultrasonic irradiation at room temperature; (2.2) without ultrasonic irradiation at 40 °C; (3.2) with ultrasonic irradiation at room temperature.

conditions was 73% after 200 min. Thus, no significant changes occurred in the nitrate conversion reactions in

which only temperature increases were applied, at least in the range from room temperature to 40 °C.

The chemical effects promoted by ultrasonic irradiation in aqueous solutions, under proper conditions, may lead to the formation and collapse of gas or vapor bubbles inside the solution. Collapsing bubbles are extremely useful, for they cause local temperature and pressure to rise up to 5000 K and 800 atm, respectively [17]. Ultrasonic irradiation of pure water in the presence of oxygen and nitrogen can generate nitrite and nitrate and increase the acidity [19]. To attain any significant degree, the reaction whereby nitrate transforms into nitrite requires O_2 . Thus, in the absence of the oxygen gas, no NO_x^- is detectable, since the dissociation of oxygen is a major initial step. Also, there is evidence that the nitrate/nitrite ratio increases over time [19]. Therefore, ultrasonic irradiation applied in the reduction of nitrate to nitrite by hydrazine can be considered an artifact. To avoid this problem we used fresh boiled water to prepare all reaction solutions. The graphs in Fig. 1 – typical plots for our experimental runs – reveal that the nitrate and nitrite concentrations displayed a mirror-like relationship, as expected, supporting the idea that, in the dark and oxygen free, the nitrite resulting from the reaction originated solely from the reduction of nitrate.

In each experiment, the initial concentration of nitrate was $0.6 \text{ mg N}(\text{NO}_3^-) \text{ l}^{-1}$ and an excessive amount of hydrazine–Cu(II) reagent was added, causing the reaction to behave in a first-order dependence in relation to nitrate.

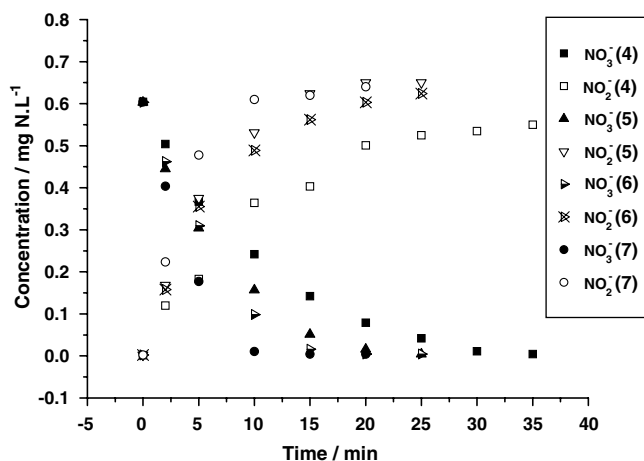


Fig. 3. Reduction of nitrate to nitrite by hydrazine–copper reagent in the dark, under different ultrasonic irradiation powers: (4) 3.0245 W; (5) 5.6990 W; (6) 8.9796 W; (7) 14.0357 W.

Table 1
Experimental conditions and rate constants for Experiments from 1 to 3

Experiment	$[\text{N}(\text{NO}_3^-)]_0 / \text{mg l}^{-1}$	Temperature/°C	Ultrasonic irradiation	Power/W	Reaction time/min	Conversion/%	Rate constant/ min^{-1}
1	0.6	Rt	No	–	200	68	5.90×10^{-3}
2	0.6	40	No	–	200	73	6.40×10^{-3}
3	0.6	Rt	Bath	0.9083	200	92	1.33×10^{-2}

Rt = room temperature.

Table 2
Experimental conditions and rate constants for Experiments from 4 to 7

Experiment	$[\text{N}(\text{NO}_3^-)]_0/\text{mg l}^{-1}$	Temperature/ $^{\circ}\text{C}$	Ultrasonic irradiation	Power/W	Reaction time/min	Conversion/%	Rate constant/ min^{-1}
4	0.6	Rt	Probe	3.0245	30	97	1.36×10^{-1}
5	0.6	Rt	Probe	5.6990	20	97	1.95×10^{-1}
6	0.6	Rt	Probe	8.9796	15	97	2.55×10^{-1}
7	0.6	Rt	Probe	14.0357	10	98	3.59×10^{-1}

Rt = room temperature.

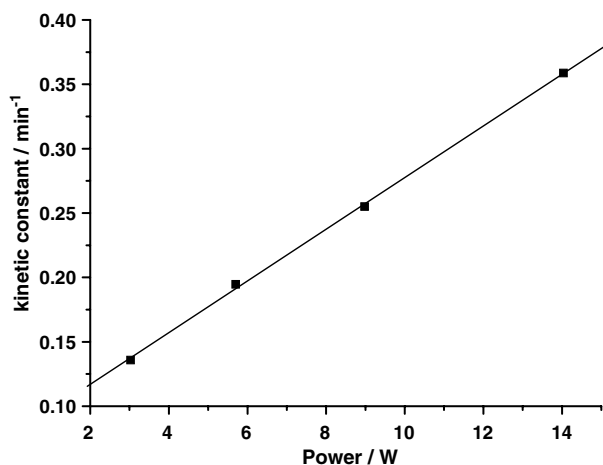


Fig. 4. Correlation between the kinetic constants and ultrasonic power.

This finding was obtained graphically by plotting $\ln[\text{N}(\text{NO}_3^-)]$ versus the reaction time (Fig. 2).

Table 1 summarizes the rate constants determined graphically for the various conditions employed here. These values yield the following half-lives ($t_{1/2} = 0.693/k$, where k is the kinetic constant for first-order dependence in relation to nitrate): $t_{1/2}$ (silent reaction) = 1.96 h; $t_{1/2}$ (in ultrasound cleaning bath) = 0.86 h, both reactions conducted at room temperature, and $t_{1/2}$ (silent reaction, at 40°C) = 1.80 h. Assuming that the reactions are completed in five half-lives, the reaction without ultrasonic irradiation is completed in 9.80 h at room temperature and in 9.02 h at 40°C . Thus, the 24 h wait for the reaction to be completed, as recommended in Refs. [3,4], is unnecessary. The assay performed under ultrasonic irradiation revealed that the reaction was completed in 4.3 h at room temperature. These results demonstrate that ultrasonic irradiation itself increased the electron transference reaction by 2.2-fold. These results show that in comparison to its silent reaction, the procedure using ultrasonic irradiation was much faster and afforded higher yields.

The acoustic power of the ultrasonic bath used here was determined calorimetrically to be 0.9083 W (Table 1). Another parameter considered in this study was the power of ultrasonic irradiation. In order to evaluate the influence of ultrasound intensity in the reaction rate, the reduction of nitrate to nitrite by hydrazine–Cu were conducted under different ultrasonic irradiation powers using an ultrasonic probe. In these experiments the same conditions of the silent

reactions and the reactions carried out in ultrasonic cleaning bath were kept. The powers of the ultrasonic irradiation for each experiment were set in: (4) 3.0245 W; (5) 5.6990 W; (6) 8.9796 W; (7) 14.0357 W. The acoustic powers were also measured calorimetrically. Fig. 3 shows the kinetic curves obtained for experimental runs and in all cases the reaction rates were increased. If we compare the experiments when the powers were set in 3.0245 W (the lowest power) and 14.0357 W (the highest power), we can see that in the former case, nitrate was completely converted to nitrite after 35 min, while no nitrate was detected after 10 min in the latter case; the other way around of the silent reaction, which was completed in about 10 h. These results demonstrate that high intensity ultrasonic irradiation itself can increase the electron transference reaction by ~ 60 -fold in comparison to its silent reaction. Fig. 3 (7) reveals that after 10 min, the concentration of nitrite remains unmodified, this is a sign that nitrite was not lowered by ultrasound at these conditions.

The results for experiments from 4 to 7 performed under different ultrasonic irradiation powers are also summarized in Table 2.

The strong influence of the ultrasonic waves in the reduction reaction rates can be sustained by an excellent linear correlation ($R^2 = 0.9993$) between the kinetic constants, obtained graphically by time dependence of $\ln[\text{N}(\text{NO}_3^-)]$, and the ultrasonic powers (Fig. 4).

4. Conclusions

Our assessment of different metal ions as catalysts revealed Cu(II) was the most effective one for this reaction. The results of this work indicated that ultrasonic irradiation increased the reaction rate, decreasing the reaction time from about 10 h, in the silent reaction, to 10 min when the ultrasonic irradiation power was set in 14.0357 W. The strong influence of the ultrasonic irradiation in the reduction reaction rates can be sustained by an excellent linear correlation ($R^2 = 0.9993$) between the kinetic constants and the ultrasonic powers. Moreover, the nitrate conversion also increased from 68%, in the silent reaction, to 98% when the reaction was conducted at highest ultrasonic power evaluated in this work. These results have demonstrated that the high intensity ultrasound is very beneficial for this reduction reaction to proceed in good yield and in short reaction time in comparison to its silent reaction.

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