inorganics

MDPI

Article

Copper (II)-Catalyzed Oxidation of Ascorbic Acid: Ionic Strength Effect and Analytical Use in Aqueous Solution

Anastasia E. Murekhina [1], Daniil N. Yarullin [1], Maria A. Sovina [2], Pavel A. Kitaev [2] and George A. Gamov [1,*]

[1] Department of Inorganic Chemistry and Technology, Ivanovo State University of Chemistry and Technology, 153000 Ivanovo, Russia; oxt705@isuct.ru (A.E.M.); yarullin@isuct.ru (D.N.Y.)
[2] Municipal Autonomous Institution "Lyceum No. 21", 153012 Ivanovo, Russia; sovina.mary@yandex.ru (M.A.S.); pavel.kitaev@yandex.ru (P.A.K.)
* Correspondence: ggamov@isuct.ru; Tel.: +7-(915)-821-85-62

Abstract: Copper is an important metal both in living organisms and in the industrial activity of humans, it is also a distributed water pollutant and a toxic agent capable of inducing acute and chronic health disorders. There are several fluorescent chemosensors for copper (II) determination in solutions; however, they are often difficult to synthesize and solvent-sensitive, requiring a non-aqueous medium. The present paper improves the known analytical technique for copper (II) ions, where the linear dependence between the ascorbic acid oxidation rate constant and copper (II) concentration is used. The limits of detection and quantification of the copper (II) analysis kinetic method are determined to be 82 nM and 275 nM, respectively. In addition, the selectivity of the chosen indicator reaction is shown: Cu^{2+} cations can be quantified in the presence of the 5–20 fold excess of Co^{2+}, Ni^{2+}, and Zn^{2+} ions. The La^{3+}, Ce^{3+}, and UO_2^{2+} ions also do not catalyze the ascorbic acid oxidation reaction. The effect of the concentration of the common background electrolytes is studied, the anomalous influence for chloride-containing salts is observed and discussed.

Keywords: copper (II); ascorbic acid; kinetics; rate constant; secondary salt effect; limit of detection; limit of quantification

Citation: Murekhina, A.E.; Yarullin, D.N.; Sovina, M.A.; Kitaev, P.A.; Gamov, G.A. Copper (II)-Catalyzed Oxidation of Ascorbic Acid: Ionic Strength Effect and Analytical Use in Aqueous Solution. *Inorganics* **2022**, *10*, 102. https://doi.org/10.3390/inorganics10070102

Academic Editor: Vladimir Arion

Received: 14 June 2022
Accepted: 15 July 2022
Published: 18 July 2022

1. Introduction

Copper is one of the most important elements for living organisms, primarily as a catalytic metal in biological systems. It allows many crucial proteins in plants, fungi, and mammalians to function properly [1], including transcriptional regulators, cell transporters and receptors, oxidoreductases, electron transfer proteins, monooxygenases, etc. [2]. Although it is involved in all the biological functions in monovalent form, only Cu^{2+} is bioavailable in significant amounts [3]. The adverse effects of increased copper intake or metal accumulation caused by gene disorder (Wilson's disease) are also known [1,2]. Copper is a dangerous pollutant of water bodies [4,5] and drinking water, which could be contaminated from copper containers [6]. This shows the importance of copper (II) control in aqueous medium.

Ascorbic acid (AAH_2^0) is an essential vitamin, which is used for prophylaxis and treatment of scurvy, and it also possesses several other valuable biological properties. Most of these properties are related to the antioxidant properties of AAH_2^0. For example, its role as a cofactor of many enzymes (e.g., hydroxylases that belong to the Fe^{2+}–2-oxoglutarate-dependent families of dioxygenases [7]) is substantiated by the vitamin's capability of maintaining iron in the ferrous state, thereby ensuring the full activity of this class of enzymes [7]. Another important member of ascorbate-dependent enzymes is the hypoxia-inducible transcription factor (HIF) [7], the upregulation of which is closely related to the development of many forms of cancer [8]. It is not surprising that ascorbic acid was considered a promising compound for preventing or slowing cancer development [9–13],

while the oxidized form of AAH_2^0—dehydroascorbic acid (DHA)—proved useless in inhibiting HIF activity and thus reducing the malignant potential in advanced melanoma [13]. Pro-oxidant action of vitamin C on the complex of copper (II) with 2-phenyl-4-(butylamino)-naphtholquinoline-7,12-dione—which might accelerate the lipid peroxidation in tumors—was also reported [14]. Ascorbic acid shows some antibacterial potential in vitro against both Gram-negative and Gram-positive microorganisms [15] and might be of some use in septic shock treatment; however, rather due to improvement of microvascular function and peripheral tissue perfusion, as well as vasopressor requirement than antibacterial potential (see the recent discussion in *Crit. Care* [16–19]). The possible effect of ascorbic acid on wound healing due to proliferative action [20] and the prevention of Alzheimer's disease due to the efficient binding of AAH_2^0 to amyloid-β oligopeptide [21] is also being studied.

However, another valuable property of ascorbic acid (also related to the antioxidant activity) is of special interest to us. Ascorbate monoanion (AAH^-)—which exists predominantly in neutral media considering $pK_{a1} = 4.14$ and $pK_{a2} = 11.28$ [22]—has a low reduction potential (0.19 V for the pair of DHA/AAH^- at pH 3.5). However, the rate of redox reaction in absence of catalysts (for example Cu^{2+} [23] or Fe^{2+} ions) is very low as the reaction is spin-forbidden [24]. Only ascorbate dianion (AA^{2-}) is capable of auto-oxidation in the air without trace d-metal ion. The fact that the rate of ascorbic acid oxidation depends on the concentration of metal cations is used in analytical chemistry for kinetic determination of some metals concentrations [25–27]. Moreover, ascorbic acid consumption is one of the assays used in atmosphere analysis to quantify aerosol oxidative potential, which better predicts adverse health outcomes than particle mass [24,28,29]. This consumption correlates positively with the total concentrations of iron and copper in ambient aerosol [24,30]. Analytical devices that use the pair Cu^{2+}/ascorbic acid for the generation of the particles for antioxidant essay [31] and pesticide determination [32], for example, are also known.

An important aspect of both biochemical and analytical applications of ascorbic acid is their complexation with biologically and environmentally relevant cations including Cu^{2+}, and such research has been started recently [33,34].

In the present paper, we intend to improve the known technique of quantitative analysis of copper (II) ions using the kinetic method (namely, the kinetics of ascorbic acid oxidation induced by Cu^{2+} ions). In particular: (1) the effect of the ionic strength set by four indifferent electrolytes (NaCl, $NaClO_4$, KNO_3, KCl) will be tested; (2) the effect of other d-metal ions such as Ni^{2+}, Zn^{2+}, and Co^{2+} on the possibility of quantitative determination in aqueous solution using ascorbic acid oxidation as an indicator reaction will be studied. It is worth noting that other analytical methods of copper (II) determinations are known, including the classic ones (chelatometry, coulometry, etc.) and several fluorimetric sensors designed recently (see, e.g., just a few of them in papers [35–48]). However, the relatively low sensitivity and selectivity are the disadvantages of the classic techniques, and a preconcentration of copper (II) is required to achieve the nanomolar limit of detection using atomic absorption spectroscopy [49–52], while the fluorescent sensors are often difficult to synthesize. Moreover, luminescent probes require a non-aqueous solvent for analytical determination, which limits their applicability. Kinetic analysis is as labor intensive as the other methods (since calibration plots are necessary for quantitative analysis in every technique) but also free from the mentioned drawbacks.

2. Results and Discussion

2.1. Quantitative Analysis of Copper (II) Ions Using Ascorbic Acid Oxidation as an Indicator Reaction

The dependence of the conditional rate constant of ascorbate oxidation on the copper (II) concentration is close to linear at any studied ionic strength value, which is set using the various indifferent electrolytes (Figures 1 and S1). R^2_{adj} varies from 0.92 to 0.99, the

worst value of R^2_{adj} = 0.8624 is observed for the highest concentration of NaCl causing the significant slowing of the oxidation reaction.

The following process is considered for the kinetic measurements:

$$\text{Ascorbic acid} \rightarrow \text{Dehydroascorbic acid; k}' \quad (1)$$

where "Ascorbic acid" and "Dehydroascorbic acid" represent all the coexisting protonated species of the reduced (ascorbate) and oxidized (dehydroascorbate) forms of the acid. In other words, $C_{total}(AAH_2^0) = [AAH_2^0] + [AAH^-] + [AA^{2-}]$; C_{total} (DHA) = $[DHA^0] + [DHA^-]$.

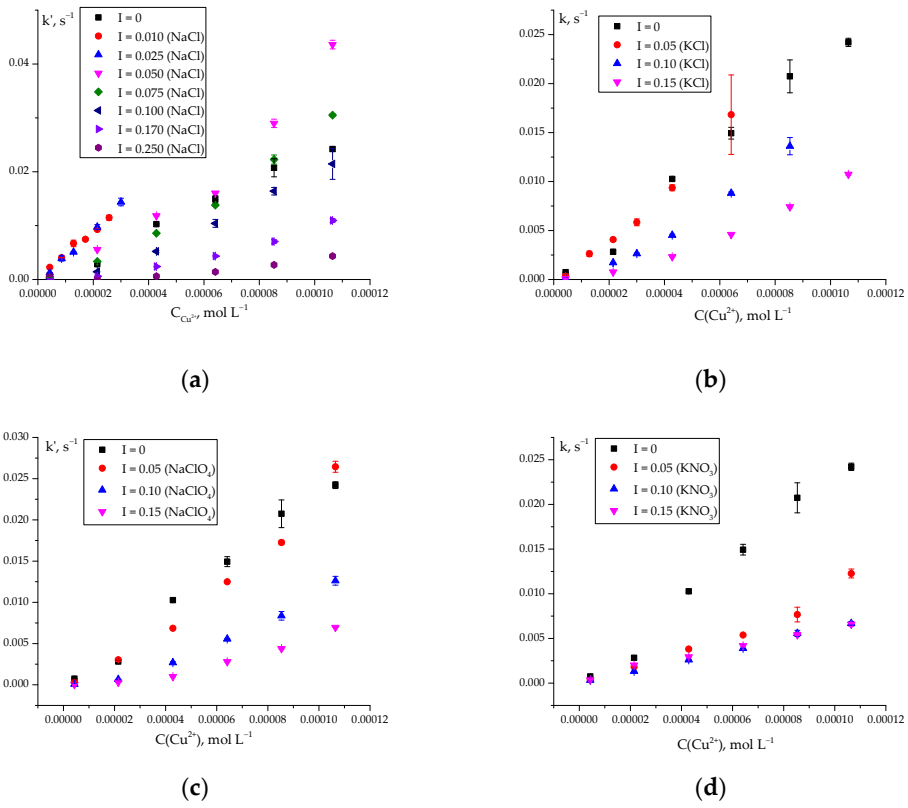


Figure 1. Dependence of apparent rate constant of ascorbic acid oxidation on copper (II) ion concentration at different ionic strength values set by: (**a**) NaCl; (**b**) KCl; (**c**) $NaClO_4$; (**d**) KNO_3.

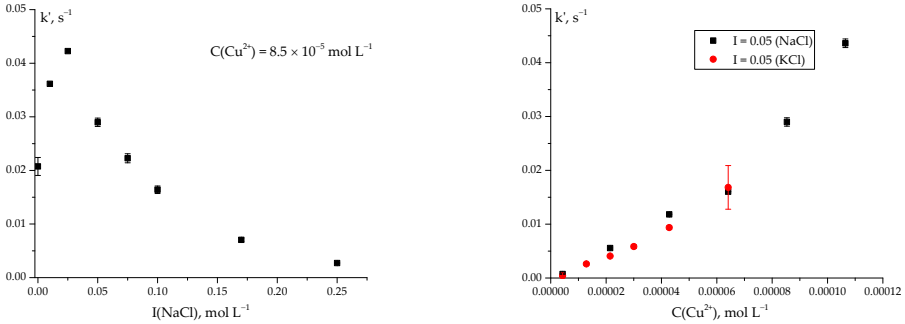
These calibration plots (Figures 1 and S1) allow one to find the unknown concentration of copper (II) ions. The limits of detection (LOD) and quantification (LOQ) could be calculated using the standard 3σ and 10σ rule [53]. However, since the analytical response in this case is a conditional rate constant instead of absorbance or fluorescence intensity, a corresponding modification of the method [53] is required. The lowest rate constant that could be calculated from the UV–vis spectral data corresponds to the lowest detectable change in the absorbance during 600 s of kinetic experiment. In the absorbance range of the 0 to 1, the error of experimental A measurements claimed by the manufacturer is 0.002 units. Therefore, the lowest detectable change in absorbance—which can be referred to the catalytic action of the copper (II) ions—is 0.004 units (because the error can be made at the beginning and the end of the kinetic experiment). The lowest rate constant corresponding to that low alteration of A is equal to $6.68 \cdot 10^{-6}$ s^{-1}, which gives a LOD of 82 nM and a LOQ of 275 nM at zero ionic strength considering k'_{cat} = 243.08 s^{-1} (k'_{cat}, the rate constant of the catalytic reaction is equal to the rate of hypothetical reaction of ascorbic acid

oxidation, where C_{cat} = 1 mol L^{-1}, and is equal to the slope in Figure S1a). In our opinion, it is a good result in comparison even with more complex fluorimetric techniques using sophisticated chemosensors. Although the kinetic method using ascorbic acid oxidation shows higher LOD for Cu^{2+} than 20 nM [35], 4.7 nM [36], and 24.5 nM [37] and slightly higher than LOD of inductively coupled plasma-atomic emission spectroscopy (74 nM [54]), this technique is more sensitive than the reported fluorescent ones [38–48], atomic absorption spectroscopy (AAS) [49] (however, preconcentration methods [50–52] improves the sensitivity of AAS by 10 times compared to the proposed method) and atomic emission spectroscopy [54]). Another advantage of the proposed method is its probable applicability for determining the equilibrium concentration of copper (II) ions in complex systems containing ligands (including biomacromolecules such as nucleic acids and proteins), i.e., for studying the coordination equilibria and determining the stability constants of Cu^{2+} complexes. It should be noted, however, that the interactions between copper (II) ions and biomacromolecules can be studied only in an absence of other ligands, in the limited range of ionic strength value and using the buffer, which does not bind metal ions into complexes.

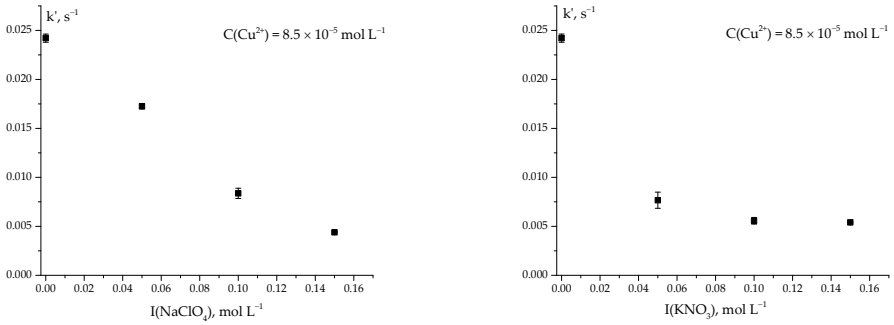
The LOD and LOQ can be further improved if the small ionic strength value is set using chloride-containing electrolyte, which leads to a steeper slope.

2.2. Effect of the Nature and Concentration of Background Electrolytes on the Rate of Ascorbic Acid Oxidation

The initial set of experiments, with NaCl utilized as an indifferent electrolyte, showed that the effect of salt concentration on the apparent rate constant of vitamin C auto-oxidation is non-linear (Figure 2a). Moreover, the decrease in the NaCl content did not make the observed value of k′ closer to that determined for zero ionic strength. The first small addition of this background electrolyte to the system accelerates the indicator reaction, and only I(NaCl) ≥ 0.1 mol L^{-1} decreases the rate constant. We performed additional experiments with other often used background electrolytes (KCl, $NaClO_4$, KNO_3) to figure out whether it is the common property of the electrolytes or the unique peculiarity of NaCl or every salt bearing chloride ion.



(**a**) (**b**)



(**c**) (**d**)

Figure 2. Dependence of the apparent rate constant of ascorbic acid oxidation at fixed $C(Cu^{2+})$ = 8.5 × 10^{-5} mol L^{-1} on the concentration of the background electrolyte: (**a**) NaCl; (**c**) $NaClO_4$; (**d**) KNO_3. (**b**) Dependence of apparent rate constant of ascorbic acid oxidation on copper (II) ion concentration at fixed ionic strength value 0.05 mol L^{-1} set by NaCl and KCl.

Our results give some evidence that the latter is true: 0.05 M KCl makes the indicator reaction of ascorbic acid autoxidation as quick as 0.05 M NaCl (Figure 2b). Other salts used to set the ionic strength value also give the non-linear dependence of k′ on the electrolyte concentration (Figure 2c,d).

The oxidation of ascorbic acid induced by copper (II) ions is a complex sequence of the reactions influenced in a different manner by the ionic strength. The shifts of chemical equilibria caused by the variation in concentration of background electrolyte may also change the equilibrium concentrations of components involved, both directly (since equilibrium constants depend on ionic strength value; primary salt effect) and indirectly (due to the reactions between background electrolyte and the involved components; secondary salt effect). Zhou et al. [23] give the general mechanism of vitamin C oxidation in the presence of Cu^{2+} ions; however, the most detailed description of the reactions occurred in the studied system is provided in a report [24]. Using this complex chemical model, the authors [18] determined the apparent rate constants for the following general processes.

$$Cu^{2+\prime} + AAH_2^0 + O_2 = Cu^{2+\prime} + DHA + H_2O_2;\ k' = 7.7 \times 10^4 \quad (2)$$

$$Cu^{2+\prime} + AAH^- + O_2 = Cu^{2+\prime} + DHA + H_2O_2 - H^+;\ k' = 2.8 \times 10^6 \quad (3)$$

where DHA is dehydroascorbic acid, $Cu^{2+\prime}$ represents all species involving copper (II) ions including free Cu^{2+} ions and $CuOH^+$, $CuCl^+$, and $CuCl_2$ as well as Cu^{2+} complexes with ascorbic acid of different composition [34,55,56] etc.

The secondary salt effect leading to the change in ascorbic acid dissociation degree can be neglected for two reasons. First, the variation in the dissociation constants of vitamin C with the ionic strength alteration up to 0.25 mol L^{-1} (NaCl) is negligible [22]. AAH_2^0 and AAH^- coexist in roughly equimolar quantities when the ascorbic acid is dissolved in the distilled water [22]. Second, the monoascorbate anion undergoes oxidation much more readily than ascorbic acid [24]. Therefore, the loss in its concentration would be refilled via instantaneous dissociation of AAH_2^0 caused by the shifted chemical equilibrium between two species. There are limited data on dehydroascorbic acid dissociation [57]; however, it is unlikely that a small change in ionic strength value could significantly alter the pH value of the solution.

The secondary salt effect on the composition of the mixture of different copper (II) species is more complex.

First, the variation of the ionic strength may influence the coordination equilibria between Cu^{2+} and ascorbic acid considering a relatively large yield of the complex under our experimental conditions (1.6 × 10^{-6} to 3.4 × 10^{-5} mol L^{-1} of $[CuAAH_2]^{2+}$ for $C^0(Cu^{2+})$ = 4.3 ×

10^{-6} and 1.06×10^{-4} mol L^{-1}, respectively, taking into account the stability constants from [41,42]). If the results of Ritacca et al. are used for the calculation of equilibrium compositions, the predominant complexes are $[CuAA_3]^{4-}$ when copper (II) ions is taken in a short supply, and $[Cu(OH)AA]^-$ if the total concentration of Cu^{2+} exceeds that of ascorbic acid. The catalytic action of the complexes is probably less pronounced than that of free copper (II), and alteration of the ionic strength leading to the shift of coordination equilibria towards the reactant should accelerate the reaction of auto-oxidation of vitamin C, while the stabilization of the complexes should slow the process of AAH_2^0 destruction.

Second, NaCl is truly indifferent only towards Cu^{2+} ions as the chloride complexes of copper (II) are very weak [24,58]. It does not apply to the chloride complexes of copper (I) [59], which plays an important role in the Fenton-like process according to the following equations [24]:

$$Cu^{2+\prime} + HO_2\cdot = Cu^+ + O_2 + H^+;\ k' = 10^8 \quad (4)$$

$$Cu^{2+\prime} + H_2O_2 = Cu^+ + O_2^- + 2H^+;\ k' < 1 \text{ for } Cu^{2+}, CuOH^+ \text{ and } CuSO_4;\ k' = 70 \text{ for } CuCl^+ \text{ and } CuCl_2 \quad (5)$$

$$Cu^+ + O_2 \leftrightharpoons Cu^{2+} + O_2\cdot -;\ k_{1\prime} = 4.6\cdot 10^5;\ k_{-1\prime} = 8 \times 10^9 \quad (6)$$

$$Cu^+ + OH\cdot = Cu^{2+} + OH^-;\ k' = 3 \times 10^9 \quad (7)$$

$$Cu^+ + H_2O_2 = Cu^{2+} + OH\cdot + OH^-;\ k' < 100 \quad (8)$$

$$Cu^+ + H_2O_2 = Cu^{3+} + 2OH^-;\ k' = 61 \quad (9)$$

$$Cu^+ + Cu^{3+} = 2Cu^{2+};\ k' = 3.5 \times 10^9 \quad (10)$$

$$Cu^+ + HO_2\cdot = Cu^{2+} + H_2O_2 - H^+;\ k' = 2.3 \times 10^9 \quad (11)$$

$$Cu^+ + O_2\cdot^- = Cu^{2+} + H_2O_2 - 2H^+;\ k' = 9.4 \times 10^9 \quad (12)$$

The rate constants use s^{-1} time scale.

Chloride complexes of copper (I) also undergo the oxidation [60,61]:

$$CuCl + H_2O_2 = CuCl^+ + OH^- + OH\cdot;\ k' = 3.7 \times 10^3 \quad (13)$$

$$CuCl_2^- + H_2O_2 = CuCl^+ + OH^- + OH\cdot + Cl^-;\ k' = 190 \quad (14)$$

$$CuCl + O_2 = Cu^{2+} + O_2^-;\ k' = 19 \quad (15)$$

$$CuCl_2^- + O_2 = Cu^{2+} + O_2^-;\ k' = 0.12 \quad (16)$$

$$CuCl_3^{2-} + O_2 = Cu^{2+} + O_2^-;\ k' = 0.44 \quad (17)$$

Gonzales-Davila et al. [62] deduced an equation expressing the correlation between the logarithm of the rate constant of copper (I) oxidation and pH, temperature, and ionic strength value. This equation appears to be valid only within the parameters used for its derivation (pH 7.2 to 8.5; T of 278.2 to 308.2 K; I(NaCl) 0.1 to 0.7 mol L^{-1}). However, under different experimental conditions, at constant pH and temperature it may also have the form proposed [62]:

$$\lg k' = \lg k_0' + B\sqrt{I} + CI \quad (18)$$

where k′ and $k_{0'}$ are the rate constants at given and zero ionic strength, respectively; I is an ionic strength value, B and C are the empirical constants.

We tried to use Equation (18) to describe the experimental data (Figure 2a), and the expression also seems valid to the results (Figure 3):

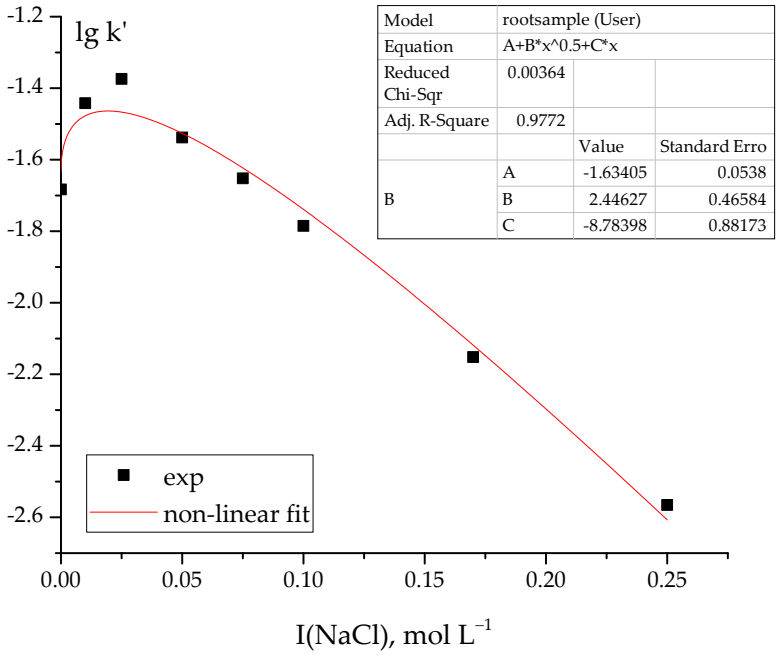


Figure 3. Non-linear fitting of the dependence of the logarithm of apparent rate constant of ascorbic acid oxidation at fixed $C(Cu^{2+}) = 8.5 \times 10^{-5}$ mol L^{-1} on the concentration of the background electrolyte.

This might be an indirect indication of the fact that the anomalous chloride concentration effect on the conditional rate constant of vitamin C oxidation in the presence of copper (II) ions might be explained by the changes in rate constant of copper (I) oxidation. However, no less than three objections could be raised against this conclusion: (i) taking the logarithm of the dimensional value is, strictly speaking, prohibited; (ii) taking the logarithm is recognized as some sort of mathematical trickery (see, e.g., [63]), which helps greatly in smoothing the dependence (which, otherwise, might be indicative of additional factors influencing it); (iii) the possibility of the same equation use for two dependencies does not mean any link between them (although does not exclude such an option either).

Other indifferent electrolytes ($NaClO_4$, KNO_3) cause a decrease in the apparent rate constant of ascorbate autooxidation induced by copper (II) ions. It can indicate that the rate-limiting stage of this reaction is an interaction between ions with the opposite charges, and the primary salt effect slows the overall process.

2.3. Selectivity of Copper (II) Quantitative Analysis Using Ascorbic Acid Oxidation as an Indicator Reaction

We tested the effect of cations such as Ni^{2+}, Co^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, and UO_2^{2+} on the apparent rate constant of ascorbic acid, and observed none. These cations do not catalyze the destruction of vitamin C. Figure 4 shows the possibility of using ascorbic acid for the copper (II) concentration determination when some other cations are also present in the solution:

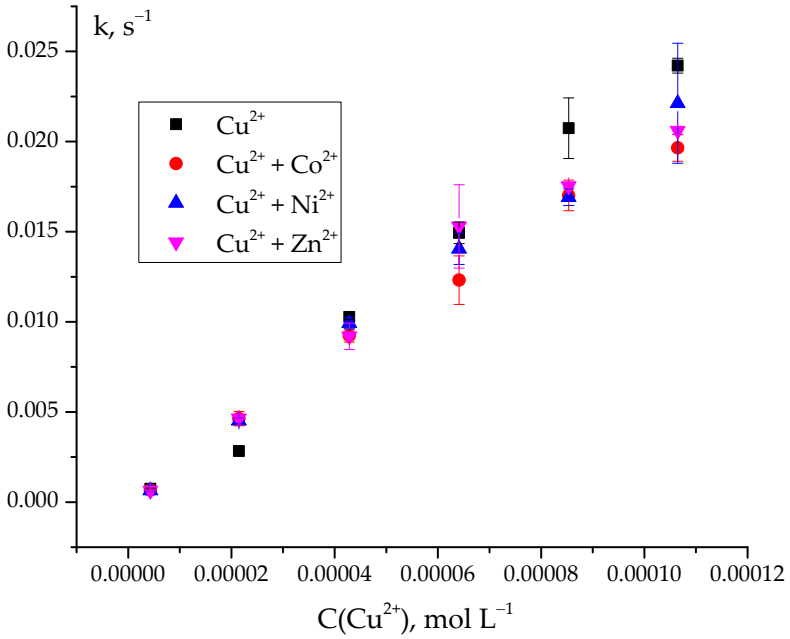


Figure 4. Dependence of apparent rate constant of ascorbic acid oxidation on Cu^{2+} concentration at zero ionic strength and in the presence of Co^{2+}, Ni^{2+}, and Zn^{2+} (10^{-4} mol L^{-1}).

The differences between dependencies of the conditional rate constant on the copper (II) concentration with and without additions of other d-metal ions are negligible. They do not interfere with the quantitative analysis of Cu^{2+} in an aqueous solution.

Finally, we tried to analyze the aqueous solution containing simultaneously Ni^{2+}, Co^{2+}, Zn^{2+} (concentration of each 10^{-4} mol L^{-1}), and Cu^{2+} of 2.15×10^{-5} mol L^{-1} concentration. Three experiments returned the value of copper (II) concentration $2.12 \pm 0.11 \times 10^{-5}$ mol L^{-1}, which corresponds to a recovery of $98.6 \pm 5.1\%$.

3. Materials and Methods

3.1. Chemicals

Ascorbic acid (Khimreaktiv, Nizhnii Novgorod, Russia), $Cu(NO_3)_2$ (Acron Organics, New Jersey, USA), NaCl, KCl, $NaClO_4$, KNO_3, $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Co(NO_3)_2$, $Zn(NO_3)_2$ (all Khimreaktiv, Nizhnii Novgorod, Russia), $La(NO_3)_3$, $Ce(NO_3)_3$, and $UO_2(CH_3COO)_2$ (all RedkiiMetallRF, Novosibirsk, Russia) were used without additional purification. Copper (II), nickel (II), cobalt (II), zinc (II), lanthanum (III), cerium (III), and uranyl (VI) concentrations in the stock 0.01 M solutions were determined chelatometrically. Only freshly prepared solutions of ascorbic acid were used. The concentration of ascorbic acid was determined spectrophotometrically using the literature values of the molar extinction coefficients [22]. All solutions were prepared using bidistilled water ($\kappa = 3.6$ μS/cm, pH = 6.7). The necessary amounts of background electrolytes were weighted using Shimadzu laboratory balances (AUW220, Shimadzu, Japan) with an error of weighing of 5×10^{-5} g.

3.2. Copper (II) Assay

An aliquot (1 to 25 μL) of aqueous solution of $Cu(NO_3)_2$ of the concentration of 0.01161 mol L^{-1} concentration, which corresponds to the final concentration of 4.3×10^{-6} to 1.06×10^{-4} mol L^{-1} was added to 2.7 mL of aqueous solution of ascorbic acid (1.5×10^{-4} mol L^{-1}) and the necessary background electrolyte placed in a standard quartz cell with an

optical path length of 1 cm, and quickly shaken. The absorbance of the resulting mixture was then recorded every 10 s during 600 s at a wavelength of 265 nm, which corresponds to the maximum absorbance of ascorbic acid and negligible absorbance of dehydroascorbic acid and other possible products/semi-products. Kinetic measurements were performed on the Shimadzu1800 double-beamed spectrophotometer (Shimadzu, Columbia, MD, USA).

The dependence of absorbance on time was then recalculated using the literature values of molar extinction coefficients of ascorbic acid [22], and the resulting data 'ascorbic acid concentration' vs. 'time' were processed using Kinet software [64] to obtain the apparent rate constant of the ascorbic acid oxidation. All kinetic experiments were at least triplicated; the error bars in Figures 1, 2, and 4 represent the standard deviations calculated for the corresponding sample size.

The rate of the catalytic monomolecular reaction is expressed as

$$\frac{dC_A}{d\tau} = -\mathrm{k}'C_A \tag{19}$$

where

$$\mathrm{k}' = \mathrm{k}_0 + \mathrm{k}_{cat}\,C_{cat} \tag{20}$$

where k′ is an observed apparent rate constant; k_0 is a rate constant of the reaction in the absence of catalyst; k_{cat} is a rate constant of the catalytic reaction; C_{cat} is a catalyst concentration.

As it follows from Equation (20), the observed rate constant is linearly dependent on the catalyst concentration.

4. Conclusions

In the present contribution, we gave a further development of the kinetic analytical technique for quantitative determination of copper (II) ions using copper-induced autooxidation of ascorbic acid as an indicator reaction. The method is cheaper in comparison with copper (II) analysis using the fluorescent chemosensors. Moreover, it does not require non-aqueous solvents. In addition, kinetic analysis shows limit of detection and quantification as low as those characteristic for some fluorimetric techniques (82 and 275 nM, respectively). Kinetic analysis is also selective, allowing Cu^{2+} detection in solutions containing Ni^{2+}, Co^{2+}, and Zn^{2+} ions as well.

The effect of the ionic strength set by four different background electrolytes (NaCl, KCl, KNO_3, $NaClO_4$) on the rate of oxidation of vitamin C is also studied. Chloride-containing salts taken at low concentrations (up to 0.1 mol L^{-1}) accelerate the ascorbate destruction reaction, while a higher electrolyte content causes slowing of the process. This can probably be explained by the formation of chloride complexes of copper (I), whose oxidation rate differs from that of the free cation.

We believe that the perspective of the present work is using the kinetic analysis to study the interactions of copper (II) ions with biomacromolecules (proteins, DNA), which is difficult to perform using other methods. However, the limits of the proposed technique should be kept in mind: (1) other ligands capable of Cu^{2+} binding should be absent; (2) buffer agent should be indifferent towards cation; (3) the possible ionic strength value is limited (which is not the biggest problem because the blood serum ionic strength is 0.15 mol L^{-1}).

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/inorganics10070102/s1, Figure S1: Linear fitting of the dependencies of an apparent rate constant of ascorbic acid autooxidation induced by Cu^{2+} ions in aqueous medium at ionic strength set by: (a) none, 0 mol L^{-1}; (b) NaCl, 0.01 mol L^{-1}; (c) NaCl, 0.025 mol L^{-1}; (d) NaCl, 0.05 mol L^{-1}; (e) NaCl, 0.075 mol L^{-1}; (f) NaCl, 0.10 mol L^{-1}; (g) NaCl, 0.17 mol L^{-1}; (h) NaCl, 0.25 mol L^{-1}; (i) KCl, 0.05 mol L^{-1}; (j) KCl, 0.10 mol L^{-1}; (k) KCl, 0.15 mol L^{-1}; (l) $NaClO_4$, 0.05 mol L^{-1};

(m) $NaClO_4$, 0.10 mol L^{-1}; (n) $NaClO_4$, 0.15 mol L^{-1}; (o) KNO_3, 0.05 mol L^{-1}; (p) KNO_3, 0.10 mol L^{-1}; (q) KNO_3, 0.15 mol L^{-1}.

Author Contributions: Conceptualization, G.A.G.; Methodology, G.A.G.; Validation, A.E.M., D.N.Y., M.A.S. and P.A.K.; Formal analysis, D.N.Y. and G.A.G.; Investigation, A.E.M., D.N.Y., M.A.S. and P.A.K.; Resources, G.A.G.; Data curation D.N.Y. and G.A.G.; Writing—original draft preparation, G.A.G.; Writing—review and editing, G.A.G.; Visualization, G.A.G.; Supervision, G.A.G.; Project administration, G.A.G.; Funding acquisition, G.A.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Higher Education of the Russian Federation, grant number FZZW-2020-0009, 075-15-2021-671, as part of ionic strength effect studies; and Council on grants of the President of the Russian Federation, grant number MK-923.2022.1.3, as part of determination of sensitivity and selectivity of the analytical technique.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data used are given either in the text of the present paper or the Supplementary Materials.

Acknowledgments: The resources of the Center for Shared Use of Scientific Equipment of the ISUCT (with the support of the Ministry of Science and Higher Education of Russia, grant number 075-15-2021-671) were used to perform the experiments.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of ascorbic acid, NaCl, KCl, $NaClO_4$, KNO_3, $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Co(NO_3)_2$, $Zn(NO_3)_2$, $La(NO_3)_3$, $Ce(NO_3)_3$, and $UO_2(CH_3COO)_2$ are available from elsewhere.

References

1. Osredkar, J.; Sustar, N. Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance. *J. Clin. Toxicol.* **2011**, *S3*, 0495. https://doi.org/10.4172/2161-0495.S3-001.
2. Festa, R.A.; Thiele, D.J. Copper: An essential metal in biology. *Curr. Biol.* **2011**, *21*, R877–R883. https://doi.org/10.1016/j.cub.2011.09.040.
3. Kaim, W.; Rall, J. Copper—A "Modern" Bioelement. *Angew. Chem. Int. Ed.* **1996**, *35*, 43–60. https://doi.org/10.1002/anie.199600431.
4. Leal, P.P.; Hurd, C.L.; Sander, S.G.; Armstrong, E.; Fernandez, P.A.; Suhrhoff, T.J.; Roleda, M.Y. Copper pollution exacerbates the effects of ocean acidification and warming on kelp microscopic early life stages. *Sci. Rep.* **2018**, *8*, 14763. https://doi.org/10.1038/s41598-018-32899-w.
5. Luo, Y.; Rao, J.; Jia, Q. Heavy metal pollution and environmental risks in the water of Rongna River caused by natural AMD around Tiegelongnan copper deposit, Northern Tibet, China. *PLoS ONE* **2022**, *17*, e0266700. https://doi.org/10.1371/journal.pone.0266700.
6. Manne, R.; Kumaradoss, M.M.R.M.; Iska, R.S.R.; Devarajan, A.; Mekala, N. Water quality and risk assessment of copper content in drinking water stored in copper container. *Appl. Water Sci.* **2022**, *12*, 27. https://doi.org/10.1007/s13201-021-01542-x.
7. Du, J.; Cullen, J.J.; Buettner, G.R. Ascorbic acid: Chemistry, biology and the treatment of cancer. *Biochim. Biophys. Acta Rev. Canc.* **2012**, *1826*, 443–457. https://doi.org/10.1016/j.bbcan.2012.06.003.
8. Jun, J.C.; Rathore, A.; Younas, H.; Gilkes, D.; Polotsky, V.Y. Hypoxia-Inducible Factors and Cancer. *Curr. Sleep Med. Rep.* **2017**, *3*, 1–10. https://doi.org/10.1007/s40675-017-0062-7.
9. Block, G. Vitamin C and cancer prevention: The epidemiologic evidence. *Am. J. Clin. Nutr.* **1991**, *53*, 270S–282S. https://doi.org/10.1093/ajcn/54.6.1310s.
10. Patterson, R.E.; White, E.; Kristal, A.R.; Neuhouser, M.L.; Potter, J.D. Vitamin supplements and cancer risk: The epidemiologic evidence. *Canc. Causes Control* **1997**, *8*, 786–802. https://doi.org/10.1023/a:1018443724293.
11. Gey, K.F. Vitamins E plus C and interacting conutrients required for optimal health. *BioFactors* **1998**, *7*, 113–174. https://doi.org/10.1002/biof.5520070115.
12. Loria, C.M.; Klag, M.J.; Caulfield, L.E.; Whelton, P.K. Vitamin C status and mortality in US adults. *Am. J. Clin. Nutr.* **2000**, *72*, 139–145. https://doi.org/10.1093/ajcn/72.1.139.
13. Fischer, A.P.; Miles, S.L. Ascorbic acid, but not dehydroascorbic acid increases intracellular vitamin C content to decrease Hypoxia Inducible Factor -1 alpha activity and reduce malignant potential in human melanoma. *Biomed. Pharmacother.* **2017**, *86*, 502–513. https://doi.org/10.1016/j.biopha.2016.12.056.

14. Selyutina, O.Y.; Kononova, P.A.; Koshman, V.E.; Fedenok, L.G.; Polyakov, N.E. The Interplay of Ascorbic Acid with Quinones-Chelators—Influence on Lipid Peroxidation: Insight into Anticancer Activity. *Antioxidants* **2022**, *11*, 376. https://doi.org/10.3390/antiox11020376.
15. Mumtaz, S.; Mumtaz, S.; Ali, S.; Tahir, H.M.; Kazmi, S.A.R.; Mughal, T.A.; Younas, M. Evaluation of antibacterial activity of vitamin C against human bacterial pathogens. *Braz. J. Biol.* **2021**, *83*, e247165. https://doi.org/10.1590/1519-6984.247165.
16. Rosengrave, P.; Spencer, E.; Williman, J.; Mehrtens, J.; Morgan, S.; Doyle, T.; Van Der Heyden, K.; Morris, A.; Shaw, G.; Carr, A.C. Intravenous vitamin C administration to patients with septic shock: A pilot randomised controlled trial. *Crit. Care* **2022**, *26*, 26. https://doi.org/10.1186/s13054-022-03900-w.
17. Muzaffar, S.N.; Saran, S.; Siddiqui, S.S. Vitamin C therapy in septic shock. *Crit. Care* **2022**, *26*, 87. https://doi.org/10.1186/s13054-022-03965-7.
18. Lee, H.J.; Kim, O.; Baek, M.S.; Kim, W. Vitamin C for septic shock in previous randomized trials: Implications of erroneous dosing, timing, and duration. *Crit. Care* **2022**, *26*, 61. https://doi.org/10.1186/s13054-022-03946-w.
19. Lavillegrand, J.; Raia, L.; Urbina, T.; Hariri, G.; Gabarre, P.; Bonny, V.; Bige, N.; Baudel, J.; Bruneel, A.; Dupre, T.; et al. Vitamin C improves microvascular reactivity and peripheral tissue perfusion in septic shock patients. *Crit. Care* **2022**, *26*, 25. https://doi.org/10.1186/s13054-022-03891-8.
20. Yi, Y.; Wu, M.; Zhou, X.; Xiong, M.; Tan, Y.; Yu, H.; Liu, Z.; Wu, Y.; Zhang, Q. Ascorbic acid 2-glucoside preconditioning enhances the ability of bone marrow mesenchymal stem cells in promoting wound healing. *Stem Cell Res. Ther.* **2022**, *13*, 119. https://doi.org/10.1186/s13287-022-02797-0.
21. Sampaio, I.; Quatroni, F.D.; Lins, P.M.P.; Nascimento, A.S.; Zucolotto, V. Modulation of beta-amyloid aggregation using ascorbic acid. *Biochimie* **2022**, *200*, 36–43. https://doi.org/10.1016/j.biochi.2022.05.006.
22. Gamov, G.A.; Yarullin, D.N.; Gudyrina, M.A.; Pogodina, E.I.; Medvedeva, A.S.; Zavalishin, M.N. Protonation of L-Ascorbic Acid in an Aqueous Solution at T = 298.2 K, p = 0.1 MPa, and I = 0.10−5.0 mol L−1 (NaCl). *J. Chem. Eng. Data* **2022**, *67*, 1358–1364. https://doi.org/10.1021/acs.jced.2c00034.
23. Zhou, P.; Zhang, J.; Zhang, Y.; Liu, Y.; Liang, J.; Liu, B.; Zhang, W. Generation of hydrogen peroxide and hydroxyl radical resulting from oxygen-dependent oxidation of L-ascorbic acid via copper redox-catalyzed reactions. *RSC Adv.* **2016**, *6*, 38541–38547. https://doi.org/10.1039/c6ra02843h.
24. Shen, J.; Griffiths, P.T.; Campbell, S.J.; Utinger, B.; Kalberer, M.; Paulson, S.E. Ascorbate oxidation by iron, copper and reactive oxygen species: Review, model development, and derivation of key rate constants. *Sci. Rep.* **2021**, *11*, 7417. https://doi.org/10.1038/s41598-021-86477-8.
25. Mottola, H.A.; Perez-Bendito, D. Kinetic Determinations and Some Kinetic Aspects of Analytical Chemistry. *Anal. Chem.* **1994**, *66*, 131–162. https://doi.org/10.1021/ac00084a007.
26. Crouch, S.R.; Cullen, T.F.; Scheeline, A.; Kirkor, E.S. Kinetic Determinations and Some Kinetic Aspects of Analytical Chemistry. *Anal. Chem.* **1998**, *70*, 53–106. https://doi.org/10.1021/a1980005s.
27. Cerda, V.; Gonzalez, A.; Danchana, K. From thermometric to spectrophotometric kinetic-catalytic methods of analysis. A review. *Talanta* **2017**, *167*, 733–746. https://doi.org/10.1016/j.talanta.2017.02.004.
28. Campbell, S.J.; Utinger, B.; Lienhard, D.M.; Paulson, S.E.; Shen, J.; Griffiths, P.T.; Stell, A.C.; Kalberer, M. Development of a Physiologically Relevant Online Chemical Assay to Quantify Aerosol Oxidative Potential. *Anal. Chem.* **2019**, *91*, 13088–13095. https://doi.org/10.1021/acs.analchem.9b03282.
29. Bates, J.T.; Fang, T.; Verma, V.; Zeng, L.; Weber, R.J.; Tolbert, P.E.; Abrams, J.Y.; Sarnat, S.E.; Mulholland, J.A.; Russell, A.G. Review of Acellular Assays of Ambient Particulate Matter Oxidative Potential: Methods and Relationships with Composition, Sources, and Health Effects. *Environ. Sci. Technol.* **2019**, *53*, 4003–4019. https://doi.org/10.1021/acs.est.8b03430.
30. Godri, K.J.; Harrison, R.M.; Evans, T.; Baker, T.; Dunster, C.; Mudway, I.S.; Kelly, F.J. Increased Oxidative Burden Associated with Traffic Component of Ambient Particulate Matter at Roadside and Urban Background Schools Sites in London. *PLoS ONE* **2011**, *6*, e21961. https://doi.org/10.1371/journal.pone.0021961.
31. Moreno, M.T.; Mellado, J.M.R.; Medina, A. Rapid Electrochemical Determination of Antioxidant Capacity Using Glassy Carbon Electrodes Modified with Copper and Polyaniline. Application to Ascorbic and Gallic Acids. *Bioint. Res. Appl. Chem.* **2022**, *13*, 23. https://doi.org/10.33263/BRIAC131.023.
32. Wang, Y.; Zhu, F.; Yin, L.; Qu, G.; Ma, D.; Leung, C.; Lu, L. Ratiometric fluorescent detection of pesticide based on split aptamer and magnetic separation. *Sensors Actuat. B Chem.* **2022**, *367*, 132045. https://doi.org/10.1016/j.snb.2022.132045.
33. Cesario, D.; Furia, E.; Mazzone, G.; Beneduci, A.; De Luca, G.; Sicilia, E. Complexation of Al^{3+} and Ni^{2+} by L-Ascorbic Acid: An Experimental and Theoretical Investigation. *J. Phys. Chem. A* **2017**, *121*, 9773–9781. https://doi.org/10.1021/acs.jpca.7b10847.
34. Ritacca, A.G.; Malacaria, L.; Sicilia, E.; Furia, E.; Mazzone, G. Experimental and theoretical study of the complexation of Fe^{3+} and Cu^{2+} by L-ascorbic acid in aqueous solution. *J. Mol. Liq.* **2022**, *355*, 118973. https://doi.org/10.1016/j.molliq.2022.118973.
35. Anand, T.; Sivaraman, G.; Chellappa, D. Quinazoline copper(II) ensemble as turn-on fluorescence sensor for cysteine and chemodosimeter for NO. *J. Photochem. Photobiol. A Chem.* **2022**, *281*, 47–52. https://doi.org/10.1016/j.jphotochem.2014.02.015.
36. Tian, M.; He, H.; Wang, B.; Wang, X.; Liu, Y.; Jiang, F. A reaction-based turn-on fluorescent sensor for the detection of Cu(II) with excellent sensitivity and selectivity: Synthesis, DFT calculations, kinetics and application in real water samples. *Dyes Pigm.* **2019**, *165*, 383–390. https://doi.org/10.1016/j.dyepig.2019.02.043.

37. Arslan, F.N.; Geyik, G.A.; Koran, K.; Ozen, F.; Aydin, D.; Elmas, S.N.K.; Gorgulu, A.O.; Yilmaz, I. Fluorescence "Turn On–Off" Sensing of Copper (II) Ions Utilizing Coumarin–Based Chemosensor: Experimental Study, Theoretical Calculation, Mineral and Drinking Water Analysis. *J. Fluoresc.* **2020**, *30*, 317–327. https://doi.org/10.1007/s10895-020-02503-4.
38. Kim, G.; Choi, D.; Kim, C. A Benzothiazole-Based Fluorescence Turn-on Sensor for Copper(II). *J. Fluoresc.* **2021**, *31*, 1203–1209. https://doi.org/10.1007/s10895-021-02752-x.
39. Silpcharu, K.; Soonthonhut, S.; Sukwattanasinitt, M.; Rashatasakhon, P. Fluorescent Sensor for Copper(II) and Cyanide Ions via the Complexation−Decomplexation Mechanism with Di(bissulfonamido)spirobifluorene. *ACS Omega* **2021**, *6*, 16696–16703. https://doi.org/10.1021/acsomega.1c02744.
40. Selvan, G.T.; Varadaraju, C.; Selvan, R.T.; Enoch, I.V.M.V.; Selvakumar, P.M. On/Off Fluorescent Chemosensor for Selective Detection of Divalent Iron and Copper Ions: Molecular Logic Operation and Protein Binding. *ACS Omega* **2018**, *3*, 7985–7992. https://doi.org/10.1021/acsomega.8b00748.
41. Wang, B.; Xu, W.; Gan, K.; Xu, K.; Chen, Q.; Wei, W.; Wu, W. On the synthesis and performance of a simple colorimetric and fluorescent chemosensor for Cu^{2+} with good reversibility. *Spectrochim. Acta A Mol. Biomol. Spec.* **2022**, *277*, 121245. https://doi.org/10.1016/j.saa.2022.121245.
42. Gauthama, B.U.; Narayana, B.; Sarojini, B.K.; Kodlady, S.N.; Sangappa, Y.; Kudva, A.K.; Raghu, V. A versatile rhodamine B-derived fluorescent probe for selective copper(II) sensing. *Inorg. Chem. Comm.* **2022**, *141*, 109501. https://doi.org/10.1016/j.inoche.2022.109501.
43. Sawminathan, S.; Munusamy, S.; Manickam, S.; Kulathulyera, S. A simple quinazolinone-isophorone based colorimetric chemosensor for the reversible detection of copper (II) and its application in real samples. *J. Mol. Struct.* **2022**, *1257*, 132633. https://doi.org/10.1016/j.molstruc.2022.132633.
44. Heo, J.S.; Suh, B.; Kim, C. Selective detection of Cu^{2+} by benzothiazole-based colorimetric chemosensor: A DFT study. *J. Chem. Sci.* **2022**, *134*, 43. https://doi.org/10.1007/s12039-022-02037-1.
45. Isaad, J.; El Achari, A. Water-soluble coumarin based sequential colorimetric and fluorescence on-off chemosensor for copper(II) and cyanide ions in water. *Opt. Mater.* **2022**, *127*, 112275. https://doi.org/10.1016/j.optmat.2022.112275.
46. Giri, P.K.; Samanta, S.S.; Mudi, N.; Shyamal, M.; Misra, A. Highly Sensitive 'on–off' Pyrene Based AIEgen for Selective Sensing of Copper (II) Ions in Aqueous Media. *J. Fluoresc.* **2022**, *32*, 1059–1071. https://doi.org/10.1007/s10895-022-02929-y.
47. Lv, M.; Bian, L.; Wu, W.; Xu, Z.; Wang, Y.; Zhao, X.; Xu, Z.; Fan, Y.; Yan, L. Thiocarbazone-appended coumarin: An easily accessible ratiometric fluorescent chemosensor for multianalyte (Zn^{2+} and Cu^{2+}) systems. *Corol. Techn.* **2022**, *138*, 157–167. https://doi.org/10.1111/cote.12579.
48. Isaad, J.; El Achari, A. Sequential colorimetric sensor for copper (II) and cyanide ions via the complexation−decomplexation mechanism based on sugar pyrazolidine-3,5-dione. *J. Mol. Struct.* **2022**, *1252*, 132151. https://doi.org/10.1016/j.molstruc.2021.132151.
49. Kim, B.; Choi, H. Determination of Copper by Atomic Absorption Spectrophotometry After Solid-Liquid Separation by Adsorption of its 1-Nitroso-2-Naphthol Complex onto Microcrystalline Benzophenone. *Anal. Lett.* **1999**, *32*, 995–1009. https://doi.org/10.1080/00032719908542872.
50. Ouyang, Z.; Chen, Z.; Mou, H. Determination of copper by Flame Atomic Absorption Spectrometry using Flow Injection Online Preconcentration with Double Micro-columns. *Rev. Anal. Chem.* **2010**, *29*, 51–58. https://doi.org/10.1515/REVAC.2010.29.1.51.
51. Sahan, S.; Sahin, U. Determination of Copper(II) Using Atomic Absorption Spectrometry and Eriochrome Blue Black R Loaded Amberlite XAD-1180 Resin. *Clean Soil Air Water* **2010**, *38*, 485–491. https://doi.org/10.1002/clen.200900303.
52. Bagherian, B.; Chamjangali, M.A.; Evari, H.S.; Ashrafi, M. Determination of copper(II) by flame atomic absorption spectrometry after its perconcentration by a highly selective and environmentally friendly dispersive liquid–liquid microextraction technique. *J. Anal. Sci. Tech.* **2019**, *10*, 3. https://doi.org/10.1186/s40543-019-0164-6.
53. Thomsen, V.; Schatzlein, D.; Mercuro, D. Limits of Detection in Spectroscopy. *Spectroscopy* **2003**, *18*, 112–114.
54. Tan, M.; Sudjadi; Astuti; Rohman. Validation and quantitative analysis of cadmium, chromium, copper, nickel, and lead in snake fruit by Inductively Coupled Plasma-Atomic Emission Spectroscopy. *J. Appl. Pharm. Sci.* **2018**, *8*, 44–48. https://doi.org/10.7324/JAPS.2018.8206.
55. Jameson, R.F.; Blackburn, N.J. Role of copper dimers and the participation of copper(III) in the copper-catalysed autoxidation of ascorbic acid. Part II. Kinetics and mechanism in 0.100 mol dm^{-3} potassium nitrate. *J. Chem. Soc. Dalton Trans.* **1976**, *6*, 534–541. https://doi.org/10.1039/DT9760000534.
56. Davies, M.B. The copper(II) catalyzed reaction of L-ascorbinc acid with tris(oxalato)cobaltate(III) ion in aqueous solution. *Inorg. Chim. Acta.* **1984**, *92*, 141–146. https://doi.org/10.1016/S0020-1693(00)80011-0.
57. Kriss, E.E.; Kurbatova, G.T. Vanadium(IV) complexation with ascorbic and dehydroascorbic acids. *Zhurn. Neorg. Khim.* **1976**, *21*, 2368–2374. (In Russian).
58. Powell, K.J.; Brown, P.L.; Byrne, R.H.; Gajda, T.; Hefter, G.; Sjöberg, S.; Wanner, H. Chemical speciation of environmentally significant metals with inorganic ligands Part 2: The Cu^{2+}-OH^-, Cl^-, CO_3^{2-}, SO_4^{2-}, and PO_4^{3-} systems (IUPAC Technical Report) *Pure Appl. Chem*. **2007**, *79*, 895–950. https://doi.org/10.1351/pac200779050895.
59. Xiao, Z.; Gammons, C.H.; Williams-Jones, A.E. Experimental study of copper(I) chloride complexing in hydrothermal solutions at 40 to 300 °C and saturated water vapor pressure. *Geochim. Cosmochim. Acta* **1998**, *62*, 2949–2964. https://doi.org/10.1016/S0016-7037(98)00228-2.

60. Nicol, M.J. Kinetics of the oxidation of copper(I) by hydrogen peroxide in acidic chloride solutions. *South Afr. J. Chem.* **1982**, *35*, 77–79. https://doi.org/10.10520/AJA03794350_1171.
61. Yuan, X.; Pham, A.N.; Xing, G.; Rose, A.L.; Waite, T.D. Effects of pH, Chloride, and Bicarbonate on Cu(I) Oxidation Kinetics at Circumneutral pH. *Environ. Sci. Technol.* **2012**, *46*, 1527–1535. https://doi.org/10.1021/es203394k.
62. Gonzalez-Davila, M.; Santana-Casiano, J.M.; Gonzalez, A.G.; Perez, N.; Millero, F.J. Oxidation of copper(I) in seawater at nanomolar levels. *Marine Chem.* **2009**, *115*, 118–124. https://doi.org/10.1016/j.marchem.2009.07.004.
63. van de Weert, M. Fluorescence Quenching to Study Protein-ligand Binding: Common Errors. *J. Fluoresc.* **2010**, *20*, 625–629. https://doi.org/10.1007/s10895-009-0572-x.
64. Available online: http://www.chem.msu.su/rus/teaching/KINET2012/ (accessed on 13 June 2022).