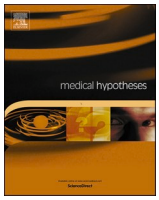
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Medical Hypotheses 160 (2022) 110775

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy



Copper acetate aerosols: A possible tool complementary to vaccination in fight against SARS-CoV-2 and variants replication

Roger Deloncle [a,*], Olivier Guillard [b], Alain Pineau [c], Gérard Lesage [a]

[a] School of Pharmacy, Tours University, Tours 37200, France
[b] School of Medicine and Pharmacy, Poitiers University, Poitiers 86022, France
[c] School of Pharmacy, Nantes University, Cedex 1, Nantes 44035, France

ARTICLE INFO

Keywords:
COVID 19
SARS-CoV-2 variants
Aerosols
Amino acids complexes
Copper acetate
Nasal sprays

ABSTRACT

In SARS-CoV-2, at the S1/S2 furin cleavage site, a four amino acid insert (P-R-R-A) not found in closely related corona viruses, has been shown to facilitate entry into respiratory epithelial cells and promote virus transmission, infectivity and virulence. By cupric aerosol treatment, complexation of these four amino acids (-P-R-R-A-), at the spike (S) protein site will lead to a conformational change possibly impeding SARS-CoV-2 replication process in the respiratory track. Since these four amino acids yield strong and stable copper complexes, subsequent to a steric hindrance, this complexation will disturb the furin-like protease cleavage at the spike protein site as it has been recently shown in vitro with copper gluconate.

The compilation of stability constants for copper amino-acid complex formation, showing values of the same order of magnitude for all the twenty proteinogenic amino-acids demonstrate thermodynamically that copper amino-acid chelation for SARS-CoV-2 virus will not be affected by mutations leading to amino acid exchanges in the spike protein region. Given its low toxicity, and its very low stability formation constant, copper acetate is proposed rather than copper gluconate for possible cupric aerosol or nasal spray treatments aimed at impeding SARS-CoV-2 multiplication. It will open different medical perspectives, complementary to vaccination, in the fight against COVID 19 native virus, variants and future mutants.

Introduction

Since the end of 2019, subsequent to the spread throughout the world of the causative agent SARS-CoV-2, a severe acute respiratory syndrome, COVID 19, has taken on epidemic proportions, with dramatic consequences from the health, economic and social standpoints. In the observed SARS-CoV-2 structure, a four amino acid insert (-P-R-R-A-) facilitating viral entry into respiratory epithelial cells through their ACE2 receptor has been found at the spike (S) protein site. This insert, at the interface between the S1 receptor binding and the S2 fusion subunits, is responsible for a new potential protease cleavage site for furin-like proteases [1].

In addition to this structural fracture, the carboxylic and amine groups in these amino acids have free electronic doublets on the nitrogen or oxygen atoms that may be involved in metal complex formation by semi-polar bindings. Acting as electron donors, free electronic doublets yield amino acids as Lewis bases that can be involved in coordination links with Lewis acids such as transition 3d metals. As interactions have been suspected between viral infections and trace elements [2], metal hybridization can lead to the formation of metallic complexes with the 3d transition metals, mainly copper, which is able to complex with amino acids. With a single 4 s electron filling responsible for special redox properties, this metal appears as an interesting element having an antiviral activity, and it might be the best 3d transition metal for a possible aerosol or nasal spray treatment.

In the human body, copper is present as Cu^{+} (cuprous) and oxidized Cu^{2+} (cupric) compounds; it is an intermediary for electron transfer in redox reactions and can produce free radicals by Haber-Weiss and Fenton reactions. The antiviral activities of Cu^{2+} ions seem to be effective inhibitors for viral entry and replication, mRNA and capsid protein degradations during the viral life cycle, and it may be hypothesized that copper and copper-complexes have beneficial antiviral action with regard to ROS-mediated virus death [3]. In this respect, a recent review on COVID19 has evoked the role of copper in association with antiviral treatments against SARS-CoV-2 [4], and it has also been shown that on copper-coated surfaces, the virus was inactivated within two hours [5].

* Corresponding author.
E-mail address: roger.deloncle@univ-tours.fr (R. Deloncle).

https://doi.org/10.1016/j.mehy.2022.110775
Received 1 November 2021; Received in revised form 17 December 2021; Accepted 10 January 2022
Available online 25 January 2022

All of these facts demonstrating copper toxicity against SARS CoV-2 might be linked to the property of copper to bind and complex with the four amino acids in the insertion sequence at the spike protein junction. To stop SARS-CoV-2 pulmonary cell penetration, it appears possible to disturb spike furin-like protease cleavage by means of copper amino acid complexation through copper aerosolization in the immediate vicinity of the virus.

Hypothesis

Copper amino acid complex formation

Copper complexation with amino acids can be schematized as follows:

Equilibrium I $Cu^{++} + AA \leftrightarrow [Cu\text{-}AA]$ $K_1 = |[Cu\text{-}AA]|/|Cu^{++}| \times |AA|$

Equilibrium II $[Cu\text{-}AA] + AA \leftrightarrow [Cu(AA)_2]$ $K_2 = |[Cu\text{-}(AA)_2]|/|[Cu\text{-}AA]| \times |AA|$

and when considering the four amino acids (-P-R-R-A-) involved in the spike protein site insert, the stability formation constants are respectively:

Proline:	$\log \beta_1 = 9{,}40$ [6]	$\log \beta_1\beta_2 = 16{,}60$ [7]
Arginine:	$\log \beta_1 = 7{,}38$ [8]	$\log \beta_1\beta_2 = 13{,}66$ [8]
Alanine:	$\log \beta_1 = 8{,}15$ [9]	$\log \beta_1\beta_2 = 14{,}95$ [9]

These stability constants in the formation of copper amino acid complexes demonstrate that copper proline, copper arginine and copper alanine chelates are highly stable, and one can expect similar results if the amino acids are included in a peptide sequence such as the SARS CoV-2 spike protein site. In order to form complexes with these spike protein amino acids, the copper ion would need to be in the immediate vicinity of the virus, and complementarily to vaccination, copper aerosol treatment appears to be a possible solution. The electronic structures of the amino acids inserted in the spike (S) protein are given in Fig. 1.

On oxygen and nitrogen atoms, free electronic doublets may be involved in semi-polar bonds with copper ion, yielding a formation of chelated copper structures. This chelation is possible either with each single amino acid from the spike (S) peptide insert (-P-R-R-A-), or with several of the four amino acids in a single virus sequence. It is also possible for copper to bind with several amino acids from a second nearby viral molecule. (Fig. 2).

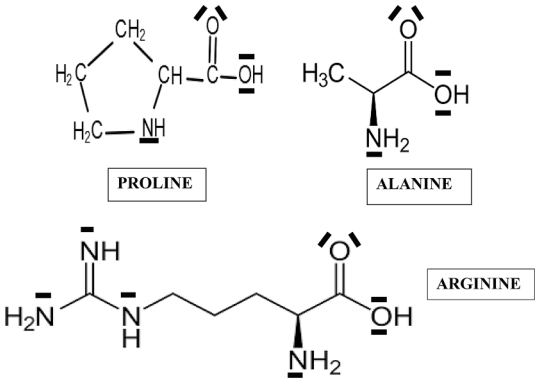


Fig. 1. The amino acids inserted in the SARS-CoV-2 spike protein junction. *The bold hyphens indicate the presence of free electronic doublets on nitrogen or oxygen atoms.*

This copper chelation of the four amino acid inserted in the spike region could be schematized as follows (Fig. 3).

Concerning mutations for COVID 19, it appears that variants of SARS-CoV-2 result from amino acid exchanges on virus surface proteins and that copper complexation of these surface amino acids might also hinder virus penetration into pulmonary cells. In a paragraph below, "thermodynamics on copper amino acid complexes", considerations on stability constants for the twenty exchangeable proteinogenic amino acids demonstrate that this copper complexation leading to highly stable chelates will always be possible, even with viruses that have mutated.

A possible copper aerosol treatment for SARS-CoV-2 variants.

Since the beginning of the pandemic COVID-19, several deletions such as Δ69/70 in Denmark, Δ144/145 in Great Britain, or Δ242/244 in South Africa have been reported and throughout the world a number of SARS-CoV-2 mutations have been identified, such as the Chinese mutation H49Y in which a histidine amino acid has been substituted for a tyrosine residue on position 49. In the United States of America, similar substitutions have also been found for example in G476S and V483A

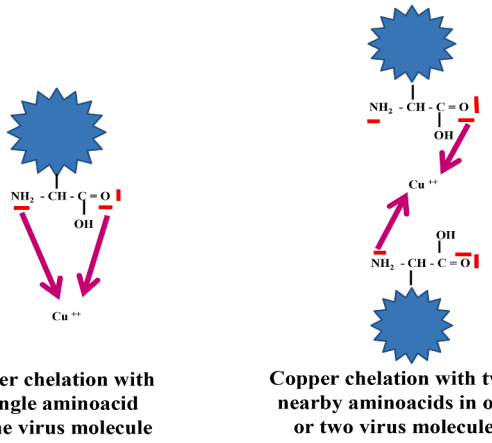


Fig. 2. Copper chelation of the amino acids inserted in the SARS-CoV-2 spike protein junction with a single virus or with a second nearby virus molecule. *The red bold hyphens indicate the presence of free electronic doublets on nitrogen or oxygen atoms and pink arrows semi polar bonds to copper.*

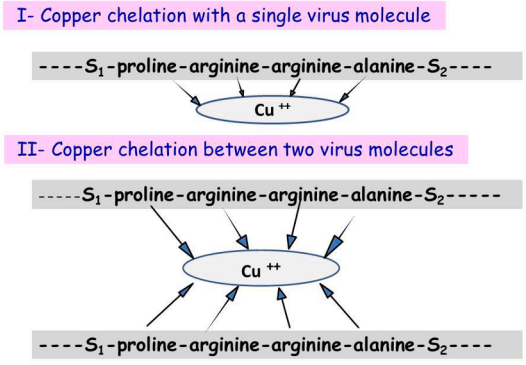


Fig. 3. Possible effects on SARS-CoV2 structure by Cu-aerosol treatment. *The arrows represent possible semi polar bonds from amino acids to copper.*

variants where glycine and serine have been exchanged on site 476, or valine and alanine on position 483. In the Californian L452R variant an arginine substitution for leucine has been reported on site 452, and in the New York variant S477N, serine and asparagine have been swapped on site 477. In Brazil and Hawaii in the mutant E484K a glutamic acid has been exchanged with a lysine residue on site 484 and in Nigeria a P681H mutant able to increase S protein production by infected cells, has been identified with a proline histidine exchange on 681 position. In Europe three main mutations have been detected V367F, D614G and N501Y with a valine-phenylalanine substitution on position 367, a glycine-aspartic acid exchange on 614 or asparagine-tyrosine mutation on 501 sites. This last variant, which was reported in England in December 2020, with supplementary mutations in Australia and New Zealand such as K417N (lysine to asparagine on site 417) or E484K (glutamate to lysine on site 484), appears to be responsible for an increase in contagiousness and speedier transmission of the disease. In the same way since October 2020, a double mutation L452R and E484Q has been reported for the Indian variant B.1.617 which was responsible for a record in contamination in this country. In more, all over the world since the beginning of 2021, for B.1.617 species, other mutations and deletions increasing transmission, infectivity and viral virulence have been described, such as a double substitution L452R and T478K two simple substitutions P681R and T19R and a double deletion (157–158 Δ) in a virus known as the delta viral variant. More recently in the B1.1.529 new variant "omicron" first detected in november 2021 in southern Africa no less than twenty mutations or deletions have been found such as N501Y asparagine-tyrosine exchange able to increase viral transmission.

While virus mutations during multiplication is a frequent phenomenon one may wonder whether or not the mutation might be able to modify the efficiency of vaccines developed from the original SARS-CoV-2 virus if these vaccines are prepared against sequences from regions likely to give rise to mutations. Questions can also be asked about the copper amino acid aerosol treatment proposed in the present paper as a mean of impeding SARS-CoV2 replication. The answer to this second question will be given when considering the stability constants for copper (II) amino acid complexes.

Table 1
Stability constants formation for copper cupric amino acid complexes.

AMINO ACID		log β = log (K1 . K2)	Reference
Alanine	A	15.85	[9]
Arginine	R	13.66	[8]
Aspartic acid	D	15.9	[10]
Asparagine	N	14.29	[11]
Cysteine*	C	*	[12]
Glutamic acid	E	14.91	[8]
Glutamine	Q	13.4	[8]
Glycine	G	13.9	[9]
Histidine	H	17.5	[13]
Isoleucine	I	14.68	[8]
Leucine	L	14.69	[8]
Lysine	K	13.7	[14]
Methionine	M	14.08	[8]
Phenylalanine	F	17.56	[6]
Proline	P	17.83	[6]
Serinddde	S	14.01	[15]
Thddreonine	T	14.01	[8]
Tryptophan	W	15.32	[8]
Tyrosine	Y	17.53	[6]
Valine	V	14.25	[9]

* It is not possible to give a value for a cupric-cysteine complex formation since Cu^{++}, subsequent to a dismutation process, oxidizes the cysteine thiol group into cystin. A stability constant about log β = 10 is given for a cuprous Cu^{+} cystein complex [12].

Thermodynamics on copper amino acid complexes

On compiling the scientific literature about the subject, one can find that the values given for these stability constants had not the same meaning according to all researchers. We found that log β_2 could be the stability constant relative to equilibrium II only or relative to the two successive equilibria I + II. (see paragraph above) Though side chains might be involved in copper amino acid chelation, we have chosen to report on Table 1 the global stability constant for the succession of chemical equilibria I + II. It leads to the formation of the complex [Cu-$(AA)_2$] with a resulting stability constant being the product of the stability constants K_1 and K_2. This makes possible a comparison between all the proteinogenic amino acids that might be exchanged in SARS-CoV-2 variants.

On examining these data, it appears that stability constants for these very highly stable copper complexes are in the same magnitude order whatever the amino acid indicating that substitution of an amino acid for another will not affect the copper chelation of amino acids in the spike region.

Concerning the N501Y "English mutant", an asparagine residue has been substituted for a tyrosine amino acid in position 501, and we are convinced that a such amino-acid substitution on the spike protein site will not affect the effectiveness of a cupric aerosol treatment. The stability complex constant log β = 14.29 [11] for copper asparagine and log β = 17.53 [6] for copper tyrosine, leading to a more stable copper chelate, indicate that the copper aerosol impeding of the spike furin-like cleavage will not be disturbed by this amino acid exchange in the spike region. In the same way, in the New York variant S477N where serine and asparagine are exchanged on site 477, it appears that copper chelation will not either be hindered in an aerosol treatment since stability constants log β = 14.29 [11] for copper asparagine and log β = 14.01 [15] for copper serine are almost identical.

In the Indian variant B.1.617 already found in april 2021 in Europe, a mutation has been reported between a leucine and an arginine residues (log β = 14.69 and 13.66 respectively) on site 452 and a swapping on site 484 between glutamic acid and glutamine (log β = 14.91 and 13.40 respectively). More recently in France a variant derived from clade 19B known as "Henri Mondor variant" circulates in different regions of France [16]. In this variant two deletions have been described and among eighteen amino acid exchanges, only eight substitutions have been found in the spike protein domain. In this spike region, the substituted amino acid with the lowest copper chelate stability constant has been observed in a N501Y asparagine-tyrosine mutation (log β = 14.29 and 17.53 respectively) similar to the above English mutant. Concerning the amino acid exchanges observed for the Henri Mondor mutant and the Indian variant, stability constants for copper chelates are all of the same order of magnitude and over than 10^{13}. This fact indicates that such mutations still leading to highly stable copper complexes will not be able to hinder the copper aerosol viral replication blocking.

Copper aerosols and nasal sprays for improved breathing

In France, copper-enriched hypertonic or isotonic nasal sprays have been proposed to improve breath in case of colds, rhinitis or sinusitis. The main salt in these aerosols is penta-hydrated copper sulphate and the inhaled volume for each spray is estimated at 0.3 mL. These nasal sprays are widely used and perfectly tolerated and no annoying deleterious effect has ever been reported as requiring a report to pharmacovigilance services.

Concerning pulmonary diseases, it has also been shown that a copper deficit might be responsible for emphysema and clinical trials involving copper-heparin inhalation have been proposed by a Dutch team to restore better breathing [17]. It should be noted that no deleterious side effect has ever been reported with this type of copper heparin treatment. Moreover, inhalation experiments with nebulized ^{99m}Tc-heparin have demonstrated that deposits in the lungs seem to be remarkably low and

that less than 15% of the nebulizer charge reaches the mouth, while 7.6% reaches the lower respiratory tract [18]. Though heparin anticoagulant properties have been used for treatment of SARS-CoV-2 infection [19], copper-heparin inhalation would probably not be the best choice for complexing the four amino acid insert (-P-R-R-A-) in the spike (S) protein site. In fact, the stability constant in heparin copper complex formation is log β = 4 [20], meaning that it is not a very strong stable copper chelate, and though copper might possibly be exchanged from copper heparinate and chelate the four amino acids inserted in the SARS-CoV-2 spike protein junction, it would be better, to introduce copper near the virus in its free ionic form Cu^{++} in order to avoid competitive chelating reactions.

Considering these two applications of "copper aerosols" for improved breathing, it appears necessary to determine the best copper salt for a COVID aerosol or nasal spray treatment. On the earth, copper is found in various chemical forms: inorganic, organic or complexed salts. Inorganic copper salts such as sulphate or chloride result from neutralization of $Cu(OH)_2$, a weak base with strong acids. Inorganic copper salts are subjected with water to hydrolysis and to resultant "strong acid" liberation in the medium. This factor can explain the toxicity of these mineral salts by corrosion of mucous membranes. In organic soluble salts (malate, lactate, pyroglutamate, gluconate, acetate), the metal is bound to weak organic acids such as those found in food. In these forms, copper is well-assimilated and basic acid balance depends on the pKa of the organic acid. The third form of copper is complexed with amino acids or chelating agents and has no effect on basic acid balance.

Concerning copper homeostasis, it has been estimated that a daily intake of 0.75 mg can maintain a stable copper balance in a 70-kg adult [21]. In humans, copper toxicity depends on its chemical nature and the route of exposure. Copper is a respiratory irritant and inhalation exposure may cause irritation of the respiratory tract, including corrosion of mucous membranes [22]. Other signs and symptoms of inhalation exposure to copper salts include congestion of mucous membranes and ulceration of the nasal septum [23] and the oral emetic dose for copper sulphate is 250 mg [24]. Subsequent to these different studies, the safe upper limits for copper intake have been set at 10 mg/day for women and 12 mg/day for men [25].

Hypothesis evaluation

Choice of copper acetate for an aerosol COVID treatment

In order to complex with copper, the four spike-insert amino acids (-P-R-R-A-), inorganic copper salts are to be avoided, insofar as subsequent to hydrolysis they may release strong acids and attack the pulmonary mucosa. Moreover, copper amino acid complexes are destroyed in an acidic medium whereas on the contrary dissolved copper Cu^{2+} has a high affinity with α-amino acids in neutral and alkaline aqueous environments [26]. Copper complexes should likewise be avoided since nothing is known about the stability constants of copper with the four amino acid peptide (-P-R-R-A-) in the spike insert. It will result the formation of a more stable copper complex between the (-P-R-R-A-) insert and the copper chelate added within the aerosol.

Finally, in light of these informations, the best choice to hamper SARS-Cov2 replication is to select copper salts yielding copper in its ionic form Cu^{++} in solution. This will be possible with organic acids such as acetic, gluconic, lactic, pyroglutamic, or malic acids (pKa = 4.76, 3.86, 3.86, 3.61, and 3.46 respectively), which are naturally found in the body, possibly limiting their toxicological effects. In this way, in vitro with gluconate salt on concentrations above 200 μM, it has recently been shown [27] that copper was able to mitigate SARS-CoV-2 infection in Vero E6 cells though this effect is abolished in solution in the presence of albumin. In fact copper gluconate wouldn't be the best choice for use in a copper treatment since the stability constant for copper gluconate is log β = 36.6 [28]). A quick calculation for a 200 μM copper gluconate solution indicate a free copper Cu^{++} concentration about 5.8 10^{-13} M, demonstrating that copper will not mainly predominate in the free ionic form Cu^{++} in such solutions. This value log β = 36.6 is much higher than those for copper amino-acid chelates (Table 1) meaning that it will not be possible to exchange this metal from copper gluconate for chelating amino acids in the SARS-Cov2 spike virus region. Concerning all the organic acids mentioned above, on the other hand it appears that acetic acid having the greatest pKa will present the least aggressive acidic properties against the mucous membranes, and probably the fewest toxicological effects. In more the stability constant log β = 1.76 for copper acetate [29], allows copper to be mainly in the free ionic form Cu^{++} in solution. A similar quick calculation as made for a 200 μM copper gluconate indicate for copper acetate in solution a free copper Cu^{++} concentration about 3.05 10^{-3} M altogether compatible with chelation of amino acids in the virus spike region.

Experimental data

In order to verify the possible toxicological acidic effects of a copper acetate aerosol on the respiratory tract, we have measured the pH of an isotonic (NaCl 0.9%) copper acetate aerosol solution prepared according to the safe upper limits for copper (12 mg/day for men [25]). For a copper acetate aerosol treatment, it can be estimated that a maximum volume of 10 mL of aerosol may be inhaled daily. This will occur with an upper limit for copper of 12 mg/day for men to a maximum concentration of 1.2 g copper per liter in the nebulizer aerosol solution.

Since the measured pH of a such solution (pH = 4.32) is not compatible with a physiological aerosol treatment we have tried to enable a pH increase, without copper hydroxide $Cu(OH)_2$ precipitation, by diluting and alkalizing the isotonic copper acetate solutions. The relevant results are given in Table 2 and the corresponding volumes for safe upper limits of copper (12 mg/day) in humans have been calculated.

Since skin pH approximates pH 5.5, it appears possible to use copper acetate solutions in aerosol nebulizers or nasal sprays. For copper acute toxicity by oral administration in rats, a lethal dose LD50 of 501 mg/kg has been given for copper acetate and 1710 mg/kg for cupric gluconate [30].Transposition of this copper acetate value for a 70 kg man result to a 35 g copper acetate lethal dose.

In a commercial hypertonic copper nasal spray solution with a given inhaled volume approximating 0.3 mL for each inhalation, we have measured a pH = 6.49. A quick calculation with a copper acetate aerosol spray solution 0.1 g/L in copper, demonstrates that it needs between three and four hundred inspirations a day to reach the maximum safe upper limit for a daily copper intake (12 mg/day [25]). For nebulizing aerosol solutions, analogous calculations can allow an estimation of the optimal duration of a safe aerosol session.

In the future, subsequent to mutations, it will probably be possible to SARS CoV-2 to be modified by amino acid exchange in the spike region leading to new viruses variants. It will then be necessary to verify with the new variant mutations if the stability constant with another amino acid still matches with a highly stable copper complex able to impede the SARS-CoV-2 replication by copper aerosolization. This could be done by consulting the above Table 1 or better the compilation tables on Stability constants of metal-ion complexes [31].

Table 2
How to increase pH for copper isotonic aerosol acetate solutions without copper hydroxide precipitation.

isotonic copper acetate Cu g/L	measured pH	$Cu(OH)_2$ pH précipitation	volume corresponding to 12 mg Cu daily
1.2	4.32	5.23	10 mL
0.5	5.30	5.82	24 mL
0.25	5.63	6.17	48 mL
0.1	5.97	6.30	120 mL
0.05	6.42	–	240 mL

Consequences of the hypothesis and discussion

In conclusion, since the objective of copper acetate aerosolization is first to impede SARS-CoV-2 replication, it is clear that such a treatment may lead to clinical perspectives that are not in opposition but complementary to vaccination. While the herd immunity expected from mass vaccination of the population will not be possible before several months, for lack of vaccine and virus mutations, it appears that copper acetate aerosol or nasal spray treatments, could also help in the fight against virus spread. Though copper is a constituent of physiological proteins such as ceruloplasmine, copper enzymes or redox proteins, it must also be noticed that it may have toxicological effects towards macromolecules and RNA [32]. Copper acetate, beeing a salt from a weak acid, is however not subject to strong acid hydrolysis and has consequently a low toxicity. It overcomes aggressiveness towards mucous membranes due to strong acid liberation in the upper pulmonary tract as may occur with mineral salts such as copper sulphate. In solution, copper acetate predominates as free Cu^{++} ions that facilitate the copper complexation of amino acids inserted on viral proteins. The compilation of stability constants for copper amino acid complexes demonstrates that amino acid exchanges such as those observed for virus variants will not disturb copper amino acid complexation in the virus spike region.

Since we have shown that a copper acetate nasal spray 0.1 g/L in copper corresponds to physiological pH values and requires between three and four hundred inspirations a day to reach the maximum safe upper limit for a daily copper intake, such a copper acetate solution (0.1 g/L copper) could be used for safe aerosol treatments and to an even greater extent in nasal spray filling for asymptomatic positive COVID patients in order to impede virus dissemination.This might complement vaccination in an effort to definitively eradicate COVID 19 and help a return to a practically normal life

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the Spike protein of SARS-CoV-2 is essential for infection of human lung cells. Molec. Cell 2020;78(4):779–84. https://doi.org/10.1016/j.molcel.2020.04.022.

[2] Romàrio de Jesus J, Araùjo AT. Understanding the relationship between viral infections and trace elements from a metallomics perspective. implications for COVID-19.Metallomics 2020;12:1912. https://doi.org/10.1039/d0mt00220h.

[3] Ishida T. Antiviral activities of Cu^{2+} ions in viral prevention, replication, RNA degradation and for antiviral efficacies of lytic virus, ROS-mediated virus, copper chelation. World Sci. News 2018;99:148–68.

[4] Andreou AA, Trantza S, Filippou D, Sipsas N, Tsiodras S. The Potential Role of copper and N- acetylcysteine (NAC) in a Combination of Candidate Antiviral Treatments Against SARS-CoV-2. In vivo. Review (COVID-19) 2020;34:1567–88. https://doi.org/10.21873/invivo.11946.

[5] Hutasoit TN, Kennedy B, Hamilton S, Luttick A, Rahman Rashid RA, Palanisamy S. Sars-CoV-2(COVID-19) inactivation capability of copper-coated touch surface fabricated by cold spray technology. Manuf. Let. 2020;25:93–7. https://doi.org/10.1016/mfglet.2020.08.007.

[6] Tanaka M, Tabata M. Stability constants of metal(II) complexes with amines and aminocarboxylates with special reference to chelation Bull Chem Soc.Jpn 2009; 82 (10):1258-1265. doi:10.1246/BCSJ.82.1258.

[7] Zolotarev YA, Kurganov AA, Semechkin AV, Davankov VA. Determination of stability constants of fixed-site complexes of copper(II) ions and sorbed Copper-L-proline complexes with an asymmetric resin containing L-proline groups. Talanta 1978;25:499–504. https://doi.org/10.1016/0039-9140(78)80083-6.

[8] Hallman PS, Perrin DD, Watt AE. The computed distribution of Copper (II) and Zinc (II) ions among seventeen amino acids present in human blood. Biochem. J 1971; 121:549–55. https://doi.org/10.1042/bj1210549.

[9] Dogan A, Köseoglu F, Kiliç E. The stability constants of copper complexes with some α-aminoacids in dioxin-water mixtures. Anal Biochem 2001;295:237–9. https://doi.org/10.1006/abio.2001.5205.

[10] Bottari E, Festa MR, Jasionowska R. Copper (II) complexes with aspartate and glutamate. Polyhedron 1989;8(8):1019–27. https://doi.org/10.1016/s0277-5387(00)81114-6.

[11] Gergely A, Nagypal I, Farkas E. Thermodynamic relations of parents and mixed complexes of asparagine and glutamine with copper(II). J. Inorg. & Nucl. Chem 1975;37:551–5. https://doi.org/10.1016/0022-1902(75)80371-X.

[12] Rigo A, Corazza A, di Paolo ML, Rosetto M, Ugolini R. Scarpa M Interaction of copper with cysteine stability of cuprous complexes and catalytic role of cupric ions in anaerobic thiol oxidation. J. Inorg. Biochem 2004;98:1495–501. https://doi.org/10.1016/jinorgbio.2004.06.008.

[13] Deschamp P, Kukkarni PP, Gautam-Basak M, Sarkar B. The saga of copper(II)–L-histidine. Coord Chem Rev 2005;249(9–10):895–909. https://doi.org/10.1016/j.ccr.2004.09.013.

[14] Yamauchi O, Odani A. Stability constants of metal complexes of amino acids with charged side chains. Part 1 Positively charged side chains (Technical Report). IUPAC. 1996;68(2):469–96 (p 485).

[15] Sharma V. The stability constants of metal complexes of serine and threonine. Bioch. Biophys. Acta 1967;148(1):37–41. https://doi.org/10.1016/0304-4165(67)90276-0.

[16] Fourati S, Decousser JW, Khouider S, N'Debi M, Demontant V, Trawinski E, et al. Novel SARS-CoV-2 Variant Derived from Clade 19B Emerg. Infect. Dis. 2021;27(5): 1540–3. https://doi.org/10.3201/eid2705.210324.

[17] R. Janssen R, Wouters EFM ,Janssens W , DaamenWF Hagedoorn P, de Wit HAJM et al. Copper-Heparin Inhalation Therapy To Repair Emphysema: A Scientific Rationale Int. J Chron. Obstruct Pulmon Dis. Dovepress 2019; 14: 2587-2602. doi . org/10.2147/COPD.S228411.

[18] Bendstrup KE, Chambers CB, Jensen JI, Newhouse MT. Lung deposition and clearance of inhaled (99m)Tc-heparin in healthy volunteers. Am. J. Resp. Crit. Care. Med. 1999;160(5):1653–8. https://doi.org/10.1164/ajrccm.160.5.9809123.

[19] Hippensteel Joseph A, LaRiviere Wells B, Colbert James F, Langouët-Astrié Christophe J, Schmidt Eric P. Heparin as a therapy for COVID-19: current evidence and future possibilities. Am. J. Physiol. Lung Cell. Mol. Physiol 2020;319 (2):L211–7. https://doi.org/10.1152/ajplung.00199.2020.

[20] Mukherjee DL, Park JW, Chakrabarti B. Optical properties of Cu(II)complexes with heparin and relative glycosaminoglycans. Arch. Biochem. Biophys 1978;191(1): 393–9. https://doi.org/10.1016/0003-9861(78)90103-0.

[21] Shike Moshe, Roulet Michel, Kurian Regina, Whitwell Jocelyn, Stewart Sandy, Jeejeebhoy Khursheed N. Copper metabolism and requirements in total parenteral nutrition. Gastroenterol 1981;81(2):290–7. https://doi.org/10.1016/S0016-5085(81)80060-1.

[22] Reigart JR , Roberts JR Copper Compounds. Recognition and Management of Pesticide Poisonings, 5th ed. U.S. Environm. Prot. Agency, Off Prev., Pest.& Tox. Subst. Off. Pest.Prog., U.S. Gov.Print. Off. Washington, DC 1999; 145-146.

[23] Schienberg HI Copper, alloys, and compounds . Encycl. Occup. Health and Safety, 3rd ed.; Parmeggiani, L. Ed. Int. Labour Office: Geneva, Switerzland. 1983; 546-548.

[24] Romeu-Moreno A, Aguilar C, Arola LL, Mas A. Respiratory toxicity of copper. Environ. Health Perspec. 1994;102(s.3):338–40. https://doi.org/10.1289/ehp.94102s3339.

[25] Institute of Medicine (US) Panel on Micronutrients. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc.Washington, DC: Natl. Acad. Press (US) 2001.

[26] Norio Kitadai Dissolved divalent metal and pH effects on Amino Acid polymerization: A thermodynamic evaluation. Orig. Life Evol.Biosph. 2017; 47: 13–37. doi:10.1007/s11084-016-9510-5.

[27] Rodriguez Killian, Saunier Florian, Rigaill Josselin, Audoux Estelle, Botelho-Nevers Elisabeth, Prier Amélie, et al. Evaluation of in vitro activity of copper gluconate against SARS-CoV-2 using confocal microscopy-based high content screening. JTEMB 2021;68:126818. https://doi.org/10.1016/j.jtemb.2021.126818.

[28] Sawyer DT. Metal gluconate complexes Chem. Rev 1964; 64(6): 633-643. doi. org10.1021/cr 60232 a003.

[29] Bunting John W, Thong Kain Men. Stability constants for some 1:1 metal-carboxylate complexes. Can. J. Chem 1970;48(11):1654–6. https://doi.org/10.1139/v70-273.

[30] Acute oral toxicity, OECD 401 method, OECD guidelines for the testing of chemicals Section 4 Edition OCDE Paris 1987. https//doi.org/10.1787/9789264040113-en.

[31] Sillén LG , Martell AE , Schwarzenbach G. Stability constants of metal-ion complexes Special publication n°17 (1964) The Chem. Soc. Ed., Burlington House London W 1.

[32] Linder Maria C. The relationship of copper to DNA damage and damage prevention in humans Mut. Res./Fund. & Molec. Mech. Mutag 2012;733(1-2):83–91. https://doi.org/10.1016/j.mrfmmm.2012.03.010.