

Disease Prevention Control

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The preferred agent for the eradication of external parasites from marine fish and some freshwater fish is copper. Still, despite a long history of use, many discordant recommendations of expert aquarists betray a limited understanding of the basic chemistry of copper in the marine aquarium.

Atoms, the elemental units of matter consist of a positively charged nucleus that is surrounded by a spherical field of orbital negatively charged electrons. Nuclei display a broad spectrum of attractive force for outer electrons, and those elements with strongly attracting nuclei tend to accept extra electrons and become negatively charged ions (for example, chloride), while those with weak nuclei tend to donate electrons and become positively charged ions (copper). Usually, these ions will neutralize their charges by electrostatic association and form salts or ionic compounds (copper chloride). Those elements with moderately strong nuclei do not accept electrons but share them with other moderately strong or weak nuclei, resulting in neutral, or almost neutral, compounds, respectively. When moderately strong nuclei share with weak nuclei, the electrons occupy the orbital spheres of the strong nuclei more often than they do those of the weak, resulting in polar compounds with positive and negative zones (for example, water). Polar compounds or polar segments (groups) of a compound can have electrostatic attraction for other charged groups or can share a full or partial negative charge with a weak acceptor and, thereby, form coordinate compounds or complexes. Some negatively charged ions (chloride, bromide, iodide) are also capable of sharing electrons and forming coordinate complexes.

When soluble copper salts are added to water, the salts dissociate into positively charged cations (the ionic copper) and negatively charged anions (sulfate, chloride, acetate, etc.), and these ions become hydrated, or complexed, with water molecules. This is depicted in Figure I.

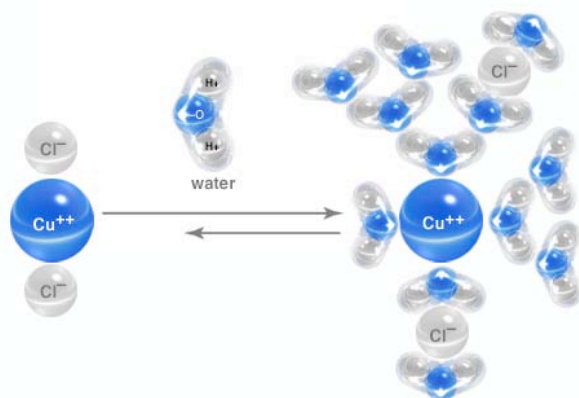


FIGURE 1

The dissolving of copper chloride in water. The symbols are Cu = copper; Cl chloride; o = oxygen; H = hydrogen. Observe that water (H₂O) is a polar substance with a positive and negative zone and that, when an ionic salt like copper chloride is added to it, water molecules, which are present in excess, arrange their negative charges around the positively charged cupric ion and their positive charges around the negatively charged chloride ions, effectively separating the components of the salt into dissociated cupric and chloride ions. The long arrow indicates that this dissociation takes place much more extensively than the reassociation of the ions (short arrow).

Water molecules are present in excess and, therefore, effectively keep the ionic copper isolated from its parent anion. Although random interaction with the parent anion does occur to a limited extent, they rapidly dissociate again. This is indicated by the long and short arrows. The equilibrium, or the direction of the activity, favors the formation of hydrated ions. In simpler terms, this is akin to taking a thousand steps forward and one backward. This is what makes the salt soluble! If the equilibrium favored the association of cation and anion rather than dissociation, the salt would be relatively insoluble.

A solution containing ionized copper that is open to the air eventually loses its copper through precipitation of an insoluble copper salt. If fish are present, this takes place even more rapidly. This is due to the absorption of carbon dioxide from air and its release by fish into water, where it dissolves and forms carbonic acid, which dissociates into hydronium and carbonate ions. Ionic copper interacts with this carbonate, and here the equilibrium favors association rather than dissociation, resulting in the precipitation of insoluble copper carbonate. This is what happens to stock solutions of copper sulfate as well as to copper salts in the freshwater aquarium.

The marine aquarium is different. It contains several anions which interact with ionic copper: chloride, sulfate, phosphate, carbonate, molybdate, borate, iodide, and bromide. The most important, both because of its high concentration and strong affinity for copper, is chloride. The chloride anion forms a four-membered complex with copper (Figure 2) and virtually inhibits any interaction of copper with carbonate. The addition of sodium chloride to an insoluble suspension of copper carbonate will cause the copper salt to dissolve. Even if copper carbonate were to form in the marine aquarium, it would readily redissolve. Precipitation of copper carbonate, then, is not a mechanism of copper loss in the marine aquarium. Because of the strong formation of the chloride-cupric complex, most copper salts added to the marine aquarium are equivalent, and, provided no filtration is used, all are much more stable than in fresh water. Why, then, does copper rapidly disappear from solution in the bottom filtered marine aquarium?

As Randy Keith demonstrated in his FAMA (Vol. 3, No. 1) article, and as could have been deduced from simple chemical considerations, copper is precipitated primarily by filtration through filterants containing magnesium carbonate. Simple experiments readily demonstrate that copper is rapidly removed from marine solutions by magnesium carbonate but not calcium carbonate. Any chemist familiar with separation

technology knows that copper, iron, zinc, and other metal ions can readily be removed from solution by adsorption on magnesium carbonate. Simple experiments also readily demonstrate that the rate of loss of copper on magnesium carbonate filterants is inversely proportional to the salinity. The chloride-cupric complex, then, inhibits, but does not prevent, the absorptive loss of copper. The consequently necessary repeated dosing when treating fish is an inconvenience that results in a dangerous accumulation of copper in the filter bed. This copper is potentially lethal to fish, makes invertebrate culture difficult or impossible, and interferes with the biological filter's full potential.

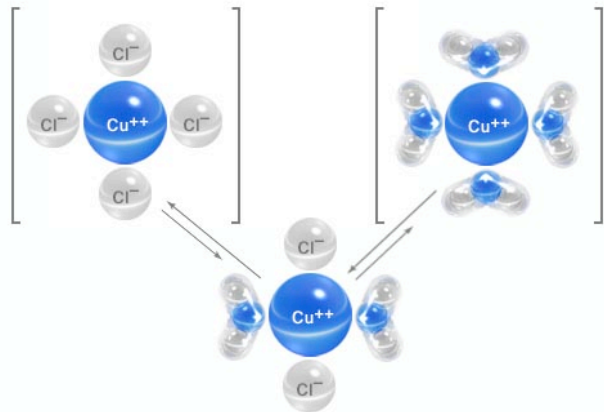


FIGURE 2

The formation of chloride-copper complex in marine water. The symbols are the same as in Figure 1. Observe that water forms a four-membered complex with copper, leaving the cupric positive charges intact. In the presence of excess chloride ions, as in the marine aquarium, chloride displaces water and forms a four-membered chloride-copper complex that has two negative charges. The arrows indicate that the reaction is reversible, but the length of the arrows show that the chloride-copper complex is the predominant form. This is true of any ionizing copper salt added to marine water.

Generally, metal ions form stable complexes and copper forms some of the most stable complexes. The water-copper complex is so stable that when a solution of copper chloride is evaporated the complex does not break down and the final substance is actually $[\text{Cu}(\text{H}_2\text{O})_2]^{++} - \text{Cl}_2$. In the presence of excess chloride ions, as in the marine aquarium, the predominant form is $[\text{CuCl}_4]^{--}$, a relatively stable complex ion that forms regardless of whether cupric chloride, sulfate, or

acetate were used. There is no sound chemical basis for asserting that copper sulfate is better or worse than copper chloride in the marine aquarium. With the water-cupric complex the link to water is through oxygen, while with the chloride-cupric complex the link is through the chloride. This link, or bond, is usually called a ligand. For copper, chloride is a stronger coordinate ligand than oxygen. Of other possible ligands, which include carbon, nitrogen, and sulfur, nitrogen is preferred by copper and, generally, will form the most stable complexes. A typical nitrogen complex of copper is ammoniated copper, $[\text{Cu}(\text{NH}_3)_4]^{++}$ (See Figure 3). Although ammoniated copper is not suitable for use in an aquarium, it is much more resistant to adsorptive loss than is the chloride complex. The concentration of the ligand is also a factor in stabilizing a copper complex: Stability increases with increasing ligand concentration. The formation of ring structures also contributes to complex stability, a good example being the ethylenediamine tetraacetate complex. This represents a special class of complexes called chelates. Chelates are very stable structures and those that are water soluble are called sequestering or inactivating agents, because they effectively isolate metal ions and render them non-reactive. Complexes and chelates may be formed through any combination of ionic, covalent, or coordinate interaction, but coordinate bonds are characteristic of complexes while covalent, or ionic, bonds are almost always involved in the formation of stable chelates. Both complexes and chelates may be negative, positive, or neutral. Negative copper complexes result from coordinate bonding with negatively charged ions, such as chloride, while positive complexes result from coordinate bonding with neutral but polar, molecules or groups, such as ammonia or amine compounds. Observe from Figure 3 that, when covalent or ionic bonds are involved, chelated copper loses its charge or ionic properties. This is characteristic of stable chelates such as EDTA, which is used in chelated copper products.

Looking now at specific recommendations for the use of copper in the marine aquarium, it is evident that the

use of any ionizing copper salt is equivalent and will yield $[\text{CuCl}_4]^{--}$, a negatively charged complex that is only moderately resistant to magnesium carbonate adsorption. The most widely recommended copper salt is cupric citrate or a copper salt combined with citric acid. Since a ring structure is formed this is a chelate, but six and seven membered rings are not exceptionally stable. Further, covalent bonds are involved, resulting in an essentially uncharged complex. This makes cupric citrate almost insoluble. It is soluble only under strongly alkaline or acidic conditions and is useful only in keeping stable stock solutions. Cupric tartrate (copper combined with Rochelle salt) forms a more stable complex, but is also insoluble under aquarium conditions. In both of these, the copper is uncharged and unavailable, except as it complexes with chloride. Usually, "chelated copper" refers to commercial forms of copper complexed with EDTA as shown in Figure 3 (next page).

This chelate is both stable and soluble under aquarium conditions. Unfortunately, it is also sequestered or inactivated, unavailable as a toxic agent either to fish or parasites. In the EDTA chelate copper is bonded through both covalent and coordinate bonds and is the center of three locked ring structures, effectively isolating any cupric charge and rendering it totally inactive. If it were not for the competition for copper from the high chloride concentration of the marine environment, it is doubtful that chelated copper would have any effectiveness. Acetic acid with a copper salt, or cupric acetate, is sometimes recommended as a more stable form of copper. This has some merit since acetate does form a complex with copper, but this is only slightly more stable than the chloride complex. Another recommendation has been to buffer acetic acid with tris (trishydroxymethylaminomethane). Although this does not appear to be recommended to stabilize copper, but to act as a buffer, the use of tris is actually one of the better recommendations to stabilize copper that have been made. Tris is an amine compound and it forms complexes with copper in the same manner that ammonia does, rendering the cop-

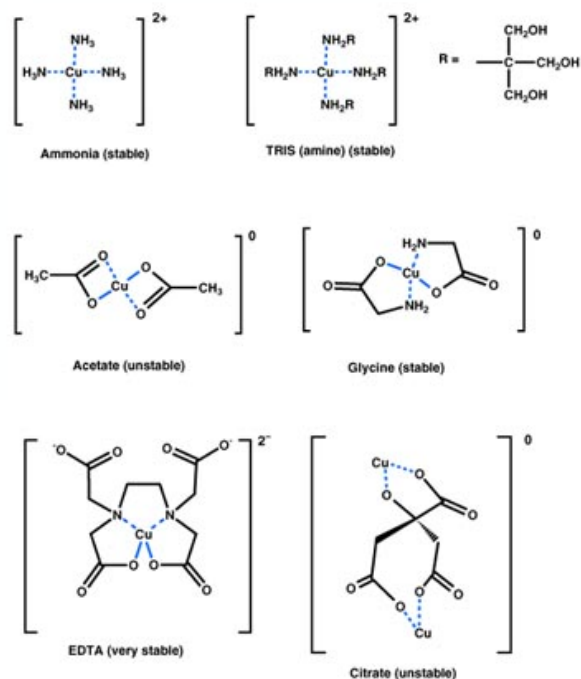


FIGURE 3

Representative copper complexes and chelates. The symbols are the same as in Figure 1 with the addition of N= nitrogen; R = any designated group such as that of "Tris." The first two complexes are that of ammonia (NH₃) and that of an ammonia analog or amine where some of the hydrogens of ammonia are replaced by any of several thousand possible R groups. One such R group yields Tris, an often used fish buffer. The rest of the complexes shown involve ring structures and are, consequently, chelates. Cupric acetate is a very unstable structure which may not actually ever exist as shown; but acetate does form a weak complex with copper. Glycine is an amino acid and occurs naturally in the aquarium; it is a good chelating agent. EDTA (ethylenediamine tetraacetate) is a very powerful sequestering agent that forms triple locked rings with copper; it, or closely related sequestering agents, are used in commercial "chelated copper" products. Citrate is an unstable weakly chelating salt used to maintain stock solutions of copper. Observe that ammonia and amine complexes retain the positive cupric charge, while the other complexes are either neutral or negatively charged. Note also that all the stable complexes are linked to copper through nitrogen.

per quite resistant to magnesium carbonate adsorption, leaving it fully charged, neither sequestered nor inactivated. Although the complex is not totally stable, it represents a remarkable improvement over other types of copper. There are numerous organic compounds that are capable of forming this type of com-

plex with copper, some more effective than others, with varying degrees of toxicity. Their use requires thorough evaluation and testing. Generally, loss of the cupric positive charge, or chelation, or both decrease the effectiveness of copper. One amine-complexed copper product that has shown excellent results in stability, effectiveness, and low order toxicity to fish is Seachem's Cupramine™

What is the mechanism of parasite and fish toxicity for copper and how does this relate to marine copper types? When this question comes up it is generally suggested that copper reacts with sulfhydryl groups, inactivating vital intracellular enzymes and other proteins. Although copper does inactivate sulfhydryl enzymes and binds proteins, it does not seem likely from current knowledge of cellular biochemistry that this is a likely mechanism. Only charged (ionic) copper is effective at the usual recommended concentrations (less than 0.3 ppm copper). Membrane biochemistry suggests it unlikely that charged copper at such low concentrations could pass through cellular membranes sufficiently to cause severe intracellular damage. Body fluid analyses of treated fish show no significant increase of copper during normal treatment. This supports the supposition that ionic copper does not pass through cellular membranes. It seems more likely that ionic copper acts as a membrane poison, binding to membrane components, causing disruption of normal membrane functions, and leading ultimately to osmotic shock. This is suggested by personal observations of apparent swelling and distortion of Cryptocaryon tomites exposed to copper. Also, the secretion response of fish and their respiratory distress when treated with copper is consistent with this interpretation.

A comparison of the negatively charged chloride-cupric complex (using copper sulfate) and the positively charged amine-cupric complex (using Cupramine™) indicates that the amine complex destroys tomites more rapidly, or at a lower concentration, than

does the chloride complex. When tomites were exposed to 0.2 ppm copper as copper sulfate, or Cupramine™, those exposed to copper sulfate required close to two hours to show evidence of 50% disruption or kill, while those exposed to Cupramine™ required about 45 minutes. Fish treated with copper sulfate showed severe distress after 12 hours at 0.4 ppm and body fluids showed increased copper. Fish treated with Cupramine™ showed severe distress after 12 hours at 0.9 ppm and little increased copper in body fluids. Recovery was also more rapid after stressing with amine-complexed copper. This indicates that positively charged amine-cupric complexes are more effective than the usual negatively charged chloride-cupric complex and that larger or positively charged molecules (organically bound amine copper) are less likely to penetrate membranes (less toxic to fish) than smaller or negatively charged molecules (chloride-cupric complex). This also suggests that, possibly, the positively charged water-cupric complex is the form active against parasites while the negatively charged chloride-cupric complex is more toxic to fish. This difference between amine-complexed copper and chloride-complexed copper probably accounts for varying experiences of success and failure in treating fish with copper, since marine aquaria contain varying amounts of natural chelates and complexing agents that will increase or decrease the effectiveness and toxicity of copper. Amino acids are good examples of such agents, as they are natural by-products of the biological environment of the aquarium, and all amino acids are powerful complexing agents. Many amine-type organics are also produced by decaying food and other decaying organic matter.

The EDTA-copper chelate passes relatively freely through membranes and fish treated with it show significant elevations of copper in body fluids. Evidence suggests slow deposition of copper in internal organs, although there are no apparent short-term toxic effects. Cryptocaryon tomites treated with 2 ppm EDTA

copper show only slight evidence of osmotic shock and the apparent kill rate is at least half that observed with 0.2 ppm ionic copper, although the eventual tomite mortality is high enough to suggest that for chelated copper the toxic mechanism may only partly be osmotic shock, the balance being some type of intracellular poisoning. It can only be assumed that the same type of poisoning takes place in fish, but that mortality does not occur simply because of the greater mass of fish as compared to parasites.

A key factor in the treatment of fish with copper is probability. Success depends on killing susceptible parasites before reinfection can occur. The proportion of parasites killed in a given time is dependent on the copper concentration and the chances of reinfection are inversely proportional to the kill rate and directly proportional to the degree of crowding and the extent of the original infestation. It is important, then, to avoid crowding and to use as high a copper concentration as possible without harming the fish. For copper sulfate, a concentration of less than 0.18 ppm has about a 75% chance of success. If maintained for 10 days. Concentrations between 0.2-0.25 ppm have a 90% chance of success, but are approaching a precarious concentration for fish, about 0.3 ppm. The effectiveness and toxicity for copper sulfate, however, is highly dependent on the pH and organic content of the water. Increasing acidity increases toxicity while increasing organic content decreases toxicity. Amine-complexed copper can be used safely at 0.3-0.5 ppm with virtually 98% chance of success. For heavy infestations of resistant parasites, it can be increased quite safely to 0.7-0.8 ppm. Chelated copper is ineffective at less than 1 ppm and at 2ppm it has about a 70% chance of success with little danger to fish. Chelated copper can be increased to 2.5-3.0 ppm, but this can be dangerous for some fish. The length of exposure for any copper type can be decreased from 10-14 days to 6-8 days, without sacrificing success, if the fish are transferred to another treatment tank after the initial 4 days.

Another area of concern to the marine aquarist is the removal of copper in case of over-dosage or at the end of treatment. Chloride-complexed copper and citrate copper fall out of solution fairly rapidly by themselves with standard bottom filtration. This can be accelerated with carbon filtration. For rapid detoxification, chelating agents have been suggested. If this is done in an emergency, only soluble chelates should be used so that they may be removed by water change later. Insoluble chelates will only precipitate the copper out of solution and leave it in the filter bed where it can cause trouble at a later time. It is unwise to use any kind of copper, or copper associated product, that will deposit copper in the filter bed. Amine-complexed copper can be rapidly removed with carbon filtration, but not with bottom filtration. Chelated copper cannot be removed with either carbon or bottom filtration. Polymeric adsorbents and ion-exchangers have also been ineffective in removing chelated copper. The only way to remove chelated copper is by water change.

Formaldehyde is often recommended in conjunction with copper treatment. It is such a highly reactive substance that commercial 37% solutions actually contain less than 0.1% free formaldehyde, the balance being the reaction product with water, methylene diol. During storage, these solutions generate significant quantities of methanol and formic acid. Despite this, there seems to be sufficient anecdotal evidence to support the use of formaldehyde with copper. Short term exposures (1/2 to 1 hour) to about 100 ppm of this powerful irritant is tolerated by fish and seems effective in forcing parasites to detach from fish. When used in a biologically filtered aquarium for an extended time (days), 10-15 ppm have been recommended. This concentration, however, is deleterious to the filter bed and will often result in 30-60% loss of nitrifying capacity. A comparison of the apparent synergistic action of formaldehyde with copper indicates that 2-6 ppm formaldehyde is just as effective as 10-15 ppm and will effect the nitrifying capacity by less than 5%. Such a low concentration of formaldehyde alone has little antibacterial, antifungal, or antiprotozoan activity.

What, then, is the mechanism of action for the combination of copper and formaldehyde? Up to now the only copper mentioned has been cupric copper, the oxidized form of copper or copper with two positive charges. There is another form of copper, the reduced form with only one positive charge, called cuprous copper. Cuprous salts are ten times more toxic to life than cupric salts. The reduction of cupric salts to cuprous salts by aldehydes in alkaline solutions is a well known reaction and is the probable mechanism for the increased effectiveness of copper in the presence of formaldehyde. Under strongly alkaline conditions weakly complexed or chelated copper is reduced fairly rapidly, while strongly complexed copper is not reduced. Close inspection of some commercial products containing formaldehyde and copper under alkaline conditions will reveal a red, brown, or yellow precipitate, cuprous oxide. Citrated copper products containing formaldehyde or another powerful reducing agent, vanadium, are usually acid solutions to prevent this reaction in the bottle. Strongly complexed or chelated copper does not react this way in the bottle. Since cuprous ions have not been detected in treated aquaria, and since formaldehyde does seem to increase copper's toxicity to parasites but not fish, informed conjecture suggests that, possibly, the reduction takes place after copper has become membrane bound. One thing is certain, however, no red, brown, or yellow precipitate from a copper product should be introduced into a marine aquarium. In marine water cuprous oxide will redissolve and, although cuprous salts are reoxidized to cupric salts fairly rapidly, it can easily bring disaster in short order.

Some self-evident, but often ignored, principles in treating fish apply particularly to the use of copper:

- (1) Unless absolutely necessary, do not treat in the display aquarium. Isolate the fish to one or more quarantine tanks instead.
- (2) If treatment in a display tank is unavoidable, do not add anything to the water that cannot be removed. This includes antibiotics, magic conditioners, drugs,

dyes, and precipitating copper salts. Although these medications fall out of solution, they have not been removed, but are only sitting in the filter bed, waiting for the worse possible time to cause trouble. No amount of carbon or miracle filtration can remove precipitated chemicals. The only kinds of copper that do not fall out of solution in the aquarium are chelated and amine-complexed copper. Amine-complexed copper is removable with carbon: chelated copper is not removable except by water change.

(3) Avoid using anything that will interfere with biological filtration. If unavoidable, use only in a quarantine tank without such a filter and make frequent water changes as indicated by ammonia levels. Chelated and amine-complexed copper do not interfere with biological filters. This is not always true of copper sulfate or citrate.

(4) Do not use anything ineffective or medication that will do more harm than good. A fish cured by death is hardly a success. Chelated and amine-complexed copper are both relatively safer than copper salts. Amine-complexed copper, however, is much more effective than chelated copper and is not as readily absorbed by fish.

(5) Always quarantine and treat new fish for prevalent infestations to minimize risk of introducing disease to display tank. Always quarantine new invertebrates as well. Quarantine should be for a minimum of two weeks. Never take a dealer's word on the disease-free condition of the fish you just purchased. Unless he personally quarantines and treats all his fish, he has no way of knowing that a fish is free of disease.