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Application of Triturated Copper Nanoparticles as an Agent for Remediation of an Azo dye, Methyl Orange

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Published: 4 December 2015

Abstract: Use of Azo dyes, having one or more azo bond (-N=N-), are more than 70% among all textile dyes used. The stability and the xenobiotic nature of reactive of these azo dyes make them recalcitrant and hence they are not totally degraded by conventional wastewater treatment processes that involve physical, chemicals or activated sludge methods. The dyes are therefore released into the environment, in the form of colored waste water. Color of the waste effluent is an important parameter for a long time. So the main criteria of removing the waste and degrade is to first remove the color of the effluent. Some common techniques which are used for degrading the chemically complex dye have several harmful side effects and they are not very cost effective and some require long retention time. So extensive research is going on to find a simple method which should obviously be a low cost process, will need less time to complete the process and will have minimum harmful side effects.
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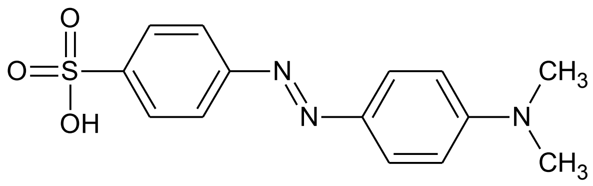
Keywords: Cuprum metallicum, methyl orange, UV-Vis spectroscopy, dye degradation, antimicrobial property

1. Introduction

Synthetically prepared colorful azo dyes are mostly used in textile, leather, pharmaceuticals, paper, and cosmetic industries. These dyes are marked as waste water pollutants. The water effluent which is coming from industrial waste, gets into the water bodies and cause extreme pollution.1Effluent containing compounds are chemically complexed and are not easily degradable for example with the help of bacteria or common chemical degradation process.



Methyl orange dye

Due to their complex structure they sometimes prevent the sunlight to penetrate inside the water bodies, because of which the life cycle of aquatic flora and fauna get hampered. Use of azo dyes, having one or more azo bond (-N=N-), are more than 70% among all textile dyes. **2.** Color of the waste effluent is an important parameter since long time. So the main criteria of removing the waste and degrade is to first remove the color of the effluent. The stability and their xenobiotic nature of reactive azo dyes makes them recalcitrant hence they are not totally degraded by conventional wastewater treatment processes that involve physical, chemicals or activated sludge methods **3**. The dyes are therefore released into the environment, in the form of colored waste water. Some common techniques which are used for degrading the chemically complex dye, are precipitation, coagulation, adsorption, flocculation, flotation, electrokinetic coagulation, silica gel, membrane filtration, ion exchange, activated carbon, NaOCl, photochemical ,electrochemical destruction, fenton's reagent ,ozonation. However, every possible side effects obtained like some techniques produce sludge and harmful by-products. Another important factor is that these methods are not very cost effective and some requires long reaction time. **4 and 5** So scientist are trying to find a simple method which should obviously be a low cost process and will need less time to complete the process. **4 and 5.**

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2. Results and Discussion

Methyl orange, 4-[4-(dimethylamino) phenylazo] benzenesulfonic acid, is an azo dye that forms orange crystals and is also used in laboratories and industries as a coloring agent having a negative impact on natural resources. **6**

Cuprum metallicum is a triturated medicine, which contains copper as a main constituent and by increasing the potency their size become in nanoform.

Conventional drugs available in the market, having antibacterial activity can lead to cause resistivity among different strains of bacteria. So research has been focusing on those materials which are new to the strains and do not have bad impact on the environment. So scientists are opting for alternative methods as antimicrobial agent. Copper nanoparticles are often used as an antibacterial agent**7**

Here cuprum metallicum can be utilized on different aspects with varied application. Different potencies of the drug (Cuprum metallicum) were used against the azo dye methyl orange and tried to degrade the effluent and function as a remediating material. Nanoparticles are very helpful as antimicrobial agent and the drug behaved as nanomedicine at higher potency **5** so we tried to find that whether at higher potency , the drug can show better result or not.

Parallelly they have been used here on pharmaceutical ground against two bacterial strains of gram positive and gram negative bacteria.

Material and methods:

Methyl orange dye was obtained from Merck, Bacterial strains i e, gram positive *Staphylococcus aureus* and gram negative

Escherichia.coli was from Microbial type culture collection and gene bank (MTCC) Institute of microbial technology, Chandigarh, India. Peptone, beef extract, yeast extract, sodium chloride, agar-agar was purchased from HiMedia. Cuprum metallicum of three different potencies were obtained as a gift from HAPCO. All the chemicals were used without purification as are all analytical grades. Throughout the experiment double distilled water was used.

Methods:

10^{-3}M Methyl orange dye was weighted and mixed with water so total solution is up to 300µl and 2ml of Cuprum metallicum of three different potencies (6C,30C,200C) were added individually, distilled water was added and make up to 4ml by volume.

Degradation of Methyl orange:

The kinetic study of the degradation of methyl orange (azo dye) with respect to time was thoroughly studied with the help of UV-Vis spectrophotometer .The time dependant degradation of the dye was investigated through change in the color and the absorbance of the dye. Just after addition of water with dye, the absorbance was measured and the sample was named as sample I. Then sample containing dye was placed in dark condition which was named as sample II. Then sample named as sample III which contained dye and water was placed under light condition only. Sample IV, V, VI contained dye and water .Then cuprum metallicum of 6C, 30C, 200C was added to IV, V, and VI and placed under exposure of visible light source. Finally absorbance was recorded at 464nm. And comparative study was carried out and checked whether cuprum metallicum would help in degradation process if the answer is yes then next query was that is 200C potency of the drug showed better result than 6C and 30C.

Antibacterial activity:

The second application was Cuprum metallicum of three different potencies were used against gram positive *S.aureus* sub species aureus (MTCC no.96) and gram negative *E.coli* DH5 alpha(MTCC no.1652) bacterial strain . The most common method of spread plate technique was used and both the strains were individually treated with Cuprum metallicum of three potencies (6C, 30C, 200C).Bacterial strains were grown in recommended liquid media which contains peptone (0.5%) beef extract (0.1%), yeast extract (0.2%), Nacl (0.5%) and distilled water.10µl of subcultured bacterial strains were inoculated in each test tube along with drug of different potencies. After overnight growth with antibacterial agent they were plated next day in an agar plate (Liquid media with 1.8% agar-agar) and colonies were counted with reference to control plate where no antibacterial agents were given..

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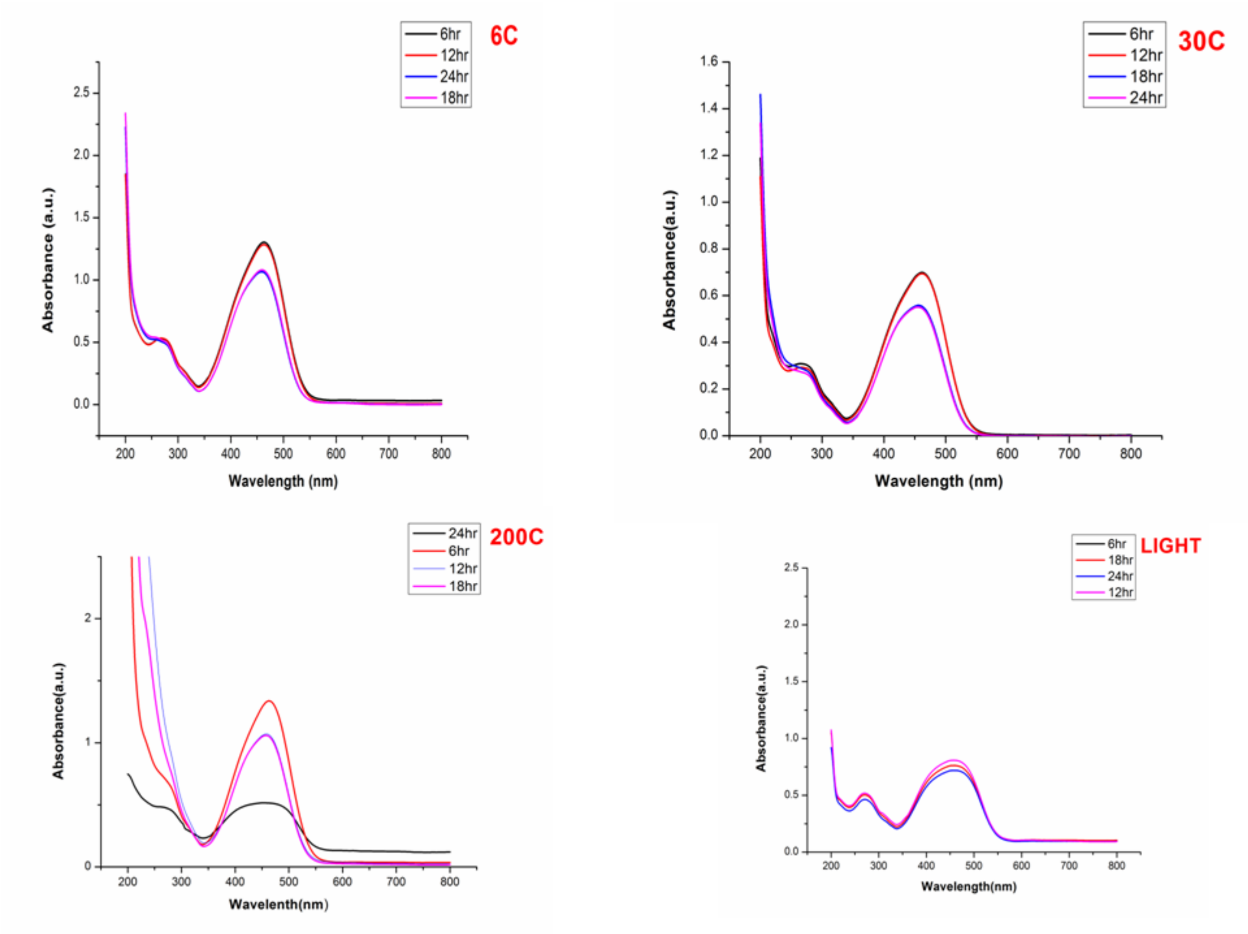

Figure 2. UV- Vis spectroscopic absorbance of dye treated with 6C, 30C, 200C and light.

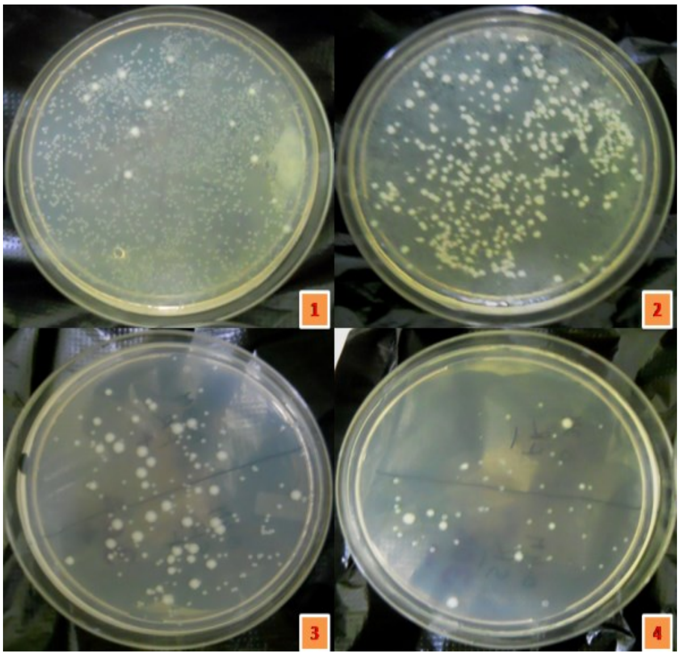

Figure 3. Antibacterial activity of cuprum metallicum for Gram negative bacteria.(*E.coli*).

Result and discussion:

Methyl orange dye treated at various conditions. Sample I showed image just after addition of dye in water. Sample II was the image taken after it was kept at dark condition for 24 hrs. Image named sample III was taken after it was placed at light condition only for 24 hrs. Sample IV, V, VI was the image of dye treated with cuprum metallicum 6C, 30C 200C respectively. The visual observation was clearly explained that color intensity of dye when treated with 200C was maximum detoriated. It means 200C work most effectively than other samples. In figure 2 we could see UV-Vis absorbance of dye individually treated with cuprum metallicum at a particular interval of time which was around 6 hrs gapping and it was for 24 hrs. Absorbance was taken at 464 nm **8**The graph named 6C,30C,200C was the graph where four absorbance of the sample contained 6C,30C,200C cuprum metallicum along with dye and also was kept under light condition were taken. Absorbance was compared with respect to time. The graph named light was the absorbance of dye when kept at light condition only. The result was when dye treated with 6C+light, and then with respect to time the absorbance was decreased at 24 hr. It means when absorbance decreased that means chemical complex also degraded with time. The degradation was maximum observed in case of 200C when compared with initial time (6hr).So it can be proved that 200C showed a very impressive result.

In figure 3 we could see four images named 1,2,3,4. Image 1 showed growth of bacteria when nothing was treated. In Image 2, 3, 4 plates were treated with 6C,30C, 200C drug of cuprum metallicum where bacterial growth was maximum restricted in case of 200C treated plate. It means 200C could easily kill bacteria of gram negative strain.

The above experiment showed a quite positive result regarding the remediation and detoxification of azo dye also the nanomedicine cuprum metallicum worked as a potent source against gram strains. According to the previous reports it has already been proved by different characterization methods that higher potencies of the drug particle act as nanoparticles as size of the particles were reduced with dilution.**9** So from the above experiment It has been found that three different potencies of cuprum metallicum were able to degrade the azo dye as with respect to time more the dye degrade more the colour intensity get lowered which was further confirmed by taking the absorbance of the dye. Previous reports were their where visible light can help to degrade dye samples through photo degradation. 10 Here it was observed that Sample VI showed maximum decrease in color intensity. Copper has always been a good and stable photo catalyst which can work better in presence of visible light.**11** It is because of the excitation of surface Plasmon resonance(SPR) which is actually oscillation of charge density that promote at the interface between metal and dielectric medium.**12** Here cuprum metallicum of three different potencies (6C, 30C, 200C) were used against two different strains of bacteria (Gram positive and gram negative).The best result was obtained against gram negative bacteria ie, *E.coli* and among different potencies of the drug, 200C of cuprum metallicum showed the best result. They can able to kill maximum no. of bacteria and few numbers of colonies were observed in the agar plate. The more the potency of the drug were used, more the antibacterial activity was shown by the drug. That means 200C showed maximum antibacterial effect and 6C showed minimum effect as compared to control where no drugs were used for treatment. The reason behind this result was copper nanoparticles is the prime component of cuprum metallicum and copper nanoparticles has multi toxicological effect against gram negative bacteria. They can generate reactive oxygen species (ROS) which cause DNA degradation, lipid peroxidation, protein oxidation in *E. coli* cells. **13**

Nanoparticle can attached to the membrane of bacterial cell by electrostatic interaction and disrupt the integrity of the membrane of the cell. At higher potency means in case of 200C the individual particles have less chances of aggregation and so each individual particle can have its own separate surface area to interact with the bacterial membrane. More the surface area more chance of interaction which leads to more toxicity. So that is why higher potency of nanomedicine showed more antibacterial activity and they simply destroy bacterial cells.**14**

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