Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

AN INVESTIGATION ON NANO METAL VITAMIN COMPLEX TO CONTROL OXIDATIVE CELL DAMAGE AND MICROBIAL SPOILAGE IN SEAFOOD

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DOI: 10.7897/2277-4572.07497

Received on: 13/06/18 Revised on: 15/07/18 Accepted on: 18/07/18

ABSTRACT

Fish is considered to be one of the most-traded food commodities worldwide with more than half of fish exports by value originating in developing countries. In order to increase its shelf life and maintain its nutritional value, texture and flavour, food preservation becomes necessary. Fish spoilage results from three basic mechanisms: Enzymatic autolysis, oxidation, microbial growth. With advancements in nanotechnology, different metals like silver, copper, zinc etc., have been engineered into a nanometre size and have attracted great research interest for use in different food industries because of their potential antimicrobial property. Vitamins like Pyridoxine, alpha tocopherol acetate, folic acid are commonly used as a dietary supplement and therapeutic agent possessing antioxidant and cell proliferation activity. Therefore, in the present research, copper oxide nanoparticles complexed with vitamin-E (MnV-C) were synthesized, and its potential to control the oxidative damage in the cells of seafood; and the microbial spoilage in seafood is investigated. All the bacterial species were found to be sensitive to the nanometal vitamin complex. The MICs of nanometal vitamin complex ranged from 1.6mg/ml to 6.4mg/ml. Maximum inhibitory zones obtained against *Bacillus cereus and Shigella* sp. were 17mm and 16mm. Thus the metal nanoparticles coupled vitamins (MnV-C) was effective against the oxidative cell damage and pathogenic microbes in seafood. This technology can be used as a preservation technique; can improve the quality fish and fish products.

Keywords: Fish spoilage, Nanometals, Vitamin complex, antimicrobial property, oxidative cell damage

INTRODUCTION

Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. World per capita fish supply reached a new record high of 20 kg in 2014. Moreover, fish continues to be one of the most-traded food commodities worldwide with more than half of fish exports by value originating in developing countries. Recent reports by high-level experts, international organizations, industry and civil society representatives all highlight the tremendous potential of the oceans and inland waters now, and even more so in the future, to contribute significantly to food security and adequate nutrition for a global population expected to reach 9.7 billion by 2050. With the ever growing world population and the need to store and transport the food from one place to another where it is needed, food preservation becomes necessary in order to increase its shelf life and maintain its nutritional value, texture and flavour. Fish is highly perishable commodity and also much in demand One-fourth of the world's food supply[1] and 30% of landed fish[2] are lost through microbial activity alone. Spoilage of food products is due to chemical, enzymatic or microbial activities[3]. During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavour and texture of the fish meat. Fish spoilage results from three basic mechanisms: Enzymatic autolysis, oxidation, microbial growth. Low temperature storage and chemical techniques for controlling water activity, enzymatic, oxidative and microbial spoilage are the most common in the industry today. Thus prevention of fish from cell damage and microbial spoilage is an essential part in preservation of seafood[4].

Nanotechnology is an emerging science and with growing use particularly in developing new materials at nanoscale levels[5]. Nanometals have been engineered and have attracted great research interest for use in different food industries. The metal nanoparticles have emerged as a potential alternative to conventional antibiotics because of their potential antimicrobial property. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. Nanotechnology increasing the shelf life of different kinds of food materials and also help brought down the extent of wastage of food due to microbial infestation[6]. Different nanometals from silver, copper, zinc, etc. can be synthesized which possess anti-microbial, anti-oxidant, anti-biotic and anti-fungal activities etc., Due to a wide presence in nature, implementation of different functions within the majority of living organisms, relatively low cost and environmental safety, copper compounds (Cu) have a high potential for their application as antibacterial agents being capable to replace silver and composites of different precious metals[7]. Cu nanoparticles are hypotoxic and show a high antibacterial effect in relation to the cells of test cultures of gram positives and gram negative organisms. Nanoparticles attached to the microbial surfaces can decrease both cell mobility and nutrient flow between the cell's exterior and interior compartments. It was reported that[8] copper has the potential to disrupt cell function in multiple ways, since several mechanisms acting simultaneously may reduce the ability of microorganisms to develop resistance against copper. The nonspecific mode of action of nanoparticles against bacteria makes them ideal candidates as antimicrobial agents with less risk of development of bacterial resistance[9]. In addition, no research has discovered any bacteria able to develop immunity to copper as they often do with antibiotics[10].

Vitamins like Pyridoxine, alpha tocopherol acetate, folic acid etc., possess antioxidant and cell proliferation activity. Oxidation occurs as a result of the reaction between atmospheric oxygen and the unsaturated fatty acids. Peroxides are intermediate products in the oxidation process, which in turn break down to odor and flavor-producing compounds. Other studies have shown that supplemental vitamin E improves the stability and flavor of veal fat, frozen poultry, milk and pork[11-14] when added to the feed over a long period of time. Vitamin E and C has ability in preventing oxidative damage of cells and repairing already existing damage[15]. Therefore, the main objective of this research is to synthesis metal nanoparticles complexed with vitamins (MnV-C), and its potential to control the oxidative damage in the cells of seafood; and the microbial spoilage in seafood is investigated.

MATERIALS AND METHODS

Synthesis of copper oxide nanoparticles[16]

Solution I: 6.9g of copper sulphate pentahydarate was dissolved in 100ml of distilled water.

Solution II: 34.6g of sodium potassium taratate and 12g of sodium hydroxide was dissolved in 100ml of distilled water.

About 50ml of solution I and 50ml of solution II was mixed together with vigorous stirring and 5g of glucose (reducing agent) was added and then the mixture was stirred vigorously was 10mins and then keep in boiling water bath at 60°C for 10mins. Then, the obtained mixture is centrifuged and washed with distilled water twice and with ethanol twice and it was air dried and the powdered substance was used for further analysis.

Preparation of Nanometal vitamin complex[17]

A series of Nanometal vitamin complex were synthesized according to the following general procedure: copper oxide nanoparticles were added gradually to magnetically stirred ethanol solution (20ml). Then, to the first reaction mixture, vitamin E oil was added and stirred carefully at about 60-80 °C till the reaction reached equilibrium. Then evaporation of the solvent (by placing the reaction mixture in a fume cupboard) and nanometal vitamin complex is synthesized.

Determination of minimum inhibitory concentration[18] (MIC)

Minimum inhibitory concentration (MIC) of nanometal vitamin complex against *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella* sp., and *Shigella* sp. was determined by standard broth macrodilution method. All the test cultures were inoculated in a sterile liquid media (Nutrient broth (Composition g/L: Peptone: 5g; Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g; Final pH (7.0 ± 0.2) and allowed to attain the growth for 24 to 48hours. To determine the MIC, a set of tubes with 1ml of Nutrient broth was added under sterile conditions. About 150µl of nanometal vitamin complex at different concentrations (25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml, 800µg/ml, 1.6mg/ml, 3.2mg/ml, 6.4mg/ml, 12.8mg/ml) was added to the tubes containing broth solution. To this above mixture, 500µl inoculum of each test cultures was added to their respective tubes. All the inoculated tubes were incubated at 37°C ± 0.2°C for 24 to 48hours. Organic solvent, methanol was used as negative control and antibacterial drug gentamicin (100µg/ml) was used as positive control.

Antibacterial activity of Nanometal vitamin complex[19]

The antibacterial activity of Nanometal vitamin complex was evaluated against the five test organisms (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella* sp., and *Shigella* sp.) by well diffusion method. Sterile Nutrient Agar (Composition g/L: Peptone: 5g; Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g, Agar 15 g; Final pH (7.0 ± 0.2) plates were prepared and allowed to solidify. About 0.1% inoculum suspensions of each of the bacterial cultures were streaked with the sterile cotton swab three times by turning the plate at 60^0 angle between each streaking. Under sterile conditions, 6mm wells were cut on the agar surface of each Nutrient Agar (NA) plates. About 50µl each of Nanometal vitamin were loaded into the well and the plates were incubated at 37ºC for 24 - 48h. The antibacterial activity was evaluated in terms of zone of inhibition around the wells of each extract in all the inoculated NA plates. The inhibition clear zones were measured and recorded in millimetre.

RESULTS AND DISCUSSION

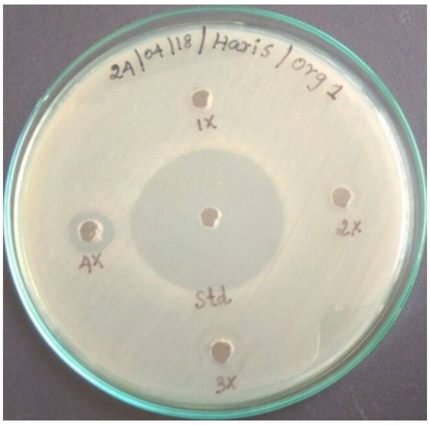
Minimal Inhibitory Concentration of Nanometal vitamin complex

The MICs of Nanometal vitamin complex against five test bacteria, *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella* sp., and *Shigella* sp. were shown in Table 2. The MICs of Nanometal vitamin complex ranged from 1.6mg/ml to 6.4mg/ml. The results were identified to be well coincided with the antibacterial activity presented in Table. All the bacterial species were found to be sensitive to the Nanometal vitamin complex. MIC of 1.6mg/ml was found to be the least concentration for test organisms *Staphylococcus aureus Bacillus cereus*. Similarly *Escherichia coli* and *Salmonella* sp. exhibited 3.2mg/ml as the MIC value. About 6.4mg/ml was found to be a satisfactory MIC value for the test organism *Shigella* sp. The obtained results in the present research were found satisfactory, supportive and also in accordance to the following literature analysis.

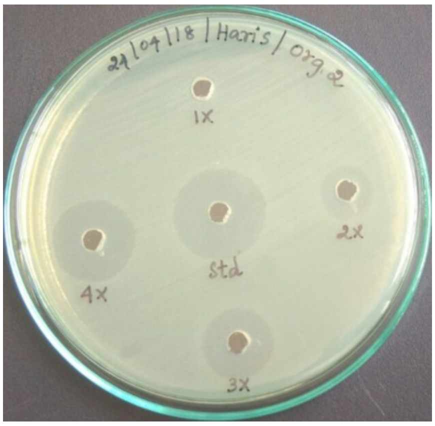
Minimal Inhibitory Concentration of Copper oxide nanoparticles were evaluated by Ruparelia *et al*., 2008[20]. Based on disk diffusion tests, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), the bactericidal effect of silver and copper nanoparticles were compared dispersed in batch cultures. Many bacterial species were found sensitive to nanoparticles. Disk diffusion tests carried out with *E. coli* and *S. aureus* showed silver nanoparticles had better bactericidal effect on bacterial species compared to the copper nanoparticles. On comparing to the other bacterial strains *B. subtilis* was found to be more sensitive to nanoparticles especially copper nanoparticles. In MIC/MBC and disk diffusion test bactericidal effect of copper nanoparticles showed a good negative correlation observed between the inhibitory zones (r2=-0.75). *E. coli* showed strain-specific variation and strain specific-variation for *S. aureus* in MIC/MBC was negligible.

Table: 1 Anti-bacterial activity of Nanometal vitamin complex against test organisms

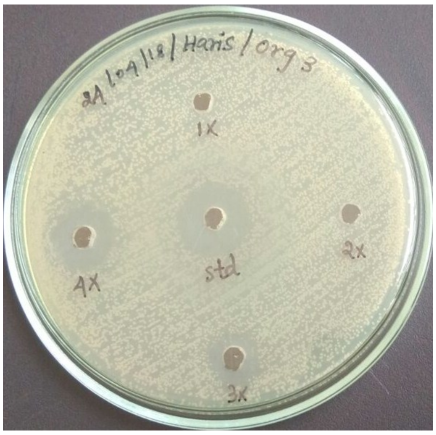
S.No	Test organism	Zone of Inhibition(in mm)				
		1x	2x	3x	4x	Ciprofloxacin
1.	*Staphylococcus aureus*	0	07	10	11	29
2.	*Bacillus cereus*	0	12	14	17	18
3.	*Escherichia coli*	0	0	09	12	15
4.	*Salmonella* sp	8	11	13	14	16
5.	*Shigella* sp	8	13	14	16	17



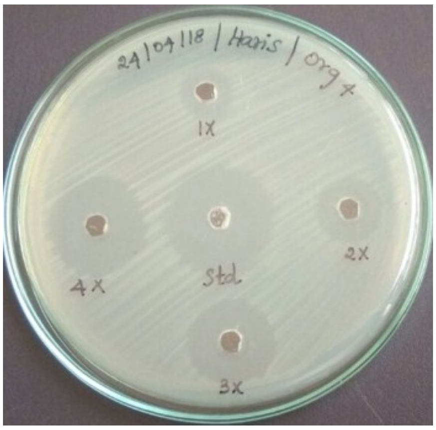
Staphylococcus aureus



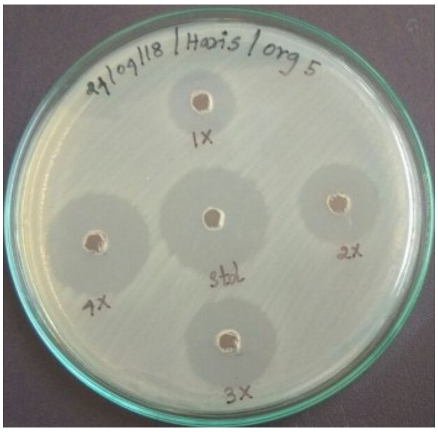
Bacillus cereus



Escherichia coli



Salmonella sp



Shigella sp

Figure 1: Antibacterial activity of Nanometal vitamin complex against different organisms

Silver and copper metal nanoparticles were synthesised by chemical processes. Silver nitrate and copper nitrate was reduced by ascorbic acid in the presence of chitosan[21]. Particle size was increased by reducing the chitosan concentration and increasing the nitrate concentration. Surface zeta potentials for silver and copper nanoparticles produced varied from 27.8 to 33.8 mV. Antibacterial activities of Silver, Copper, Silver and Copper mixtures and bimetallic nanoparticles of Silver or Copper were tested using *Bacillus subtilis* and *Escherichia coli*. The more susceptible organism among *Bacillus subtilis* and *Escherichia coli* was found to be *Bacillus subtilis*. Silver nanoparticles showed higher antibacterial activity than copper and mixtures of nanoparticles. Bimetallic Ag/Cu nanoparticles proved to possess the highest antibacterial activity with with minimum inhibitory concentrations (MIC) of 0.054 and 0.076 mg/L against *B. subtilis* and *E. coli* respectively.

Antibacterial activity of Nanometal vitamin complex

The antibacterial activity of Nanometal vitamin complex against five test bacteria, *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella* sp., and *Shigella* sp. were evaluated by agar diffusion method (Figure 1). Four different concentrations of Nanometal vitamin complex were used to determine the antibacterial activity against each organism. Maximum inhibitory zones were obtained against *Bacillus cereus and Shigella* sp at 4x concentration was 17mm and 16mm (Table.1). Thus, antibacterial activity of Nanometal vitamin complex against five test bacterial species showed promising results in the present research. During the analysis, the Nanometal vitamin complex showed good antibacterial activity against *Shigella* sp when compared to other organisms (Table: 1).

The antibacterial activity of copper nanoparticles is due to interaction with the bacterial outer membrane (peptidoglycan), causing the membrane to rupture and killing bacteria[22]. Kim *et al*., 2000[23] emphasized that the bactericidal effects observed in this study might have been influenced by the release of Cu^{2+} ions. Copper ions released by the nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death. Copper ions inside the bacterial cells may bind to deoxyribonucleic acid molecules (DNA) and become involved in cross-linking within and between the nucleic acid strands, resulting in the disorganized helical structure[24].

The antibacterial properties of Cu nanoparitcles (Cu-NPs) were investigated against *Salmonella typhi* by Anamika *et al*., 2016[25]. The Cu-NPs were prepared by the reduction of copper acetate with the help of ethylene glycol (EG), then sample was characterized by XRD for its average particle size identification. The antibacterial activity assessed by well diffusion and disc diffusion method on different concentration of nanoparticles. It was found that these Cu-NPs showed antibacterial activity in form of zone inhibition, wherein, zone of inhibition increased with increase in concentration of Cu-NPs.

Overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms and this lead to search for agents that may have antibacterial effects. Vitamin E emerged as an essential, fat-soluble nutrient in the human body and it is essential, because the body cannot manufacture its own vitamin E, so foods and supplements must provide it. The anti-bacterial analysis of vitamin E against pathogenic bacteria was investigated by Dalia Abd *et al*., 2013[26]. Results showed that gram negative bacteria were shown to be more resistant than gram positive bacteria. The resistance of gram negative bacteria towards antibacterial substances may be related to lipopolysaccharides in their outer membrane.

In a study of the effect of Vitamin E on secondary bacterial infection after influenza infection in young and old mice which was done in 2004 by Gay *et al*., [27] the result was that Vitamin E supplementation abolished the priming effect of influenza infection on *S.aureus* and the researchers concluded that vitamin E may exert its effect by number of mechanisms, including reducing reactive oxygen species (ROS), decreasing proinflammatory cytokines and adhesion molecule expression and production, increasing antioxidant and antimicrobial activity. Most of the animal studies that investigated the effect of Vitamin E on infectious diseases reported a protective effect despite the variations in the dose and duration of the supplementation, infectious organisms involved, and route of administration. Only a limited number of studies have investigated the effect of vitamin E on resistance against infections in humans.

CONCLUSION

In order to increase its shelf life and maintain its nutritional value, texture and flavour food preservation becomes necessary in storage and transport of food products. Nano metals and vitamins having antimicrobial and has potential to control the oxidative damage in the cells of seafood can be used as an alternative for the preservatives in storage of sea foods. The Nanometal vitamin complex against five test bacterial species showed promising results in the present research. The developed Nanometal vitamin complex in the present study revealed the significance of the preservation of stored sea foods preventing oxidative and microbial spoilage. Thus the metal nanoparticles coupled vitamins (MnV-C) was effective against the oxidative cell damage and pathogenic microbes in seafood which can improve the quality fish and fish products and increase their shelf life.

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How to cite this article:
Haris Variyathody and Manohar M. An investigation on nano metal vitamin complex to control oxidative cell damage and microbial spoilage in seafood. J Pharm Sci Innov. 2018;7(4): 139-143.
http://dx.doi.org/10.7897/2277-4572.07497

Source of support: Nil, Conflict of interest: None Declared