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An Analysis on Carboxymethyl Guar Gum/Ag Nanocomposite as a Promising Antimicrobial Agent

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ABSTRACT

Guar gum is one of the naturally-derived polysaccharide, which has provided an alternative to the use of synthetic non-biodegradable polymers which have a deleterious effect on the environment and human health. In the recent past, the use of these naturally derived polymers like guar gum has expatiated in various fields like research, drug delivery etc. In this paper, we have amalgamated one of the derivatives of this polymer, i.e. Carboxymethyl Guar Gum (CMGG), with silver nanoparticles to form CMGG-Silver nanocomposite. We tested this nanocomposite for its antimicrobial activity against different water samples and gram-negative bacteria, *Pseudomonas aeruginosa*. The bacterium, *Pseudomonas aeruginosa*, is involved in many types of skin and blood infections. Also, this bacterium is involved in hospital-acquired infections such as sepsis syndrome and ventilator-associated pneumonia, killing millions of people every year. We have checked the antimicrobial activity by using a varied amount of the nanocomposite. Also, we used two types of nanocomposite solution with varying concentration of CMGG while conducting the experiment. It was observed that a small volume of nanocomposite solution ranging from 500-1000 μL was found suitable for complete inhibition of *Pseudomonas aeruginosa* growth for 96-144 hours. For testing water samples, we collected samples from different locations on our campus. The nanocomposite showed promising results prohibiting the bacterial growth in these samples as well. The above observations can help us to conclude that the CMGG-Silver nanocomposite is a probable candidate for its use as an antimicrobial agent. Future experiments on different bacteria can widen the spectrum of their use in various industrial and biomedical applications.

KEY WORDS: CARBOXYMETHYL GUARGUM, GREEN SYNTHESIS, NANOPARTICLE, NANO-COMPOSITES, PSEUDOMONAS AERUGINOSA.

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INTRODUCTION

In the modern world, humans are looking for different ways to curb dangerous microbes as they have created havoc in the form of diseases. Pathogens have become a major cause of infractions in humans, animals and plants. Microbes are ubiquitous and carry various roles in the environment. Among them pathogenic bacteria have become a major challenge for researchers as many of them have become multidrug resistant. Recent studies show that many commonly occurring microbes such as

Pseudomonas aeruginosa, Staphylococcus aureus and *Streptococcus pyogenes* are associated with hospital acquired infections such as sepsis syndrome and ventilator associated pneumonia, killing millions of people every year. *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* are also responsible for most cases of food poisoning. Nanotechnology is a potent tool to match these antimicrobial compounds that can help in curbing these pathogens as the Nano stuff is dimensionally and structurally similar to most of the biological compounds which makes them one of the promising candidates to be used in biomedical research and other applications such as imaging, gene delivery systems, antimicrobial, food packaging and many more (Waren et al, 1998; Roy et al, 1999; Singh et al, 2008; Mostafa et al, 2018).

Also, antimicrobial packaging is gaining interest and is rapidly expanding with the application of nanotechnology (Sarwar et al 2018). Moreover, the hype in antimicrobial resistance against conventional antibiotics is limiting our medicinal resources at a very fast rate. Therefore, moving on to these Nano-biological materials as antimicrobial compounds can be very fruitful in the coming decades. It will break the dependence over use of synthetic compounds which is widely used in the healthcare sector as well as in the food packaging industry. Among all nanoparticles, silver nanoparticles are toxic for a wide range of microbes which makes them suitable candidates to be used as an antimicrobial agent for different applications. For instance, many of the nanocomposites made from silver are used for food packaging (Liau et al, 1997; Gupta and Silver, 1998; Nomiya et al, 2004; Batista et al, 2019). Carbohydrate silver nanocomposites have been used in antimicrobial activity because of their ability to act as a capping agent and reducing properties due to the presence of carbohydrates (Rao et al, 2012; Xia et al, 2012; Ghobashy et al, 2017) [AG6].

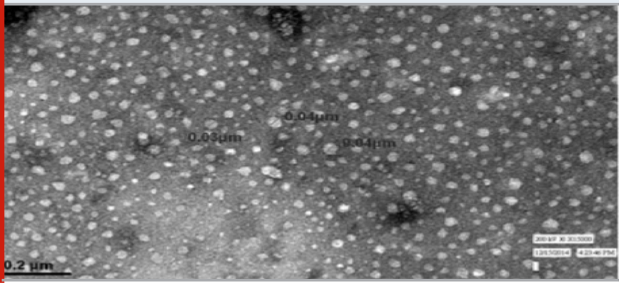
Besides the anti-bacterial nature of silver nanocomposite, biocompatibility, non-toxicity and their ability to assort with natural polymers like Collagen, Gelatin etc. have helped them to be used in tissue engineering and other medical applications like wound dressing (Aktürk et al, 2019). Also, Polymer nanocomposites have been used in water purification (Zheng et al, 2015; Pandey et al, 2017; Mukhopadhyay et al, 2020). On coming in context to the Guar gum/Ag Nanocomposites, a type of Carbohydrate nanocomposite, have also shown excellent antimicrobial activity against *L. monocytogenes, S. typhimurium* etc. and have been used as an active packaging to inhibit the growth of microorganisms (Arfat et al, 2017; Herrera et al, 2018). In an earlier paper, significance of Guar gum and their derivatives as a capping as well as a reducing agent for silver nanoparticles was mentioned (Gupta and Verma, 2014), along with the process of synthesizing Carboxymethyl Guar Gum silver Nanocomposite (CMGG/Ag NC) (Gupta and Verma, 2014). CMGG is one of the anionic semi-synthetic guar gum derivatives (Mukhopadhyay et al, 2020).

This polysaccharide is easily water-soluble. It has a wide range of applications in various industries especially the food industry (Gong et al; 2012; Gupta et al, 2013; Priyadarsini and Biswal, 2020). Lack of data to prove the antimicrobial property of the nanocomposite against different bacteria is one of the reasons we took up this study. This can help in widening the use of this nanocomposite against bacteria like *Pseudomonas aeruginosa*. Testing our nanocomposite against water samples collected from different locations in our college is a step forward to show that this nanocomposite can have multiple areas of application. It helps us in rationalizing that the CMGG-Silver nanocomposite can be used as an alternative to chlorination for water purification as the process of chlorination can itself lead to the generation of certain harmful compounds.

MATERIAL AND METHODS

Silver nitrate ($AgNO_3$) was purchased from Hi Media Laboratories Pvt. Ltd. Carboxymethyl guar gum was brought from Hariom gum Industry, Gujarat, India. All aqueous solutions, used in the experimental work, made using doubled distilled (DD) water. The microbial culture of Pseudomonas aeruginosa was ordered from the National Chemical Laboratory (NCL), Pune, India in the form of slant culture. For testing the use of the nanocomposite in water purification, different water samples were collected within the Manav Rachna International Institute of Research and Studies Campus, Sector-43, Faridabad, Haryana-121004, India.

Figure 1: TEM images of CMGG/Ag NC solution 200 nm scale.



The synthesis of CMGG/Ag NC was done as reported in the literature (Gupta and Verma, 2014). CMGG powder was dissolved in 10 ml double-distilled water (DD) under constant stirring on the magnetic stirrer. After complete dissolution of CMGG in water, the temperature was raised to 90°C. Then, 10 ml of silver nitrate solution was added to the same. The solution was continuously stirred up for 100 minutes after addition. Slowly the color of mixture started changing its color. After some times the color of the solution was converted to brownish color. This confirmed the formation of the nanocomposite. For the characterization, CMGG/Ag NC morphology was characterized using Transmission Electron Microscope (Tecnai, G2- 200 KV HRTEM, SEI company, Holland).

RESULTS AND DISCUSSION

For the morphological study, CMGG/Ag NC morphological study was done using TEM images. TEM images shown in figure 1. The presence of spherical shapes in image confirmed formation of nanocomposites.

Antimicrobial activity of CMGG/Ag NC solution against *Pseudomonas aeruginosa*: Antimicrobial activity of CMGG/Ag NC was tested using the spread plate method. A broth culture was prepared for Pseudomonas aeruginosa. It was kept at 370 C in a sterile environment for 12-14 hours. Nutrient Agar medium was prepared for the growth of *Pseudomonas aeruginosa*. Two CMGG/Ag NC stocks were prepared using different concentrations of CMGG i.e. 1% and 2% (Orsu and Matta, 2020).

The Agar media was plated in seven different Petri plates. Out of these 7 plates, plate 1 was chosen as a control in which no further addition was done. 500µL of the broth culture of log-phase *Pseudomonas aeruginosa* was spread over the plated agar in the remaining six plates. Out of these six plates, different volumes of 1% CMGG/Ag NC was spread on three plates and the 2% CMGG/Ag NC was poured on the other triples. All the plates then incubated at 370 C under sterile conditions. Growth of microbes recorded with time. The observations are tabulated in Table 1.

Our results prove that the CMGG-Silver nanocomposite exhibits antimicrobial property against *Pseudomonas aeruginosa*. These results can also be viewed as an extension to the previous work of Gupta and Verma to test the antimicrobial activity of Carboxymethyl guar gum–silver nanocomposite film against E.coli and B. subtilis (Verma and Gupta, 2015). In our experiments, we used the spread plate method for the first time in order to test antimicrobial activity. The earlier attempt that was made against *E. coli* and *B.subtilis* employed the use of a nanocomposite film (Orsu and Matta, 2020).

Antimicrobial activity of CMGG/Ag NC solution for different water samples: The collected water samples were tested for the action of CMGG/Ag NC using the spread plate technique. Instead of using the bacterial culture, water samples were spread on Nutrient Agar plates and later on the CMGG/Ag NC solution was added. This time we used a standard 1 % concentration of CMGG in the nanocomposite stock solution. The plates with nanocomposite were compared against the plates containing only water samples. The growth of microbes with time is reported in Table 2 with appropriate figures (Orsu and Matta, 2020).

Note: The figures reported were observed after 24 hours of incubation.

Table 1. Anti-microbial activity against Pseudomonas aeruginosa

S.no.	CMGG conc. (%)	CMGG/AgNC (µL)	24 hrs	72 hrs	96 hrs	144 hrs	Figure no.
1	1	500	no	no	no	**	2(a)
2	1	750	no	*3	*3	**	2(b)
3	1	1000	no	**	**	**	2(c)
4	2	500	no	*2	*6	*6	2(e)
5	2	750	no	*1	**	**	2(f)
6	2	1000	no	no	no	no	2(g)
7			no				2(d)

Figure 2. (a to g): The antimicrobial activity of nanocomposite against *Pseudomonas aeruginosa*

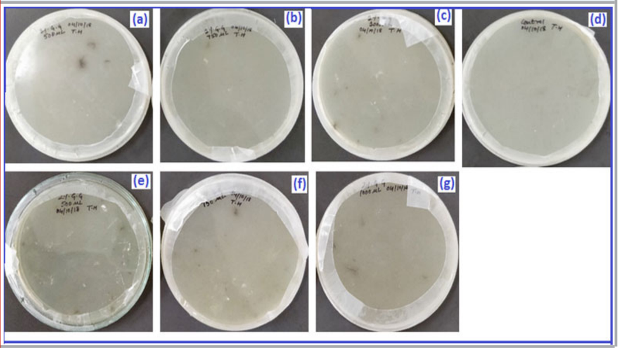
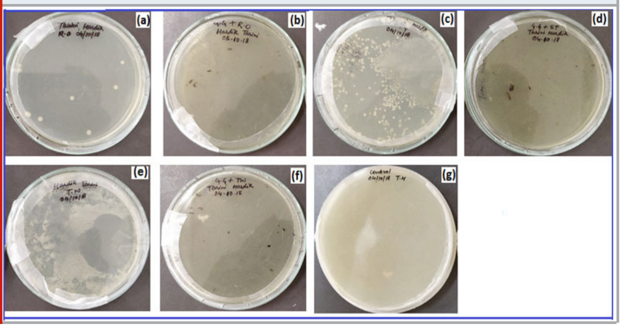


Figure 3. (a to g):Antimicrobial activity of CMGG/Ag NC solution for different water samples (references given in table 2)



The results demonstrate that this nanocomposite can have multiple areas of application. It helps us in rationalizing that the CMGG-Silver nanocomposite can be used as an alternative to chlorination for water purification as the process of chlorination can itself lead to the generation of certain harmful compounds. A recent attempt has been made to use this nanocomposite for wound healing. These types of nanocomposites can also be used in the process of drug delivery but meagre information is available on this. Another potential field of application is their use as a replacement for antibiotics which can be validated by conducting in vivo studies thereby playing an important role in curbing Antimicrobial Resistance (Orsu and Matta, 2020).

Table 2. Water sample analysis (RO: Reverse Osmosis Water, TW: Tap Water, ST: Septic Tank water Vs GG: CMGG/Ag NC solution)

S.no.	Sample	CMGG conc. (%)	CMGG/Ag NC (μL)	24 hrs	72 hrs	96 hrs	144 hrs	Figure no.
1	RO	1	500	*	*13	**	**	3(a)
2	RO + CMGG Ag NC	1	500	no	no	no	*12	3(b)
3	ST	1	500	*	**	**	**	3(c)
4	ST + CMGG Ag NC	1	500	no	no	no	*1(big)	3(d)
5	TW	1	500	**	*	**	**	3(e)
6	TW + CMGG Ag NC	1	500	no	*6	**	**	3(f)
7	Control			no				3(g)

*n refers to number of colonies, ** refers to multiple colonies. RO: Reverse Osmosis Water ,TW: Tap Water, ST: Septic Tank water , GG: CMGG/Ag NC solution

CONCLUSION

Antimicrobial activities of CMGG/Ag NC were tested against Pseudomonas aeruginosa as well as the microbial population in different water samples. Comparative study of the growth of bacteria with time has revealed the effect of CMGG/Ag NC on microbes. It was observed that a small quantity (500μL) of nanocomposite was sufficient for complete inhibition of bacterial growth up to 96 hours and a small increase in the volume of nanocomposites (up to 1 mL) was found suitable for complete inhibition of Pseudomonas aeruginosa growth up to 144 hr. From the results, it can be concluded that CMGG/Ag nanocomposites may be used as an antimicrobial agent for different purposes especially in water purification. Further research on its intake in the human body and the spectrum of its antimicrobial infection can help us to link this to in vivo treatment of bacterial infections.

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

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