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Design and fabrication of hydrogel incorporated with copper nanoparticles loaded microsponge for antimicrobial and antioxidant activity

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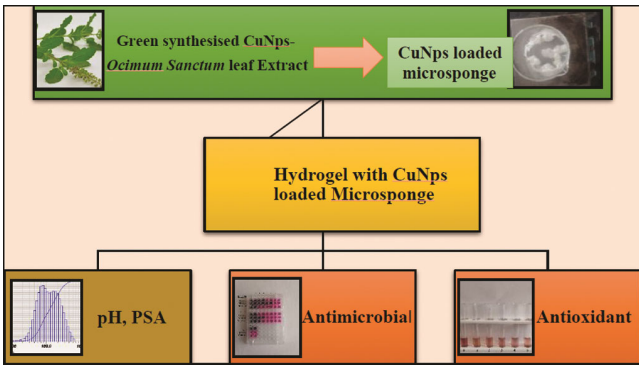
A hydrogel has been designed as an antimicrobial agent with low toxicity and reduced side effects. The hydrogel has been fabricated with green synthesised copper nanoparticles loaded microsponges. Here, the copper nanoparticles have been synthesised from the leaf extract of *Ocimum Sanctum*. This fabricated hydrogel is checked for its *p*H and particle size by particle size analyser (PSA). The best hydrogel is assessed through resazurin microtitre assay for its antimicrobial activity. Using DPPH assay, it is also checked for its antioxidant activity. The results show that the fabricated hydrogel incorporated with copper nanoparticles loaded microsponge act as an excellent antimicrobial gel and has shown good antioxidant potential.

Keywords: Antimicrobial, Antioxidant, Copper nanoparticles, Hydrogel, Microsponge

Microsponges are polymeric delivery systems that consist of an inert polymer 's porous microsphere that can trap active ingredients or drugs. Its dimensions can vary from 5 to 300µm in diameter[1,2]. A microsponge delivery system (MDS) is highly cross-linking, small sponge-like spherical particles[3] with a large porous surface that can increase stability by using biocompatible synthetic and semi-synthetic polymers[4], reduce side effects by nutritional chemistry[5] and alter the release of drugs by convergence technology[6]. It can then be incorporated into a formulated products such as a gel, cream, liquid or powder[7]. Besides acting as a reservoir for active drug, these can potentially be used for the controlled delivery of a wide variety of substances such as fragrances, emollients, sunscreens, anti-inflammatory, antifungal, antimicrobial agents[8]. It can boost the effectiveness of topically active agents with increased esthetic properties and higher product stability[9]. Recently, for the controlled release of drugs to the epidermis, this MDS has been successively addressed with assurance that the drug remains located and does not enter the systemic circulation and acts as a unique technology for the controlled release of topical agents. Moreover, numerous studies have confirmed that microsponge systems[10] are non-irritating, non-mutagenic, non-allergenic and non-toxic, with increased therapeutic efficacy and increased flexibility in the formulation[11-13].

Copper and its complexes have been used since ancient times as disinfectants because of their antibacterial and antiviral activity[14,15]. Due to the smaller size and higher surface-to-volume ratio of copper nanoparticles (CuNps) than its copper salts, it can interact with the microorganism's membrane, thereby enhancing its biocidal effect[16,17]. The stable CuNps and their applicability as biomaterial constituents are promoted in the fields of medicine, biotechnology etc. In this regard, hydrogels play a vital role in many fields such as agriculture, drug delivery, tissue engineering, water purification, contact lenses, sensors, wound dressings, etc[18]. Hydrogels are defined as hydrophilic, three-dimensional networks held together by chemical or physical bonds with water absorption capacity[19]. Hence, we have made an attempt for the first time to incorporate CuNps into microsponges and then loaded into hydrogel.

The aim of this study is to design and fabricate hydrogel incorporated with copper nanoparticles loaded microsponge (Scheme 1). In this study, we first synthesise microsponges which are loaded with green synthesised CuNps. Then this loaded microsponge is successfully incorporated into



Scheme 1

hydrogel. This loaded hydrogel is characterised by Particle Size Analyser (PSA) and determine its pH value. The best formulated hydrogel is subjected to antimicrobial and antioxidant activity.

Experimental Section

Materials

All the materials used were of analytical grade and double distilled water was used for all the synthesis.

Green synthesis of copper nanoparticles

The copper nanoparticles were prepared by using the leaf extract of *Ocimum Sanctum* and copper sulphate solution. Here, the leaf extract acts as a capping and reducing agent. The black CuNps(A) precipitate was then characterised by UV-Vis, FT-IR, SEM-EDX, XRD and followed by the evaluation of antimicrobial and antioxidant activity[20,21].

Synthesis of copper nanoparticles loaded microsponges

CuNps loaded microsponges were designed with varying proportions of ethylcellulose and polyvinyl alcohol by using the Quasi-emulsion diffusion method. The Dispersed Phase consists of CuNps(A) and EC dissolved in dichloromethane, then slowly added to PVA in an aqueous phase, stirred for 3 h under magnetic stirrer. The filtered microsponges were dried in an oven at 40–50 ·C for 24 h and stored in vacuum dessicator for further use[22]. It was then characterised by PSA, HRSEM, drug content, entrapment efficiency and the best microsponge was evaluated for its antimicrobial and antioxidant activity[23].

Formulation of hydrogel incorporated with CuNps loaded microsponge

1 g of Carbopol soaked in 100 mL of water was stirred for 2 hours with a magnetic stirrer and then has been stopped expelling air for a few minutes. 4% Triethanolamine has been added to this until a clear solution is found. Then, it is followed by the addition of (5-15%) propylene glycol and accurately weighed amount of CuNps(A) loaded Microsponge[24].

Evaluation of hydrogel incorporated with CuNps loaded microsponge

The hydrogel incorporated with CuNps(A) loaded microsponges (AHG_0 -AHG_4) have been then characterised by Particle Size Analyser (PSA) which determines its *p*H. The best loaded hydrogel (AHG_1) is evaluated for the antimicrobial and antioxidant activity.

Particle size determination

Using Particle Size Distribution Analyzer, the particle size of the loaded and unloaded hydrogel was determined. The instrument used here is the LA-950 HORIBA Laser Scattering Particle Size Distribution Analyzer. Before running the sample in the instrument, the hydrogels were dispersed in double distilled water to ensure that the light dispersal signal falls within the sensitivity range of the instrument.[25,26]

*p*H determination

The weighed amount of hydrogel has been taken in a beaker and measured its pH using digital *p*H meter.

In- vitro antimicrobial study

Determination of Minimum inhibitory concentration (MIC) using Resazurin Microtitre Assay

Preparation of resazurin solution

The resazurin solution was prepared by dissolving 270 mg in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it is a well-dissolved and homogenous solution[2].

Procedure

The test was conducted under aseptic conditions in a 96 well plates. A sterile 96 well plate was labeled.100 µL volume of hydrogel (AHG_1) was pipetted into the first well of the plate. 50 µL of nutrient broth was added to all other wells and diluted serially. 10 µL of resazurin indicator solution was added to each well. In each well, 10 µL of bacterial suspension was added. Similarly, for antifungal activity, the same set up was performed in which 50 µL of potato dextrose broth and 10 µL of fungal suspension were added on each well. Each plate was looselywrapped with cling film to ensure that bacteria are not dehydrated. The plate was incubated at 37°C for 18–24 hr. The colour change is then assessed

Table — Hydrogel with its *p*H and Particle size

Hydrogel code	*p*H	Particle size(µm)
AHG_0	6.22	133
AHG_1	6.48	117
AHG_2	6.04	129
AHG_3	6.01	118
AHG_4	6.09	121

visually. Any colour change from purple to pink or colourless were been recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value[2,20,22].

Antioxidant study

Determination of Scavenging activity by DPPH assay

DPPH-free radical scavenging assay assessed the percentage of antioxidant activity (AA per cent) of each substance. Different hydrogel concentrations (AHG_1) have been added to all of the tubes except blank. Then 3 mL of ethanol and 0.3 mL of 0.5 mM DPPH radical solution in ethanol are added. The control solution has been prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). Absorbance has been read at 517 nm after 30 min of reaction.[2,21,22] The scavenging activity percentage (AA %) is calculated using the below formula

AA % = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} × 100

Results and Discussion

Particle size and *p*H determination

The particle size of the unloaded and microsponge with CuNps loaded with hydrogel are found to be in the range of 117 µm – 133 µm. The hydrogel with lowest particle size (AHG_1) has been chosen for the biomedical applications. The *p*H **is** around 6 to 6.48 using digital *p*H meter. All these *p*H values were deemed acceptable to prevent the risk of skin irritation (Table 1).

Antimicrobial study

The best loaded hydrogel (AHG1) was chosen for antimicrobial and antioxidant activity assessment. Here, the antimicrobial activity was done by resazurin microtitre assay in which three strains (*E. coli, B. subtilis* and *C. albicans*) have been used and Streptomycin and Fluconazole used as a standard for antibacterial and antifungal activity respectively. This shows good antimicrobial activity whose MIC values are almost equal to that of loaded microsponges (Fig 1); particularly the activity of hydrogel towards

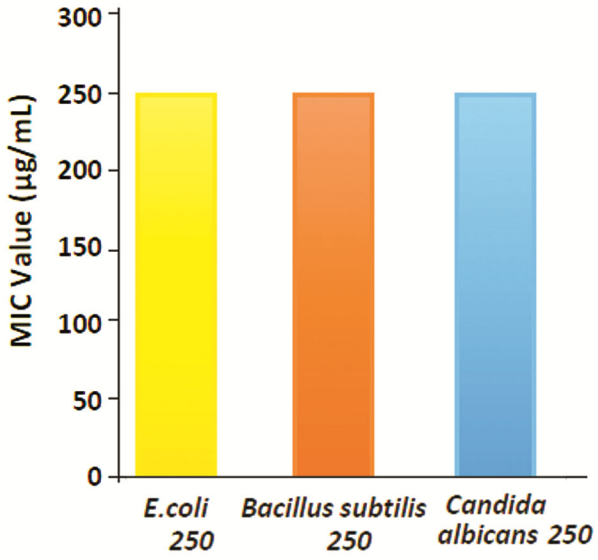


Fig.1 — MIC values from antimicrobial activity of AHG_1

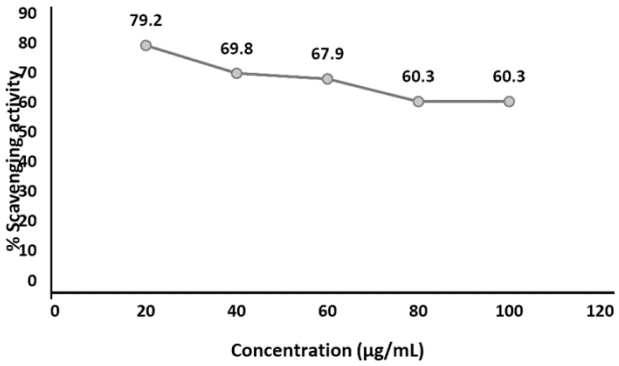


Fig. 2 — Percentage inhibition from antioxidant activity of AHG_1

C. albicans has been increased to 250 µg/mL, whereas the MIC value of the loaded microsponge is 125 µg/mL.[22]

Antioxidant study

The chosen hydrogel incorporated with CuNps microsponge has been then evaluated for the antioxidant activity by DPPH free radical scavenging assay using BHT as standard (Fig 2). It shows good scavenging activity (79.2 %) when compared to loaded microsponge (36 %).[22]

Conclusion

Microsponge delivery system has become a valuable drug delivery technology for various therapeutic applications. We have successfully synthesised hydrogel incorporated with copper nanoparticles loaded microsponge. It is then checked for the particle size and *p*H. For further study, the lowest particle size corresponding to the hydrogel has been selected as the

best hydrogel (AHG_1). All of the hydrogel formulation has a good *p*H range that has been considered non-irritant to the skin. The best hydrogel is then evaluated for antimicrobial and antioxidant activity. It acts as an excellent antimicrobial agent against all the strains chosen. The activity of hydrogel is higher is higher than that of loaded microsponge; particularly towards *C.albicans.* It also acts as a potent antioxidant agent with 79.2% of scavenging activity. The percentage inhibition value of hydrogel has been elevated when compared to that of loaded microsponge. The inference of the present work confirms that the hydrogel with CuNps(A) loaded microsponge has been well designed with good antimicrobial and antioxidant activity with less toxicity and better results. This work may be extended to the higher level of therapeutic application.

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