J. Indian Chem. Soc.,
Vol. 96, November 2019, pp. 1409-1412

Synthesis, characterization and antibacterial activity of fumaric acid incorporated silver nanoparticle hydrogel

Syed Ilias Basha[a,d], Somnath Ghosh[b], K. Vinoth Kumar[c], B. Ramesh[d], P. Hema Prakash Kumari[e] and Ethirajan Sukumar*[a]

[a]Department of Research and Development, Saveetha Institute of Medical and Technical Sciences (DeemedUniversity), Thandalam, Chennai-602 105, India

E-mail: syediliasbasha@gmail.com, drsuku3@gmail.com

[b]Department of Chemistry, Indian Institute of Petroleum and Energy, Visakhapatnam-530 003, Andhra Pradesh, India

E-mail: ghoshmnth@gmail.com

[c]VPro Biotech, 51-Arumparthapuram Main Road, Puducherry-605 110, India

E-mail: kvinothkumarphd@gmail.com

[d]Department of Pharmacology, GITAM Institute of Medical Sciences and Research (Deemed University), Rushikonda, Visakhapatnam-530 045, Andhra Pradesh, India

E-mail: ramesh_sep71@yahoo.co.in

[e]Department of Microbiology, GITAM Institute of Medical Sciences and Research (Deemed University), Rushikonda, Visakhapatnam-530 045, Andhra Pradesh, India

E-mail: drhemaprakash@gmail.com

Manuscript received online 31 August 2018, accepted 20 August 2019

Nano compounds find extensive application in many fields and in medicine, they are used widely in drug delivery system. Of late, attention is paid to their use as possible antimicrobial agents. In this study, fumaric acid (FA) has been considered for the synthesis of nano compound by incorporating it with silver nano particles (AgNps) embedded in agar-agar matrix. The nano material hydrogel so formed (FA-AgNps) has been characterized by FTIR, UV-Vis and TEM that revealed the presence of characteristic bands and absorbance maxima as well as the shapes of AgNps and FA-AgNps hydrogel. When tested against five human pathogens– *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis*, the synthesized nanoparticle hydrogel has shown significant effect against four bacteria and no activity against one strain when compared to FA, AgNps and standard drugs in the concentrations studied.

Keywords: Fumaric acid, silver nanoparticles, fumaric acid incorporated silver nanoparticle hydrogel, synthesis, characterization, antibacterial activity.

Introduction

Nanoparticles have come to stay in the development of new materials due to their novel properties that could be utilized in biology and medicine[1]. Incorporation of specific substrates with nano materials can bring out new entities for future use. The need has arisen to produce new drugs as in many diseases, microbes reveal resistance to the existing antibiotics[2]. Of late, investigations are directed towards naturally occurring small molecules as possible drug candidates. But due to solubility and related issues, these chemical entities *per se* do not go up to drug discovery stage. Hence substrate incorporated nano materials are studied for various activities including antimicrobial wound dressings[3]. Antibiotic-loaded nanoparticles were utilized to treat ocular infections[4].

Here, the naturally occurring fumaric acid (FA) has been

considered for the study. FA was first isolated from the plant *Fumaria officinalis* and many micro-organisms also produce it in small amounts[5]. It possesses simple structure. Earlier report on FA indicated inhibitory activity against *E. coli* and *Salmonella* sp.[6]. In the present study, silver nanoparticles (AgNps) were prepared by dissolving polyvinyl pyrrolidone (PVP), N,N-dimethyl formamide (DMF) and silver nitrate in clear agar-agar solution. FA was incorporated to this solution resulting in nano compound formation (FA-AgNps) which has been characterized by FT-IR, UV-Vis and TEM and tested for antibacterial activity against five human pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis*).

Results and discussion

In the synthesis of nanoparticle, univalent silver (Ag^+) ion was reduced to Ag^0 in the presence of agar-agar and DMF. The latter was preferred due to its mild reducing property and complete miscibility in water besides lack of interference with gelling mechanism of agar-agar[7]. The two step process employed in the synthesis yielded nanoparticle of size 15–25 nm.

The FT-IR of AgNps showed significant bands at 3410 cm^{-1}, 2929 cm^{-1} and 1638 cm^{-1} attributed to O-H, C-H and C-C/C=O stretching. These bands did not change their position or no new bands or trough appeared for FA-AgNps hydrogel (Fig. 1). The presence absorbance maxima (λ) at 415 nm in UV-Vis spectrum of both AgNps and FA-AgNps hydrogel are characteristic Surface Plasmon Resonance (SPR) bands for spherical AgNps. However, the absorbance intensity was slightly decreased upon the addition of FA to AgNps without changing the absorbance maxima position indicating the preserved shape and size of AgNps inside the gel (Fig. 2). TEM micrographs confirmed the above fact (Fig. 3). The shape of the AgNps was found to be more or less spherical with size in the range of 15–25 nm for both AgNps and FA-AgNps. No covalent bond was formed when FA was added to AgNps.

Table 1. Antibacterial activity of fumaric acid (FA), silver nanoparticle (AgNps) and fumaric acid incorporated silver nanoparticle hydrogel (FA-AgNps)

Bacteria	Zone of inhibition (mm)			
	FA (100 µL*)	AgNps (100 µL*)	FA-AgNps (100 µL*)	Standard
S. aureus	1.00±0.89	8.33±0.81	13.00±1.47	19.16±1.34[a]
E. coli	1.66±0.51	8.00±0.40	9.80±1.47	18.00±1.30[a]
P. aeruginosa	0.83±0.40	7.50±0.54	11.50±1.04	11.33±1.10[b]
K. pneumonia	N.A.	10.83±0.56	12.00±1.40	18.00±1.29[a]
P. mirabilis	N.A.	N.A.	0.66±0.81	12.00±1.15[a]

Each value represents mean ±SD of three readings.

*100 µL contains 0.1 mg of FA, 0.169 mg of AgNps and 0.269 mg of FA-AgNps respectively

[a]Gentamycin (10 µg); [b]Cefepime (50 µg); N.A. : No activity.

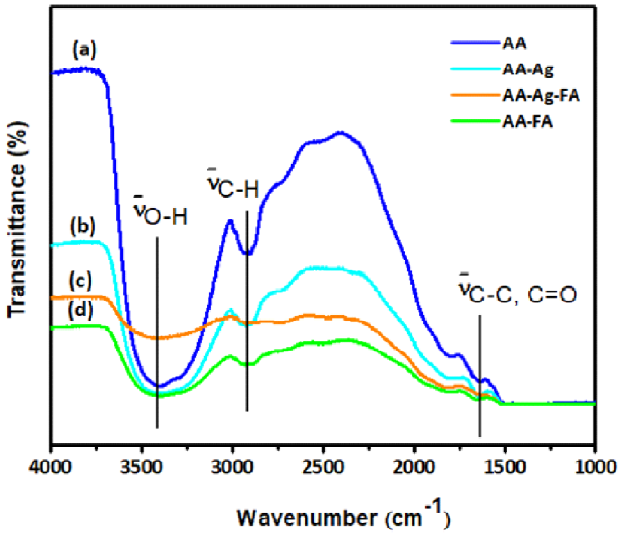


Fig. 1. FT-IR spectra of Agar-agar (AA), fumaric acid in Agar-agar (AA-FA), AgNps in Agar-agar (AA-AgNps) and fumaric acid added to AA-AgNps (FA-AgNps).

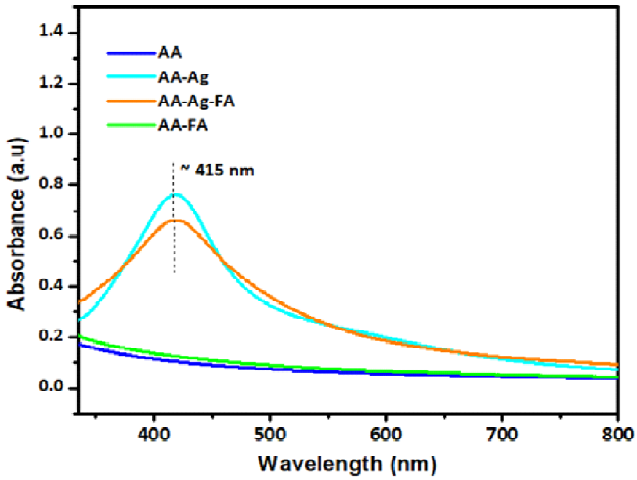
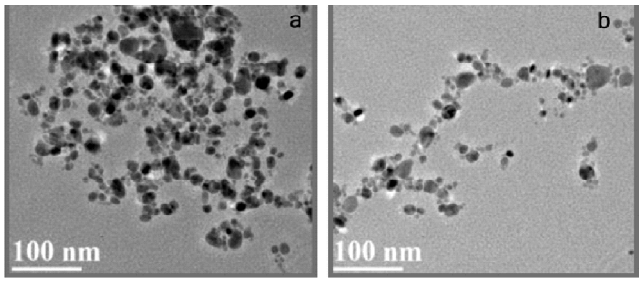


Fig. 2. UV spectra of Agar-agar (AA), fumaric acid in Agar-agar (AA-FA), AgNps in Agar-agar (AA-AgNps) and fumaric acid added to AA-AgNps (FA-AgNps).



(a) (b)

Fig. 3. TEM images of (a) AgNps and (b) FA-AgNps hydrogel.

FA-AgNps hydrogel when tested against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumonia* revealed significant effect when compared to FA, AgNps and standard antibiotics. The nano compound showed negligible inhibition against *P. mirabilis* (Table 1).

Fumaric acid is widely used in food, agriculture, pharmaceutical and chemical industries. It plays an important role in Tri-Carboxylic Acid Cycle (TCA Cycle). It has also been used as a drug[8] and also finds application as a food preservative and reported to possess antimicrobial properties[9]. It is well known that nanoparticles have distinct advantage over conventional chemical antimicrobial agents[10].

Experimental

Chemicals: Silver nitrate (99.9% $AgNO_3$, Fischer Scientific), N,N-dimethyl formamide (99.0% DMF, Finar Ltd.), polyvinyl pyrrolidone (PVP, Sisco Research Laboratory Pvt. Ltd.), Agar-agar (Hi-Media) and fumaric acid (Sigma-Aldrich, USA) were procured.

Bacterial strains: The bacterial strains used in the study were obtained from the Department of Microbiology, GITAM Institute of Medical Science and Research, Visakhapatnam, India.

Synthesis of fumaric acid incorporated silver nanoparticle hydrogel (FA-AgNps): The synthesis consists of two steps – (i) preparation of silver nanoparticles (AgNps) in Agar-agar matrix[11] and (ii) incorporation of fumaric acid into Agar-agar silver nanoparticle hydrogel formed. In the first step, 0.45 g of chloride-free Agar-agar was dissolved in 100 mL Millipore water under boiling condition till a clear solution was obtained. To this 0.1 g of PVP and 1.0 mL of DMF were added immediately followed by 0.169 g of $AgNO_3$. The contents were heated to 90°C with constant stirring for 5 h. The solution turned golden yellow confirming the formation of AgNps. In the second step, 1.0 g of FA was added in hot condition with stirring for 10 min resulting in the formation of fumaric acid incorporated silver nanoparticle hydrogel (FA-AgNps). The sample was preserved at room temperature.

Characterization of FA-AgNps hydrogel: The characterization of FA-AgNps hydrogel has been carried out using FT-IR, UV-Vis and TEM techniques.

UV-Visible spectroscopy: UV-Vis analysis was done on Cary 60 UV-Vis spectrophotometer (Agilent Technologies) using quartz cuvette. 1 mL solution of either AgNps or FA-AgNps hydrogel was dissolved in 3 mL of Millipore water and scanned over in the wavelength range of 400–800 nm.

FT-IR spectroscopy: FT-IR absorption spectrum was recorded in FT-IR Spectrum 1000 Perkin-Elmer Spectrometer.

Transmission electron microscopy (TEM): The size and morphology of AgNps and FA-AgNps were analyzed by TEM using TECNAI F 30 transmission electron microscope. Care was taken to prepare and mount the samples on the Cu grid each time to avoid any possible agglomeration of particles. The samples were prepared by similar conditions by placing a drop of well sonicated samples dissolved in water on a carbon-coated copper grid and subsequently dried in air before transferring it to the electron microscope operated at an accelerated voltage of 200 kV.

Antimicrobial activity: This has been studied by Well Diffusion method[12] in which sterile Muller-Hinton agar plates were swabbed on three axes with freshly prepared diluted culture and 6-mm wells that were bored aseptically using a sterile cork borer. The agar plugs were taken out carefully without disturbing surrounding medium. The wells were filled with 100 μL of either FA or AgNps or FA-AgNps hydrogel and allowed to stand for 1 h and incubated at 37°C for 24 h. Standard antibiotics Gentamicin (10 μg) and Cefepime (50 μg) were used for comparison. After incubation, the zones of inhibition around the samples were measured[4].

Conclusion

The fumaric acid incorporated silver nanoparticle hydrogel has been prepared for the first time and characterized. The nano compound has been studied for antibacterial property wherein it exhibited significant inhibition against four organisms out of five tested. As fumaric acid is a constituent of

the wound-healing plant *Aloe vera* (L.) Burm. F., possibly the nano compound may also be effective in treating wounds. However, a detailed study is needed to confirm this.

Acknowledgement

The authors thank the Management of GITAM Institute of Medical Sciences and Research for facilities and encouragement.

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