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Research Article

PREPARATION OF CHITOSAN STABILIZED OFLOXACIN- GOLD NANO CONJUGATE FOR THE IMPROVED ANTI BACTERIAL ACTIVITY AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Objective .In the present study, biocom[patible polymer chitosan stabilized ofloxacin- gold nanoconjugate was synthesized for the improved anti bacterial activity against clinical isolate of *E.coli* and *Staphylococcus aureus*

Methods. Chitosan stabilized oflaxacin-gold nanoparticles conjugate was prepared with aqueous solution of ofloxacin ,gold nanoparticles suspension and chitosan under optimum condition. Synthesized nanoconjugate was .characterized by scanning electron microscopy, FTIR and the synthesized nanoconjugate was tested against *E.coli* and *Staph.aureus* adopting well diffusion assay.

Result. Synthesis of chitosan stabilized ofloxacin- gold nanoconjugate was primarly confirmed by colour change of the reaction mixture, characteristic change in the FTIR pattern and size and shape by SEM. Anti bacterial activity revealed increased spectrum of antibacterial activity was recorded in chitosan stabilized ofloxacin - gold nanoconjugate against both the tested strains. The highest increase in inhibitory zone for *E.coli* and *Staph.aureus* was observed.

Conclusion. The present study suggests possible utilization of antibiotics –gold nanoconjugate as an effective anti microbial agent against the pathogenic bacteria

Keywords: chitosan,ofloxacin,nanoconjugate ,E.coli, Staphylococcus aureus

INTRODUCTION

Nanobiotechnology is a branch of biotechnology which deals with the study and application of biological and biochemical activities from elements of nature to fabricate new devices like biosensors. The term bionanotechnology is often used interchangeably with nanobiotechnology, though a distinction is sometimes drawn between the two. If the two are distinguished, Nanobiotechnology usually refers to the use of nanotechnology to further the goals of biotechnology, while bionanotechnology might refer to any overlap between biology and Nanotechnology, including the use of biomolecules as part of or as an inspiration for Nanotechnological devices[1].The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment [2,3]. Nanoparticles can be used to treat diseases that require a sustained presence of the drug at several anatomical sites [4]. Nanomaterials are of interest to defense and engineering programs because of their potential use in electronics, sensors, munitions, and energetic/reactive systems involved in the advancement of propulsion technology[5]. If formulated properly with other materials, nanomaterials may provide greater stability and efficiency for propellant system, and can pass through biological membranes, they can affect the physiology of any cell in an animal body[6,7].Different types of nanomaterials like copper,zinc,titanium,magnesium, gold[8,9] and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms[10].In addition to silver nano, gold nanoparticles are also an obvious choice due to their amenability of synthesis and functionalization, less toxicity,ease of detection and compatibility with antibiotics[11]. In the present study, enhanced antibacterial activity of chitosan coated ofloxacin–gold nanoconjugate against clinical isolate of *E.coli* and *Staphylococcus aureus* was studied.

MATERIALS AND METHODS

Synthesis and characterization of free gold nanoparticles

Gold nanoparticles were synthesized by chemical reduction of 0.01% aqueous tetrachloroauric acid with 10ml 0f 1% aqueous sodium citrate Synthesis of gold nanoparticles was confirmed by the conversion of the reaction mixture into deep pink colour and further characterization of the synthesized gold nanoparticles was carried out with determination of Plasmon absorption maxima with UV-Vis spectroscopy, particle morphology (i.e., shape and size) with Transmission Electron Microscopy (TEM) and FT-IR analysis.

Antibacterial activity of free gold nanoparticles

The antibacterial activity of free gold nanoparticles was tested against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* which were obtained from Chrompet general hospital, Chennai. The strains were maintained on nutrient agar slants. The inoculum was prepared in Nutrient broth. After 24 hours, the inoculum was spread with sterile cotton swab on Nutrient agar plates. Wells (8mm) were made using sterilized cork borer and 0.0025mg/ml, 0.0050mg/ml, 0.0075mg/ml and 0.0100mg/ml of different concentration of silver nanoparticles were added separately. Similarly, different concentration (i.e., 0.00125mg/ml, 0,00250mg/ml, 0.00375mg/ml and 0.00500mg/ml) of gold nanoparticles were added to each well. The seeded plates were incubated at 37^0C for 24 hours and the plates were observed for zone of inhibition. After the incubation period, the diameter of the zone was recorded.

Preparation and Characterization of chitosan stabilized ofloxacin- gold nanoconjugate

Chitosan was obtained from Rolex chemical industries, Mumbai and refined twice by dissolving it in dilute HOAc solution. The solution was filtered, the chitosan was precipitated with aqueous sodium hydroxide, and the precipitate was dried in vacuum at room temperature [12].The degree of deacetylation was about 85% as determined by elemental analysis, and the average molecular weight of the chitosan was 220kDa as determined by viscometric methods[13]. Chitosan stabilized ofloxacin-gold nanoparticles conjugate were prepared with aqueous solution of ofloxacin with the concentration of 3mg, 0.0025mg/ml, 0.0050mg/ml, 0.0075mg/ml

and 0.00125mg/ml, 0,00250mg/ml, 0.00375mg/ml and 0.00500mg/ml) of gold nanoparticles were mixed with 0.2% chitosan solution. The mixture was stirred under magnetic stirrer for 3hrs to get the complete homogenous mixture. The slurry thus obtained was freeze dried and used for further studies. Characterisation of nanoparticles loaded antibiotics was carried out with FT-IR, Scanning electron microscope (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) used for quantitative detection and localization of elements in the nano specimens.

Evaluation of antibacterial activity of chitosan stabilized ofloxacin –gold nanoconjugate

Synthesized nanoconjugate thus prepared were dissolved in deionized water with different concentration and evaluated against tested bacteria by well diffusion method as described earlier.

RESULT AND DISCUSSION

Synthesis of gold nanoparticles was confirmed by colour change of the reaction mixture to pink colour and broad surface Plasmon peak located at 520nm, particle size and shape with Transmission Electron microscope (TEM) as spherical particles with the size range of 25-50nm (Figour 1).

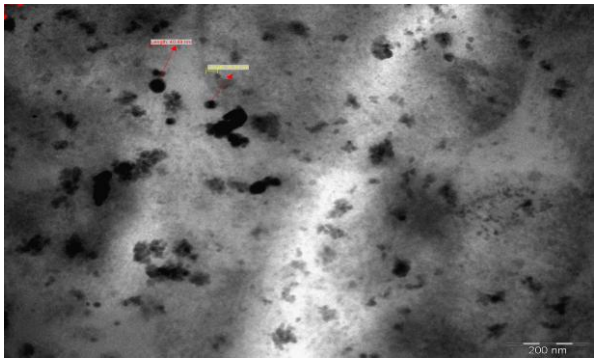

Figure 1: TEM image of synthesized gold nanoparticle

Antibacterial activity was not recorded in free gold nanoparticles. No zone of inhibition was observed in all the tested concentration such as 0.00125mg/ml, 0.00250mg/ml, 0.00375mg/ml and 0.00500mg/ml of gold nanoparticles (AuNPs) against both the tested strains (Table 1,2).

Table 1: shows

Sl. No	Concentration (mg/ml)	Zone of inhibition (mm)
1.	0.00125	00
2.	0.00250	00
3.	0.00375	00
4.	0.00500	00

Table 2: shows

Sl. No	Concentration (mg/ml)	Zone of inhibition (mm)
1.	0.00125	00
2.	0.00250	00
3.	0.00375	00
4.	0.00500	00

Antibiogram of the free ofloxacin revealed both the tested strains were susceptible and the zone of inhibition was increased as dose dependent manner. Maximum zone of inhibition was recorded in 1mg/ml and 0.01mg/ml as 38 and 36mm (Table 3,4)

Table 3: shows

Sl. No	Concentration (mg/ml)	Zone of inhibition (mm)
1.	0.25	34
2.	0.50	37
3.	0.75	39
4.	1.00	41
5.	0.025	32
6.	0.050	34
7.	0.075	37
8.	0.100	39
9.	0.0025	30
10.	0.0050	32
11.	0.0075	35
12.	0.0100	33
13.	0.0050	32
14.	0.0100	34
15.	0.0150	36
16.	0.0200	39
17.	0.0075	31
18.	0.0150	34
19.	0.0225	37
20.	0.0300	39
21.	0.0025	27
22.	0.0050	31
23.	0.0075	34
24.	0.0100	37
25.	0.00125	23
26.	0.00250	26
27.	0.00375	30
28.	0.00500	32
29.	0.00025	19
30.	0.00050	22
31.	0.00075	25
32.	0.00100	28
33.	0.0003	20
34.	0.0006	26
35.	0.0015	28
36.	0.003	30
37.	0.0045	32
38.	0.006	34
39.	0.00015	16
40.	0.0003	20
41.	0.00075	23
42.	0.0015	25
43.	0.00225	28
44.	0.003	30
45.	0.00003	00
46.	0.00006	10
47.	0.00015	18
48.	0.0003	21
49.	0.00045	23
50.	0.0006	25

Table 4: shows

Sl. No	Concentration (mg/ml)	Zone of inhibition (mm)
1.	0.25	32
2.	0.50	35
3.	0.75	37
4.	1.00	40
5.	0.025	30
6.	0.050	34
7.	0.075	36
8.	0.100	40
9.	0.0025	24
10.	0.0050	26
11.	0.0075	29
12.	0.0100	31
13.	0.0050	27
14.	0.0100	30
15.	0.0150	33
16.	0.0200	36
17.	0.0075	30
18.	0.0150	33
19.	0.0225	35

20.	0.0300	38
21.	0.0025	21
22.	0.0050	27
23.	0.0075	30
24.	0.0100	34
25.	0.00125	20
26.	0.00250	24
27.	0.00375	27
28.	0.00500	32
29.	0.00025	19
30.	0.00050	23
31.	0.00075	27
32.	0.00100	29
33.	0.0015	17
34.	0.003	21
35.	0.0045	24
36.	0.006	30
37.	0.00075	14
38.	0.0015	19
39.	0.00225	23
40.	0.003	27
41.	0.00015	00
42.	0.0003	00
43.	0.00045	00
44.	0.0006	00

No zone of inhibition was observed in 0.00003mg/ml and 0.00006mg/ml for *E-coli*. In *Staph.aureus,* 1mg/ml and 0.75mg/ml reveals distinct antibacterial activity with the zone of inhibition of 41 and 37mm respectively, no zone of inhibition was observed in 0.00015mg/ml Chitosan stabilized ofloxacin-gold nanoparticles conjugate was primarily confirmed by colour change of the reaction mixture from dark pink to pale yellow. The scanning electron microscopy study reveals chitosan stabilized ofloxacin- gold nanoparticles as spherical particles with the size range of 20 to 40nm (Figour 2).. The SEM analyzer built-in with an EDAS micro-analyzer allows a quantitative detection and localization of elements in the nano specimens. EDAS images showed the presence of elements like carbon, oxygen, nitrogen and gold in the range of 49.96, 43.17, 4.34 and 2.72% respectively (Figour 3).

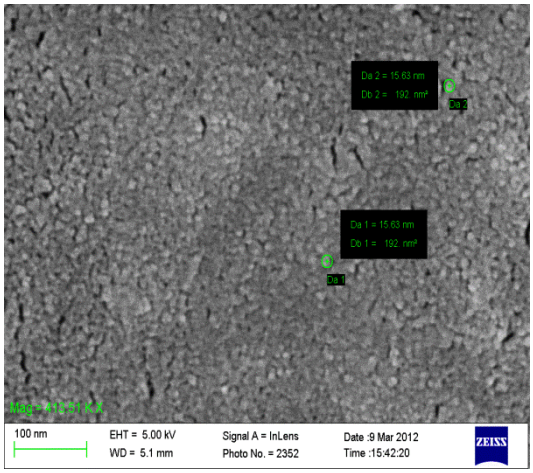

Figure 2: SEM image of chitosan stabilized ofloxacin- gold nanoconjugate

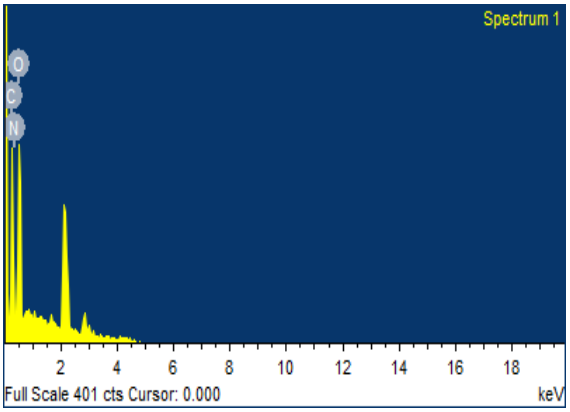

Figure 3: Energy dispersive spectroscopy (EDS) of chitosan stabilized ofloxacin gold nanoconjugate

The profiles of FT-IR spectroscopy reveals the main absorption of chitosan stabilized ofloxacin- gold nanoparticles at 3465.62cm-1, 2368.47cm-1, 2082.66 cm-1, 1638.08 cm-1 and 695.67 cm-1. When the FTIR spectrum of chitosan stabilized gold-ofloxacin were compared, it was found that almost the all the absorbed peaks were modified upon stabilization with chitosan(Figure 4,5).

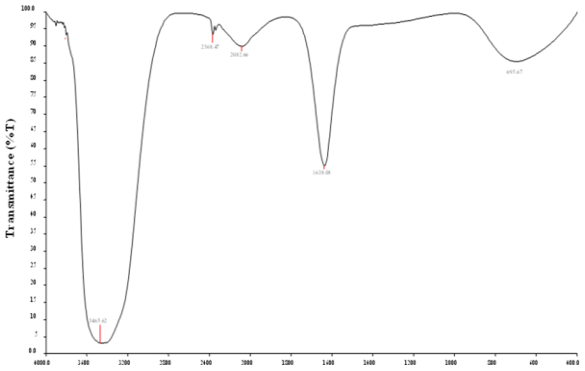

Figure 4: FTIR spectra of gold nanoparticles

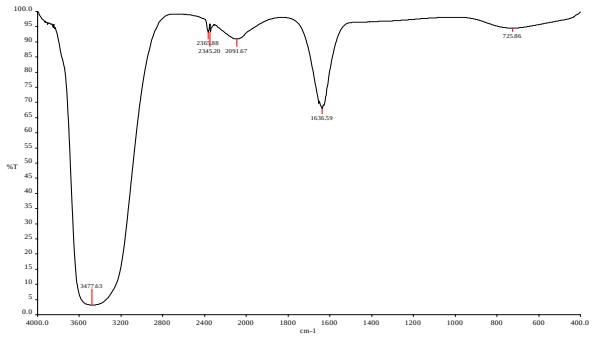

Figure 5: FTIR spectra of chitosan stabilized ofloxacin –gold nanoconjugate

It can be seen that the chitosan stabilized antibiotic nanoparticles conjugate retarded bacterial growth to a degree comparable to that demonstrated by the free antibiotic. When the free antibiotic conjugated with nanoparticles the diameter of the zone of inhibition were increased by at least two folds. Chitosan Stabilized ofloxacin-gold nanoparticles showed distinct increase in antibacterial activity against both the tested strain(Table 5,6).

Table 5: shows

Sl. No	Ofloxacin		AuNPs		Ofloxacin + AuNPs	
	Concentration (mg/ml)	Zone of inhibition (mm)	Concentration (mg/ml)	Zone of inhibition (mm)	Concentration (mg/ml)	Zone of inhibition (mm)
1.	0.00015	10	0.00125	00	0.00015 + 0.00125	25
2.	0.0003	15	0.00250	00	0.0003 + 0.00250	35
3.	0.00045	18	0.00375	00	0.00045 + 0.00375	37
4.	0.0006	20	0.00500	00	0.0006 + 0.00500	40

Table 6: shows

Sl. No	AuNPs		Ofloxacin + AuNPs	
Concentration (mg/ml)	Zone of inhibition (mm)	Concentration (mg/ml)	Zone of inhibition (mm)	Concentration (mg/ml)	Zone of inhibition (mm)
1. 0.00015	00	0.00125	00	0.00015 + 0.00125	20
2. 0.0003	14	0.00250	00	0.0003 + 0.00250	31
3. 0.00045	17	0.00375	00	0.00045 + 0.00375	38
4. 0.0006	22	0.00500	00	0.0006 + 0.00500	47

In *E-coli* the maximum inhibitionwas recorded in 0.00015+0.00125mg/ml,0.0003+0.00250mg/ml, 0.00045+0.00375mg/ml and 0.0006+0.00500mg/ml Concentration with the zone of inhibition of 25, 35, 37 and 40mm.Similar improved activity of nanoconjugate against *Staphylococcus aureus* was recorded in 0.00015+ 0.00125mg/ml, 0.0003+0.00250mg/ml, 0.00045+0.00375mg/ml, 0.0006+0.00500mg/ml Concentration with the zone of inhibition of 20, 31, 38 and 47mm In the present study ,chitosan stabilized ofloxacin gold nanoparticles recorded enhanced antibacterial activity against both the tested strains. Similar findings has been reported by Namasivayam *et al* [14,15]. Anti bacterial activity of oflaxacin and tetracycline was found to be increased with silver nanoparticles. Burygin *et al*[16] studied enhanced antibacterial activity of gentamycin -gold nanoparticles. Various polymers are now used to stabilize the metallic nanoparticles. Chitosan is the natural polymer has been reported as a polymer-based protective agent to stabilize the metal nanoparticles[17].Because of the biocompatibility,biodegradability, nontoxicity and adsorption properties of chitosan, it was used as a stabilizing agent to prepare Ag, Au and Pt nanoparticles. These chitosan- protected nanoparticles can be easily integrated into systems relevant for pharmaceutical, biomedical, and biosensor applications. Therefore, it has attracted considerable interest due to its medicinal properties, such as antifungal, antibacterial, antiprotozoal, anticancer, antiplaque, antitartar, hemostatic, wound healing and potentiates anti-inflammatory response, inhibits the growth of cariogenic bacteria, immunopotentiation, antihypertensive, serum cholesterol lowering, immune enhancer, increases salivary secretion (anti-xerostomial) and helps in the formation of bone substitute materials[18].The present study reveals the enhanced anti bacterial effect of chitosan stabilized ofloxacin gold nanoconjugate against clinical isolate of *E.coli* and *Staphylococcus aureus* would suggests the possible utilization of nanoparticles as the antimicrobial agents.

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