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Synthesis, Characterisation and Antibacterial activities of Schiff base [New fuchsin] functionalised silver nanoparticles

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Abstract: Syntheses of materials with antibacterial property are immense field of research in material science. In this paper biologically active new fuchsin based Schiff base (condensation reaction of new fuchsin and salicylaldehyde) was functionalised on green synthesized Ag nanoparticle. The Schiff base stabilized Ag nanoparticle wascharacterized by XRD, FT-IR, SEM and UV-Vis spectroscopy techniques. XRD pattern shows the formation of nanosilver with face-centered cubic structure. The FT-IR spectrum confirms the formation and stabilization of Schiff base over the Ag nanoparticle. SEM analysis confirms that particles areelongated in shape. Antibacterial activities ofprepared compounds were studied on Gram positive (*Bacillus cereus, Staphylococcus aureus*)and Gram negative (*Escherichia coli, Klebsiellapneumonia*) bacteria.The results revealed that Schiff base capped Ag nanoparticle showed high biological activity than Schiff base.
Keywords: Schiff base, Newfuchsin, XRD,FT-IR, Silver nanoparticle.

Introduction

Synthesis of Metal nanoparticles was an intriguing area in research, due to its unusual chemical and physical properties such as catalytic, electronic, optical, magnetic, as well as its application in diverse field [1].Various methods of synthesizing nanoparticles have been developed such as chemical reduction which involves the reduction of metal chlorides or nitrates by strong reducing agents[2], sonochemical[3] ,polyol[4] ,solvothermal[5] ,hydrothermal[6] ,surfactants[7] etc. All these methods pose potential environmental and biological risks. Hence, researchers focus on green method of synthesizing nanoparticles that was simple, cost effective, ecofriendly and compatible to pharmaceutical and biomedical applications [8]. A Green method of synthesizing nanoparticles was focused on three important concepts i) usage of solvent ii) reducing agent iii) stabilizing agent. Reports on synthesis of silver nanoparticles using chitosan, leaf extracts, starch[9], enzymes[10],fungus[11] asstabilizing and reducing agents have been explored well.

Biosynthesis of silver nanoparticles using biopolymers such as starch, chitosan, cellulose, protein has attracted the attention of researchers because of its industrial applications [12]. Among the available biopolymers starch was found to be non-toxic, biodegradable and costeffective. The present study investigates the effect of starch on the phase formation and stabilization of nanosilver.

Schiff base functionalized nanoparticles are of current interest because of their medicinal and pharmaceutical applications [13]. The structure and functionality of Schiff base possess excellent biological activity [14]. Sujarania et al reported on the utility of Schiff base [2-(2, 2-diphenyl ethylimino) methyl) phenols) functionalized silver nanoparticles for biomedicine [15].Recent studies have shown that the antibacterial activity of silver nanoparticles strongly depends on the nanoscale dimension (size, shape) and surface chemistry [16, 17]. In order to increase the antibacterial property, we have now integrated the antibacterial property of Schiff base with that of silver nanoparticles. Schiff base was formed using new fuchsin and salicylaldehyde. triphenylmethane derivative, Newfuchsin was being used as staining agent in medical field since many years[18]. Many reports are available in the literature on antibacterial activity of salicylaldehyde [19].Keeping in view the widespread antibacterial applications of newfuchsin and salicylaldehyde, we set out to coat it on silver nanoparticles to develop a hybrid product with enhanced antibacterial properties.

The current work reports on green synthesis of silver nanoparticles using a natural polymer starch which act as reductant and stabilizer for the nanoparticles without using any hazardous chemicals. Further, the ligand newfuchsin and salicylaldehyde was functionalized on Ag nanocolloid. Antibacterial activities of Schiff base and Schiff base functionalized Ag nanocolloid are compared.

Experimental

Chemicals

Silver nitrate (99.99%), Starch, New fuchsin(99.9%), Salicylaldehyde (99%), sodium hydroxide were purchased from Qualigens, India and used as received. Double distilled water was used throughout the studies.

Synthesis of silver nanoparticles

A 40ml of starch (1%) solution was prepared and pH was stabilized (13) using sodium hydroxide solution and heated at 60^{o}C. For the synthesis of silver nanoparticles, 20ml of Silver nitrate solution (0.001M) was slowly added to the previously prepared starch solution. The appearance of brown colloid confirms the formation of silver nanoparticles.To the silver colloid, 10ml of new fuchsin(1M)solution and 10ml of salicylaldehyde (1M) solution was successively added and stirred for 10 minutes. A portion of the colloid was collected and used for characterization.

Characterization

UV–Vis spectroscopy was used to analyze the formation of silver nanoparticles using a Hitachi Double beam spectrophotometer Model U2800, with a scanning wavelength between 200 and 900 nm. Field Emission SEM (PhilipsCM200 at 200 kV) was used to determine the shape and size of AgNPs. FT-IR analysis was executed using KBr disc technique (FT-IR spectrometer- ThermoNicolate Company Avatar 330).

Antibacterial activity

[Antibacterial activity of colloid (Nano silver capped with Schiff base and Schiff base) was studied by the disc diffusion technique. Nutrient Agar (NA) plates were seeded with 8 h broth culture of different bacteria. In each of these plates, wells were cut out using sterile cork borer. Using sterilized dropping pipettes, different concentrations (5, 10, 25 and 50 µl/well) of sample was carefully added into the wells and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37°C for18–24 h. Gentamicin (10µg) was used as positive control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone.

Results and Discussion

Synthesis of Silver nanoparticles stabilized with Schiff base

The UV-Vis absorption spectrum was shown in Fig.1.The absorbance at 405nm indicated the formation of silver nanoparticles in starch matrix. The absorbance value was in agreement to literature reports [20]. After the formation of silver colloid in starch matrix, the amine (new fuchsin) and aromatic aldehyde (salicylaldehyde) was successively added to the silver nano colloid in 1:1 molar ratio and stirred for 10 minutes. TheUV-vis spectrum of the resultant silver colloid was shown in Fig.2.The λ_{max} value of Agcolloid was shifted to 375, 256 and 210 nm. The shift in the UV absorbance was due to the chemical interaction of amine and aldehyde to Ag in starch matrix. Similar observation of absorbance peaks for Schiff base was reported [20].From the UV results, formation of schiff base over the Ag colloid was proved.

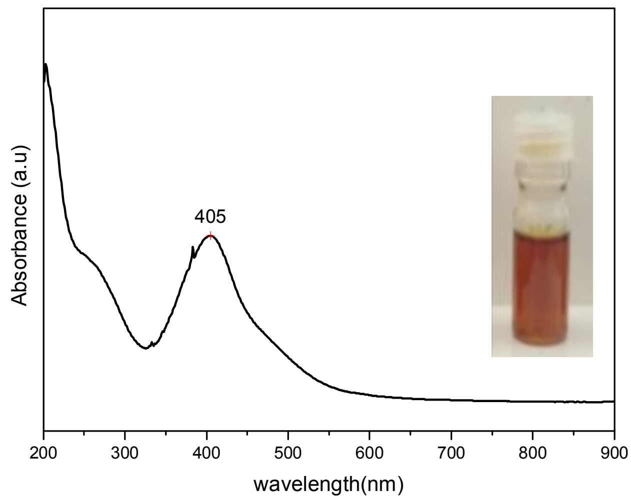


Fig.1.UV-Vis absorption spectrum of silver colloid(inset Agcolloid)

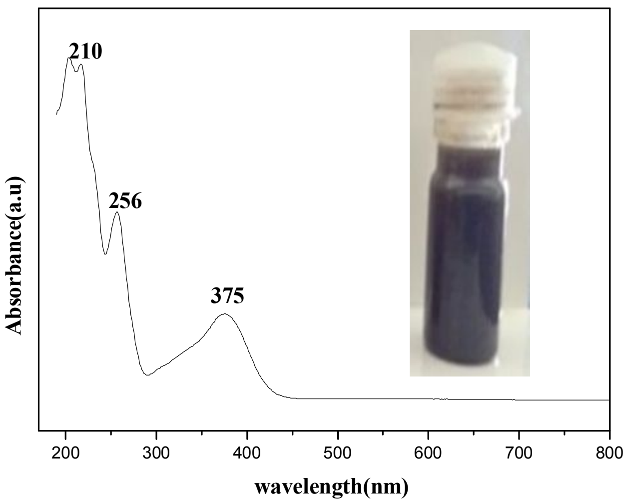


Fig.2.UV-Visabsorption spectrum of silver colloid capped with Schiff base (colloid picture)

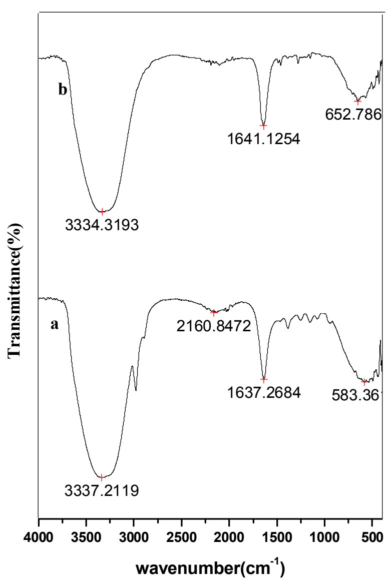


Fig.3 FT-IR spectra of Agcolloid@starch(a) and Ag colloid @starch@schiffbase(b).

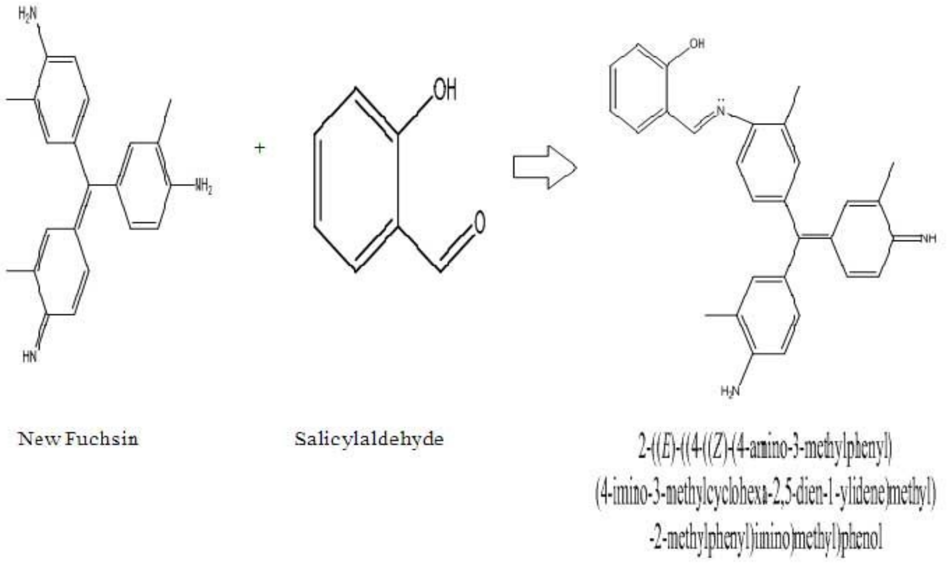


Fig.4.Scheme of Schiff base

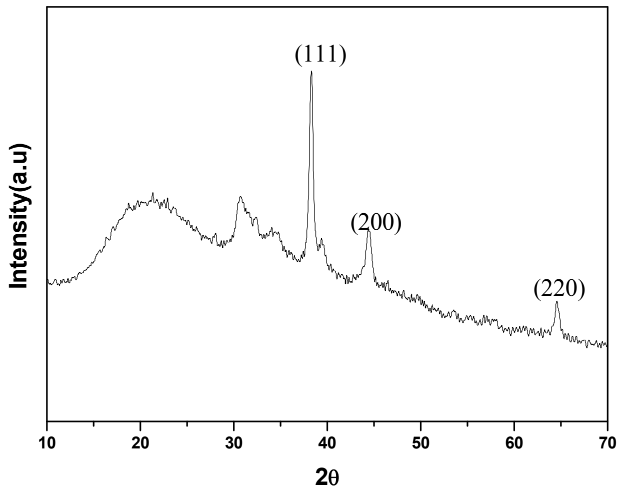


Fig.5.X-ray diffraction pattern of Schiff base capped silver colloid

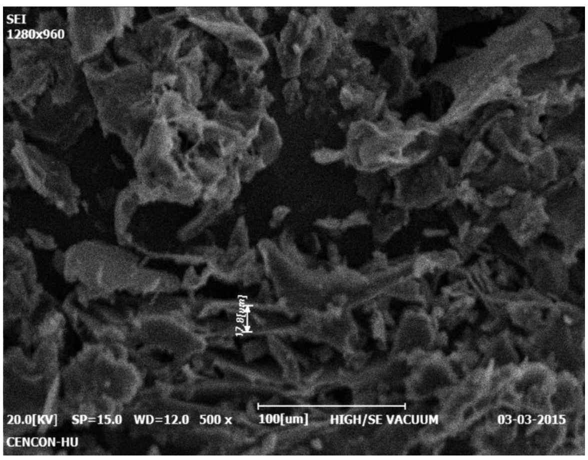


Fig.5.SEM image of Schiff base capped silver colloid

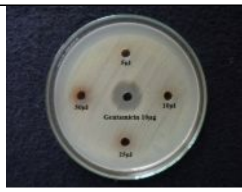
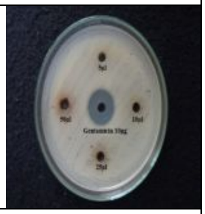
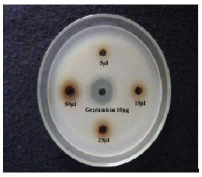
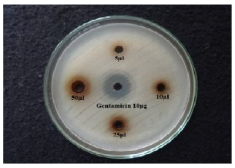
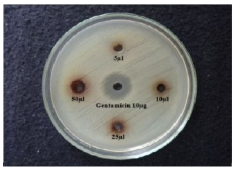
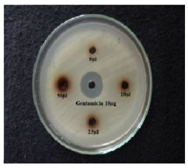
	B.c	**E.c**	***K. p***	**S.a**
Schiff base				
Silver Functio nalised Schiff Base				

Fig.6.Antibacterial effect of Schiff base and Ag@Schiff base

To confirm the possible formation of Schiff base functionalized silver colloid, the resultant colloids are centrifuged and the dried residues are analyzed by FT-IR. Comparison of the IR spectrum of starch stabilized silver nanoparticle with starch (literature reports) indicates a deviation of both hydroxyl and carbonyl group which confirms that both are involved in the formation of Ag nanoparticles. The disappearance of peaks at 1067, 1094, 1163 and 1242cm^{-1} confirm that the interaction between silver colloid and starch was through CH_2OH [21].The spectral analysis is detailed in table.1.FT-IR analysis provides an evidence to prove that starch acts as interlink between Schiff base and nanoparticles. The silver colloid stabilized by the –OH group interacts with one of the NH group of new fuchsin amine. The pH in the reaction medium facilitates the addition of –CHO group of salicylaldehyde to the free NH_2 group of amine to form Schiff base. This was confirmed by the azomethine band (C=N bond)observed in silver colloid stabilized with Schiff base at 1641 cm^{-1} [20].The IR spectra of Ag colloid stabilized with starch and Ag colloid stabilized with Schiff base are compiled in Fig.3.The proposed reaction mechanism was shown in the fig.4

Powder X-ray diffraction pattern (Fig.5) was recorded on the powder obtained after centrifuging the brown colloid. All the peaks appearing in the X-Ray diffraction pattern are indexed based on Ag (JCPDS file # 89-3722).It was reported that starch plays a major role in bio reduction of silver ions to silver nanoparticles(22).The average particle size estimated was approximately 50 nm.

Figure.6 shows the SEM images of the Ag colloid stabilized with Schiff base. SEM image showedaggregated elongatedparticles.The aggregation reveals that the stabilizing agent added was not sufficient to prevent aggregation.

Table 1. Comparison of FT-IR spectral details of starch, Ag colloid

Peak assignment	**Starch**	**Ag colloid**
-OHstr	3300-3600	3337
ν_{sym}(C–H)	2928	2972
C=O	1639	1637
C-H bending	1067	-----
C-O-H bending	1094	-----
C-O,C-Cstr	1163	-----
CH_2OH	1242	-----

Antibacterial activity

Antibacterial activities of Schiff base stabilized Ag nanocolloid and Schiff base were compared(Fig.6). Different concentrations of Schiff base stabilized Ag nanocolloid and Schiff base were injected and incubated at 37^oC for 24 hrs.. Gentamicin (10μg) was used as positive control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. Schiff base capped Ag nanoparticles and Schiff base were screened using the agar well diffusion method on both gram positive (Klebsiellapneumonia, Staphylococcus aureus) and gram negative bacteria(Bacillus cereus, Escherichia coli). We varied the concentration of samples (5,10,25,50µl) for all the four bacterial pathogens. After 24 h of incubation at 37°C, growth suppression was not observed in platesloaded with 5 and 10 µl of both the samples.The obtained experimental data clearly shown that (Table.2) Schiff base capped Ag nanoparticles showed zone of inhibition forEscherichia coli and Klebsiellapneumonia around10.00-10.58 mm for25 µlconc, while Schiff base has not showed. It has been observed that Schiff base capped Ag nanoparticles showed more antibacterial activity than Schiff base.

Table2: Inhibition Zone reports

Conc. (µl/well)	**Zone of inhibition (mm)**			
Schiff base	**B.c Bacillus cereus**	**E.c Escherichia coli**	***K. p* Klebsiellapneumoniae**	**S.a Staphylococcus aureus**
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	7.50 ± 0.00	8.00 ± 0.58	8.50 ± 0.58	0.00 ± 0.00
Ag functionalised Schiff base				
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25	0.00 ± 0.00	10.33 ± 0.58	10.00 ± 0.00	0.00 ± 0.00
50	10.67 ± 0.58	12.00 ± 0.00	12.33 ± 0.58	0.00 ± 0.00

Conclusion

The method shown here was the most convenient way to in situ synthesize and stabilize Schiff base on green synthesized silver nanoparticle. The reaction pH plays an important role for Schiff base addition reaction of new fuchsin amine and salicylaldehyde. In conclusion, a simple efficient, fast method, has been developed for the synthesis of novelSchiff bases over the silver nanoparticle. The Schiff base capped Ag nanoparticles and Schiff base were screened for their antibacterial activity against gram positive and gram negative bacteria. The zones of inhibition were determined.Schiff base capped Ag nanoparticles showed potent antibacterial activity than Schiff base.

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