Research Article

Isatin-Mediated Synthesis and Characterization of Silver Nanoparticles

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ABSTRACT

The present study discloses the synthesis of silver nanoparticles using isatin by different methods such as (i) at room temperature, (ii) variation of concentration of silver nitrate, (iii) variation of pH, (iv) at higher temperature, (v) by sonication method and (vi) solar method. Among all these methods solar method and sonication methods yielded nanoparticles quickly and easily. The synthesized silver nanoparticles were of average size 82 nm. Silver nanoparticles formed were analysed by UV-Visible spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM).

Keywords: Isatin, silver nanoparticles, XRD, SEM, FTIR.

1. INTRODUCTION

Indole ring compounds possess potent pharmacological properties such as antioxidant, antibacterial, anticonvulsant and anti-inflammatory. Isatin (indole -1H -2, 3-dione) is a versatile heterocylic molecule with indole as core molecule and find significant importance in medicinal chemistry. It is one of the constituent found in most of the drugs including antibiotics, anticancer and antidepressants. Isatin and its derivatives have profound application in wide range of products like pesticides, analytical reagents and dyes other than drugs[1, 2]. Most of the compounds of biological interest are derived from plant sources. Isatin is also a natural product obtained from the plants *Couropita guianancis Aubl*[3] and *Calanthe* discolor *Lindl*[4] belonging to the genus *Isatis*[5].

Isatin is also produced biochemically by *Altermones* sp strain inhibiting the surface of Cardiean shrimp *Palaemon macrodectylus* embryos which protect them from the pathogenic fungus *Lagenidium callinectes*[5]. The synthetic importance of Isatin has led to the extensive use of this compound in organic synthesis. Literature data reveals research focused more on the synthesis of various derivatives of isatin possessing diverse activities such as anticonvulsant, anti-inflammatory, antiviral, anticancer, antibacterial, antifungal and anti-HIV activities[6,7].

In modern development of drugs, nanotechnology plays a significant role to increase the chemical activity against the target species in diseased conditions[8]. Nanoscale materials are currently being used in electronic, magnetic, electronic, biomedical, pharmaceutical, cosmetic, energy, catalytic and materials applications[9]. Nanoparticles possess unique properties different from that of bulk products due to their size, shape and crystallographic structure with large surface to volume ratio[10]. Metallic nanoparticles are of great interest for applications ranging from antibacterial activity, catalysts, optics, and data storage[11]. Silver nanoparticles are an effective tool for killing pathogenic bacteria and hence find widespread use in clothing, cosmetics, toys, catheters and many other products[12].

Silver nanoparticles are used in many fields, as catalysts, as optical sensors, in textile engineering, in electronics, in optics, and most importantly in the medical field as bactericidal and therapeutic agents. Silver ions are also used in the formulation of dental resin composites; in coatings of medical devices; as a bactericidal coating in water filters; as an antimicrobial agent in air sanitizer sprays,

respirators, detergents, pillows, soaps, shampoos, toothpastes, washing machines *etc.*[13]. The immeasurable uses of silver nanoparticles, its widespread and mushrooming research interests has instigated this research work on synthesis of silver nanoparticles.

Metal nanoparticles can be prepared by three different routes viz., physical, chemical and biological approach. Chemical reduction is the most often practical method for the preparation of stable AgNPs in both aqueous and organic solvents[14]. Sodium borohydride and sodium citrate are the commonly used reducing agents for the synthesis of metal nanoparticles and results in particle diameters of several nanometers[15]. Nanoparticles of 10 nm sizes were obtained by electro reduction method using polyethylene glycol[16]. Sonochemical reduction method of synthesis results in silver nanoparticles of diameter 20 nm in an argon-hydrogen atmosphere[17]. Rapid synthesis of nanosilver was achieved by microwave method by increasing the concentration of poly (N-vinylpyrrolidone)[18].

El-Faham et al, synthesized AgNPs of 18–21 nm and 17–20 nm size using 3-hydrazino-isatin derivatives *viz*., 3-hydrazino-isatin [IsH] and 1-benzyl-3-hydrazino-isatin [BIsH]) in methanol as a reducing agent in an effective, rapid, and convenient manner. The AgNPs showed high antibactericidal activity against the *Bacillus subtilis, Micrococcus luteus* and *Proteus vulgaris*, as well as antifungal activities against *Saccharomyces cerevisiae*[19].

Isatin-3-thiosemicarbazone capped silver nanoparticles and Isatin-3-thiosemicarbazone was analyzed for antimicrobial assay on Human pathogenic organisms (*Salmonella typhi, Vibrio cholerae, Shigella dysenteriae, Eterococcus faecalis* and *Trichophyton rubrum*) and found that the antimicrobial screening of newly synthesized silver nanoparticles showed equal or better activity against these pathogens[20].

In an attempt to enhance the pharmacological application of isatin at nano level, an attempt has been made to attempt to synthesize the silver nanoparticles using isatin as reducing agent. Various parameters such as effect of concentration, temperature, pH and sunlight in support for the formation of silver nanoparticles were also analyzed. The synthesized nanosilver was confirmed and characterized by UV-Visible spectroscopy, XRD, SEM and FTIR analysis.

2. MATERIALS AND METHODS

2.1 Materials

Isatin AR grade was purchased from Himedia, India and silver nitrate was supplied by SD fine chemicals. Deionised water was used for making working solutions.

2.2 Experimental

2.2.1 Synthesis of silver nanoparticles using isatin under different conditions

Silver nanoparticles were synthesized by the addition of Isatin and silver nitrate (3mM) solutions in the ratio (1:5, 2:5, 3:5, 4:5 & 1:1 and 3:1, 3:2, 3:3, 3:4 & 3:5) respectively under room temperature. Higher temperature (75ºC), sonication (PCI Ultrasonics 1.5 L (H)) and solar methods were also carried out for the aforesaid concentrations of isatin and silver nitrate solution. The orange coloured solution changed to reddish brown at constant pH-7. The formation of silver nanoparticles was monitored by UV-visible spectrophotometer.

2.2.2 Synthesis of silver nanoparticles using isatin at different pH

Isatin (2ml) was treated with 5ml silver nitrate solution which was maintained at different pH (6, 7, 8, 9 and 10) and kept under room temperature. The orange colour changed to reddish brown indicates the formation of silver nanoparticles. The formation of silver nanoparticles was monitored by UV studies.

2.2.3 Separation of silver nanoparticles

The synthesized silver nanoparticles using Isatin at different conditions were separated by centrifugation (Spectrofuge 7M) at 13,500 rpm for 15 minutes. The process was repeated to obtain pure silver nanoparticles and was characterized by different spectral techniques.

2.2.4 Characterization of silver nanoparticles

The complete formation of silver nanoparticles was analyzed by Double beam UV-visible spectrophotometer-2202 (Systronics) at regular intervals of time. A drop of synthesized silver nanoparticles after centrifugation was coated on a glass plate and characterized using X-Ray Diffractometer (X' Pert Pro PANALYTICAL). Debye-Scherrer's equation was used to determine the particle size of the silver nanoparticles from the 2θ values of the X-ray diffraction peaks.

$$D = K\lambda / \beta \cos\theta$$

where,

K= constant,

λ = wavelength of the X-rays,

β = full width half maximum of the XRD peak (radians),
θ = Bragg's angle of the XRD peak.

The morphological analysis of the silver nanoparticles was investigated using TESCAN make Scanning Electron Microscope provided with Vega TC software. Further Secondary Electron Sputtering at an applied potential of 20 kV was adopted prior to recording SEM to have a clear insight to the size and shape of the nanoparticles. FTIR analysis was carried out using FTIR 8201 Shimadzu spectrometer.

3. RESULTS AND DISCUSSION

The UV-Visible spectrum of the synthesized silver nanoparticles shows absorbance band in the range of 420-430 cm^{-1} which confirms the formation of silver nanoparticles at room temperature (figure 1).

3.1 Effect of concentration of isatin

The effect of concentration of Isatin in the formation of silver nanoparticles at neutral pH is given in table 1. Rapid formation of nanosilver is obtained with 1ml of Isatin whereas 1:1 concentration of Isatin and silver nitrate gives nanosilver in 1 hour 45 minutes. Table 1 reveals silver nitrate to be reduced easily by 1ml Isatin and as the concentration of isatin is increased, there is interference between the colour of Isatin (orange) and the reddish brown colour of nanosilver which might be probably the reason for the prolonged time for appearance of the colour of nanosilver by visual comparison.

Increasing the concentration of capping agent (citrate) in solution and/or altering the pH of the aqueous dispersions of silver nanoparticles have been reported to reduce aggregation and lead to a decrease in Ag^{+} release in biological media. The efficiency of nanoparticle synthesis has been found to increase with increasing concentration of gum in the green synthesis of silver nanoparticles using gum ghatti[21].

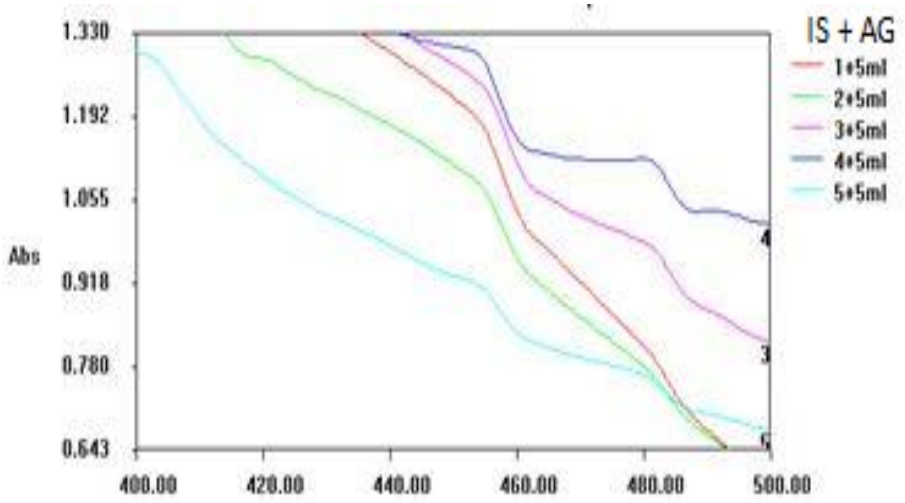

Fig. 1: UV-visible spectra of silver nanoparticles synthesised using Isatin at room temperature

Table 1: Synthesis of silver nanoparticles using Isatin at room temperature

Ratio of silver nitrate and Isatin solutions	pH	Time of formation of silver nanoparticles (min)
5:1	7.15	60
5:2	7.14	80
5:3	7.20	85
5:4	7.08	97
1:1	7.04	105

3.2 Effect of concentration of silver nitrate

A visible change in colour of the solution from orange to reddish brown was noted in 2 hours. The Surface Plasmon Resonance bands for the formed silver nanoparticles were in the range of 420-440 cm^{-1} and another band at 480 nm was observed as the concentration of the silver nitrate increases (figure 2). The time taken for the formation of silver nanoparticles is less at higher concentration of silver nitrate (table 2). As the silver nitrate concentration increases the release of silver ions may be more which results in rapid formation and the SPR band shifts to longer wavelength.

3.3 Effect of pH

The formation of silver nanoparticles at varying pH (table 3) shows that in acidic medium there is no formation of silver nanoparticles whereas at basic medium the silver nanoparticles was formed with Isatin in 12 minutes. The oxidation of isatin takes place at alkaline medium resulting in the reduction of silver nitrate to nanosilver. At acidic pH, the solutions did not yield nanosilver at room temperature and but at pH 7 (addition of NaOH), after 15 minutes sonication silver nanoparticles were formed.

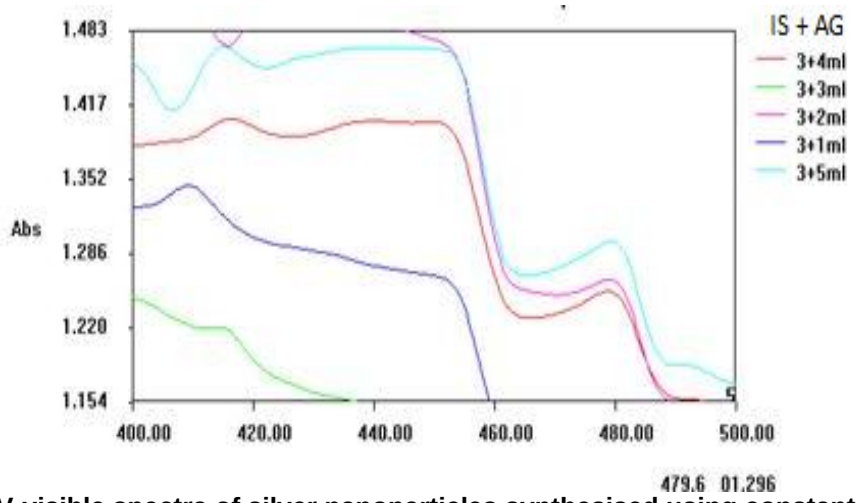

Fig. 2: UV-visible spectra of silver nanoparticles synthesised using constant concentration of Isatin at room temperature

Table 2: Synthesis of silver nanoparticles using Isatin by variation of concentration of silver nitrate

Ratio of silver nitrate and Isatin solutions	pH	Time of formation of silver nanoparticles (min)
1:3	7.23	155
2:3	7.12	140
3:3	7.28	120
4:3	7.15	115
5:3	7.07	105

Table 3: Synthesis of silver nanoparticles using Isatin by variation of pH of the solution

Concentration of Isatin (ml)	Concentration of silver nitrate (ml)	pH	Time of formation of silver nanoparticles (min)
2	5	6.19	-
2	5	7.04	12
2	5	8.21	10
2	5	9.01	8
2	5	10.01	5

The UV-visible spectrum shows that the bands were formed in the range of 440-480 cm^{-1} (figure 3). This confirms the formation of silver nanoparticles by pH variation method. In a similar study, the formation of silver nanoparticles using aspartic acid (Asp) as reductant, it is reported that there is formation of transparent silver sols in an alkaline medium. The silver nanoparticles were found to be spherical, uniform particle size and strongly depend on the (Asp). It was proposed that the oxidation of Asp occurs by the adsorbed Ag^{+} ions on the surface of silver nanoparticles[22].

3.4 Effect of temperature (75ºC)

A visible change in colour of the solution from orange to reddish brown was noted in 15 minutes at 75°C in water bath. The bands at 420-450 cm^{-1} and 480 nm were observed which confirms the formation of silver nanoparticles (figure 4). Higher temperature method aids the reduction of silver ions to stable nanosilver with agglomeration. Analysis of the formation of silver nanoparticles at various concentration of Isatin at higher temperature (table 4) shows that the concentration of Isatin (1ml) produces nanoparticles in 15 minutes whereas 1:1 solution of Isatin and silver nitrate gives nanosilver in 37 minutes. The quick formation of nanosilver compared to room temperature method may be because increasing the temperature causes an increase in the rate of reaction, increase in the particle size and shape.

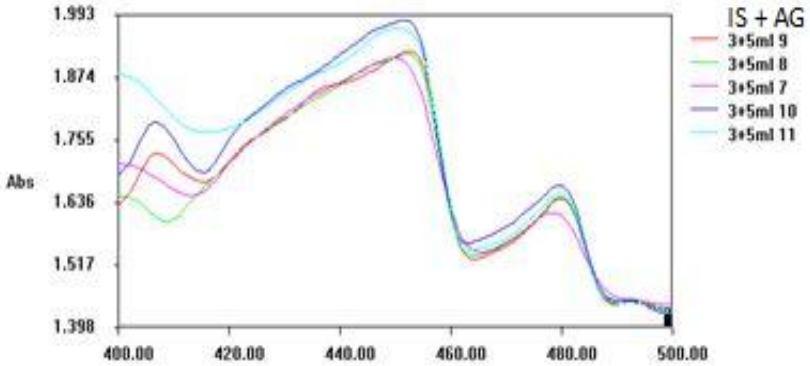

Fig. 3: UV-visible spectra of silver nanoparticles synthesised using Isatin at various pH

Table 4: Synthesis of silver nanoparticles using Isatin at higher temperature

Ratio of silver nitrate and Isatin solutions	pH	Time of formation of silver nanoparticles (min)
5:1	7.04	10
5:2	7.23	15
5:3	7.15	20
5:4	7.02	30
1:1	7.02	37

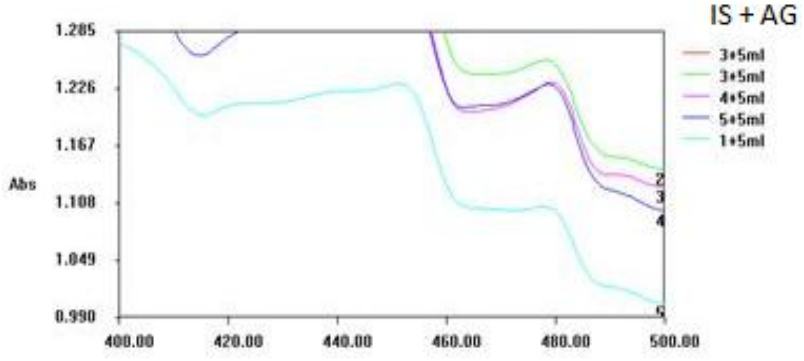

Fig. 4: UV-visible spectra of silver nanoparticles synthesised using Isatin at higher temperature

3.5 Effect of Ultrasonic waves

The results of the study of formation of nanosilver by sonication are tabulated (table 5). A visible change in colour of the solution from orange to reddish brown was noted in 1 minute under sonication. The UV-visible spectrum shows that the bands were formed in the range of 440-450 cm^{1} confirms the formation of silver nanoparticles (figure 5). The silver nanoparticles formed were stable at room temperature without flocculation of particles. This shows that silver nitrate was reduced easily by 1ml Isatin. As the concentration of Isatin is increased the time required also increases. During sonication ultrasonic waves are generated in a liquid suspension, it results in cluster breakdown or further agglomeration, as well as other effects including chemical reactions. During sonication the nucleation sites are well distributed in the micelles cores so it is easy to form silver nanoparticles easily[23].

3.6 Effect of solar radiation

The orange coloured solution changed to reddish brown within 10 minutes when exposed to sunlight. The UV-visible spectrum shows that the bands were formed in the range of 420-445 cm^{-1} and confirms the formation of silver nanoparticles (figure 6). This shows that silver nitrate was reduced easily by 1ml Isatin in the presence of sunlight. As the concentration of Isatin is increased the reaction takes place slowly.

When the solution of Isatin and silver nitrate was kept in sunlight the silver nitrate decomposes to produce microscopic grains of silver which are very small in size will react with Isatin to produce silver nanoparticles. The pH profile for the hydrolysis of isatin has shown that at pH < 3, isatin is the predominant species and at pH > 6, the ring opened isatinate is the major component. At pH values between 5 and 6, the rate of hydrolysis is first-order in hydroxide concentration or inversely proportional to the concentration of the hydronium ion, but from pH 6.5 to 10.5 it is pH independent. These results reveals the existence of a complex behaviour for the hydrolysis of Isatin. Hence the silver nanoparticles are formed from Isatin at pH above 6.

Table 5: Synthesis of silver nanoparticles using Isatin by sonication method

Ratio of silver nitrate and Isatin solutions	pH	Time of formation of silver nanoparticles (min)
5:1	7.30	1
5:2	7.23	3
5:3	7.03	20
5:4	7.15	24
1:1	7.08	32

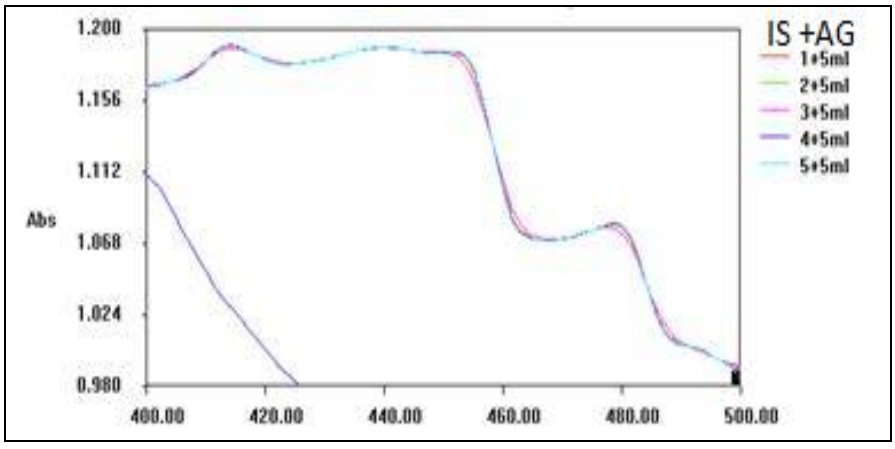

Fig. 5: UV-visible spectra of silver nanoparticles synthesised using Isatin under sonication

Table 6: Synthesis of silver nanoparticles using Isatin by solar method

Ratio of silver nitrate and Isatin solutions	pH	Time of formation of silver nanoparticles (min)
5:1	7.04	10
5:2	7.23	15
5:3	7.15	20
5:4	7.02	30
1:1	7.02	37

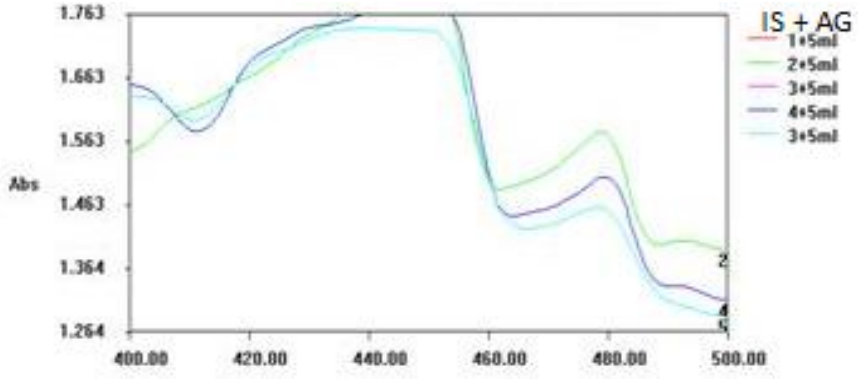

Fig. 6: UV-visible spectra of silver nanoparticles synthesised using Isatin under solar radiation

3.7 X-Ray diffraction analysis

X-Ray diffraction analysis is a tool to characterize the structure, phase, texture or even stress of crystalline materials using lattice parameters. The synthesized silver nanoparticles using Isatin shows different diffraction peaks at 29.30°, 31.81° and 66.51º (figure 7). The size of the nanoparticles was calculated by Debye Scherer's equation using FWHMs obtained from the diffraction peaks. The calculated value for the size of the silver nanoparticles is about 82 nm.

3.8 SEM analysis

SEM micrograph of synthesized silver nanoparticles by sonication method (4ml Isatin and 5ml silver nitrate) at 7.15 pH revealed spherical nanoparticles of size in the of range 130-140 nm (figure 8). The reason for the formation of larger nanoparticles (above 100 nm) may be due to increased concentration of both the isatin and silver nitrate. As the concentration increases the release of silver ions will be rapid that may sometimes lead to agglomeration.

3.9 FTIR analysis

The FTIR measurements were carried out to identify the possible functional groups responsible for the reduction of the silver ions into silver nanoparticles. The FTIR spectra of Isatin shows the peaks appeared at 3400 to 3200 cm^{-1} indicates the presence of -OH stretching. Further the peaks at 1600 to 1700 cm^{-1} corresponds to -C=C- stretching and 1500 to 1600cm^{-1} peaks indicates the presence of N-O asymmetric nitro compound (figure 9).

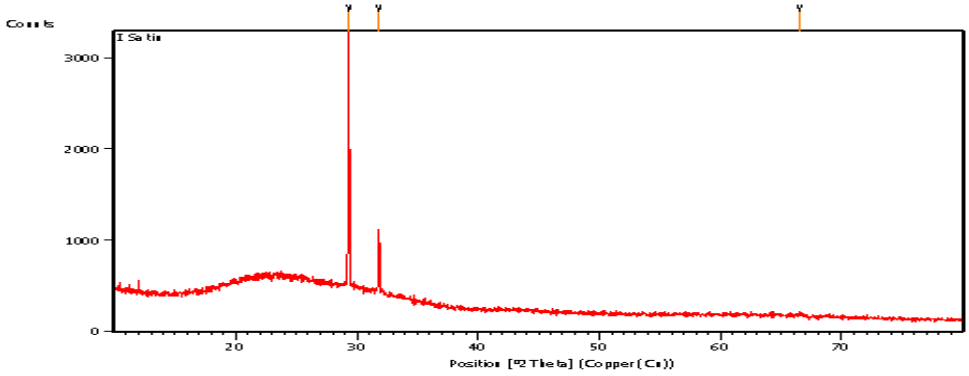

Fig. 7: XRD pattern of synthesized silver nanoparticles using isatin

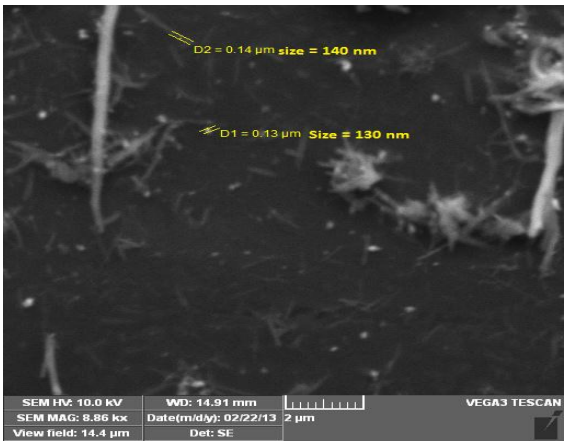

Fig. 8: SEM micrograph of synthesized silver nanoparticles using Isatin

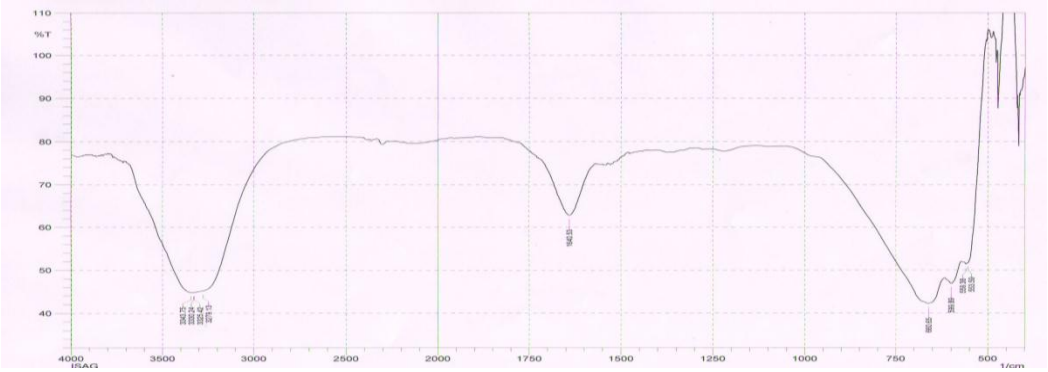

Fig. 9: FTIR spectra of the Isatin mediated silver nanoparticles solution

4. CONCLUSION

The formation of silver nanoparticles was achieved using isatin and the concentration of isatin was optimized by analyzing the reduction at various conditions. The pH of the solution and sonication method played a significant role in the synthesis of stable nanosilver. The complex behaviour of isatin at pH above 7 is responsible for the rapid reduction of silver ions. As the concentration of silver nitrate increases, the SPR bands get shift towards longer wavelength and formation of larger size nanoparticles. This is further confirmed by XRD and SEM analysis and found that particle size distribution in the range of 80-140 nm. The functional groups like –NH, -OH were involved in the formation of nanosilver as confirmed by FTIR analysis. Isatin capped silver nanoparticles may be used as lead compounds in drug discovery.

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