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# NOVEL APPROACH OF IMINO FUNCTIONALIZED SILVER NANOPARTICLES AS ANTIFUNGAL AGENT AND ADSORPTION OF Pb (II) ION FROM INDUSTRIAL EFFLUENTS

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## ABSTRACT

The Schiff base named N-(2-hydroxylbenzylidene)-2-amino pyridine [HBAP] is formed from condensation of equimolar (1:1) quantity of salicylaldehyde and 2- amino pyridine in alcoholic medium. The prepared Schiff base was characterized by elemental analysis, FTIR, UV-VIS and NMR studies. Synthesis of silver nanoparticles followed by trapping of silver nanoparticles with above mentioned Schiff base and characterized by SEM, FTIR, UV-VIS and NMR. Antifungal activity of test compounds was assessed against fungal such as *Aspergillus niger, Aspergillus nidulans* and *Candida albicans* by paper disc method at room temperature and results were compared with clotrimazole. The antifungal study revealed that silver nanoparticle coated with Schiff base showed excellent activity against *A. niger, A. nidulans,* and *C. albicans.* With the development of nanotechnology, silver nanoparticle anchored with Schiff base was used as adsorbent with sugarcane baggase as a solid phase and used in removal of heavy metal from industrial effluent. The metal ion was analyzed by AAS (Atomic absorption spectroscopy). The effects of several parameters that affect the adsorption of the metal ion including initial metal ion concentration, contact time, Schiff base weight and pH were examined.

**Keywords: A**ntifungal, Silver nanoparticles, Schiff base, Heavy metal, Adsorption.

## 1. INTRODUCTION

Schiff base is widely used in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical and biological activities. Schiff bases are synthesized from the condensation of amino and carbonyl compounds. Schiff bases have been studied extensively due to azomethine nitrogen which form coordination bond with metal. Schiff base shows antibacterial [1], antifungal [2], anticancer [3] and diuretic activities [4] due to azomethine nitrogen [5]. Heavy metals are released from various industrial plants such as electroplating, mining and leather industries, pigments, metallurgical process and textile industries. An industrial effluent contains many metal ions such as cadmium, chromium, copper, lead, zinc, manganese and iron [6-8]. Adsorption processes have been reported to be low cost alternatives for the treatment of heavy metals present in waste water. So, the development of new adsorbents with improved adsorption characteristics has remained as a significant research objective for the environmental pollution controlling processes. Many low-cost and eco-friendly nanomaterials with unique functionalities have been proposed for potential applications in detoxification of industrial effluents, groundwater, surface water and drinking water [9-12]. Silver nanoparticle acts as antimicrobial agent can be used in medical applications such as blood collecting vessels, coated capsules, band aids, biological labelling, etc [13]. Silver has also been used in filters to purify drinking water and clean swimming pool water due to its antimicrobial properties. In the present work, the Schiff base named N-(2-hydroxylbenzylidene)-2-amino pyridine [HBAP] is formed from condensation of equimolar (1:1) quantity of salicylaldehyde and 2-amino pyridine in alcoholic medium. Silver Nanoparticles were synthesized by chemical reduction method followed by coating of silver nanoparticles with above mentioned Schiff base. The structure of Silver nanoparticles coated with Schiff base is determined by UV-VIS spectra and further screened for antifungal activity of test compounds were assessed against fungal such as *A. niger, A. nidulans,* and *C. albicans* by paper disc method at room temperature. Sugarcane bagasse was benign lignocellulosic material, inexpensive (sugarcane industry waste) and rich in oxygen containing functional groups. Sugarcane bagasse was used as solid phase with imino functionalized silver nanoparticles for the extraction of heavy metal Pb (II) from industrial effluents.

## 2. MATERIAL AND METHODS

### 2.1. Instrumentation and chemicals

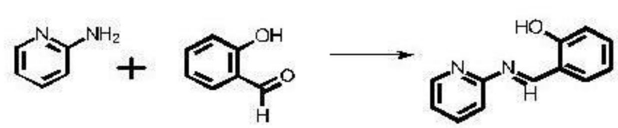
Salicylaldehyde, 2-amino pyridine, sodium borohydride and clotrimazole purchased from chemical company. Silver nitrate, sodium citrate, acetic acid and methanol purchased from sigma Aldrich chemical company. All the solvents were distilled, dried, and purified by standard procedures.

### 2.2. Synthesis of N-(2-hydroxybenzylidene)-2-amino pyridine [HBAP] schiff base

A solution of salicylaldehyde (hydroxybenzaldehyde 2.45 g, 20 mmol) in ethanol (10 ml) was mixed with solution of 2-amino pyridine (1.88 g, 20 mmol) and added few drops of formic acid. Mixture was stirred and thereafter refluxed for 7-8 hrs, the precipitate collected by filtration and recrystallized from alcohol. Table 1 shows the physical properties of Schiff base.

**Table 1: Physical Properties of Schiff base**

| | |
|---|---|
| Colour | Yellow orange crystal |
| Yield | 57% |
| Melting point | 62-64°C |
| Anal. Calculated for $C_{12}H_{10}N_2O$ | C- 72.75, H-5.02, N-14.14 |
| Found for $C_{12}H_{10}N_2O$ | C- 72.30, H-5.05, N-14.1 |




2-aminopyridine salicylaldehyde HBAP

### 2.3. Synthesis of silver nanoparticle

A large excess of sodium borohydride is needed both to reduce the ionic silver and to stabilize the silver nanoparticles. A 10 ml volume of 1.0 mM silver nitrate was added drop wise (about 1 drop second) to 30 ml of 2.0 mM sodium borohydride solution that had been chilled in an ice bath. The reaction mixture was stirred vigorously on a magnetic stir plate. The solution turned light yellow after the addition of 2 ml of silver nitrate and a brighter yellow when all of the silver nitrate had been added. The entire addition took about three minutes, after which the stirring was stopped and the stir bar removed. The clear yellow colloidal silver is stable at room temperature stored in a transparent vial for as long as several weeks or months. Reaction conditions including stirring time and relative quantities of reagents (both the absolute number of moles of each reactant as well as their relative molarities) must be carefully controlled to obtain stable yellow colloidal silver.

Chemical reduction method:

$$AgNO_3 + NaBH_4 \longrightarrow Ag + \tfrac{1}{2} H_2 + \tfrac{1}{2} B_2H_6 + NaNO_3$$

### 2.4. Trapping of silver nanoparticles by schiff base

Trapping of silver nanoparticles by Schiff base was done by anchoring the Schiff base as prepared above on the synthesized silver nanoparticles [14]. The Schiff base was successively added to the nano silver colloidal solution in and stirred for 10-15 minutes. After being stirred at moderate temperature, the colour of the solution became pale yellow to light brown. On keeping the solution overnight, the silver nanoparticles were formed and separated out from the solution by centrifugation (6,000 rpm for 10 min.) to remove ligand. The particles were cleaned with acetone and dried at room temperature for 24 hours.

### 2.5. Activation of solid phase

Sugarcane baggase (SCB) is used as solid phase in adsorption process. SCB was first washed with distilled water to remove the dust particles, then soaked overnight in NaOH solution and again washed well with DDW. SCB was then soaked in $CH_3COOH$ for 3 hrs to remove the traces of NaOH [15]. It was washed again with DDW till the wash water became colourless and then filtered, well dried, powdered and sieved before use.

### 2.6. Immobilization of Ag nanoparticles at schiff base on solid phase

The activated solid phase functionalized by an imines group was performed by methods reported previously. Then, 2 mg of the activated solid phase added in Schiff base which was dissolved in 10 ml of ethanol. The golden brownish product was dried in oven at 60 °C within 5hr [16].

### 2.7. Antifungal studies

Evaluation of Antifungal activity of test compounds were assessed against fungus such as *Aspergillus niger, Aspergillus nidulans,* and *Candida albicans* by paper disc method and results were compared with the standard drug clotrimazole.

## 3. RESULTS AND DISCUSSION

### 3.1. Characterization of schiff's base

#### *3.1.1. UV-VIS Spectral studies*

Four bands were shown (fig. 1) in ethanol medium. Band A and B at 207 nm and at 268nm wavelength respectively due to transition in aromatic ring [17]. Band C appeared at 305nm wavelength due to transition in between π orbital localized on central azomethine (-CH=N-) bond [18]. Band D appeared at 347nm wavelength due to charge transfer within the entire Schiff base molecule this bond is commonly observed in o-hydroxyl Schiff base [19] and is based on strong intramolecular H-bonding between the hydroxyl group of the salicylidine and the azomethine nitrogen [20]. In the previous published literature bands A and B appearing within the 200 -270 nm region. Bands C observed within the wavelength range 290-310 nm. Band D located within the 340 to 400 nm region can be ascribed to charge transfer within the entire Schiff base molecule.

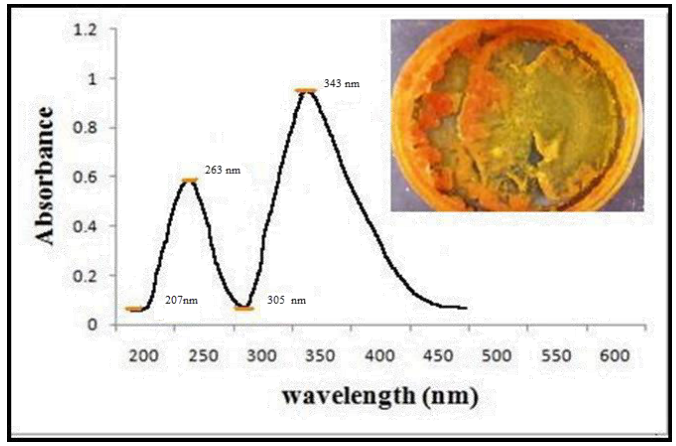



**Fig. 1: UV-Vis Spectra of Schiff base**

#### *3.1.2. FTIR Studies*

The absence of spikes above 3300 $cm^{-1}$ in figure 2 show that the primary amine has formed an imines in the ligand and cannot be absorbed within that frequency range [21].

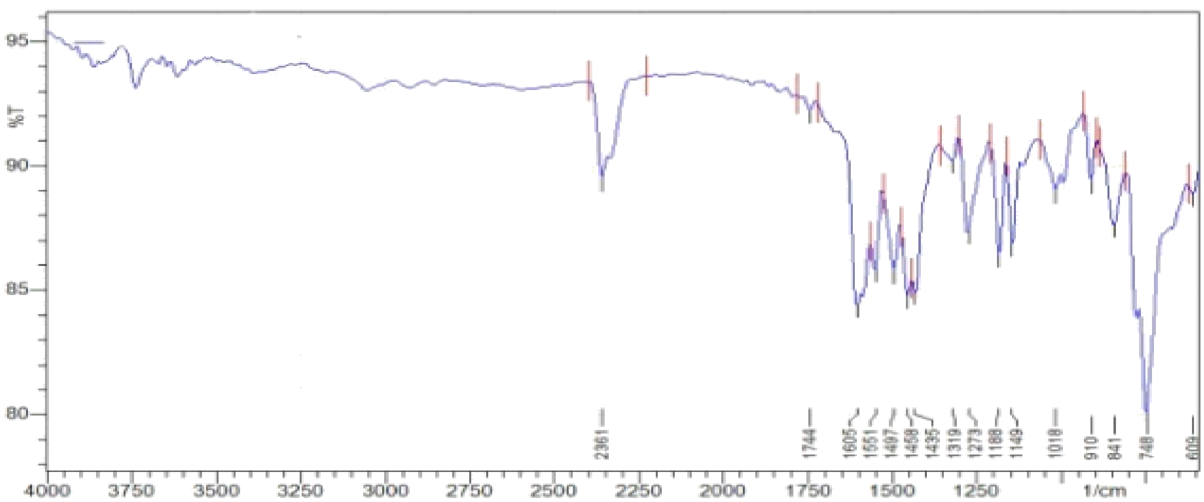



**Fig. 2: FTIR Spectra of Schiff base**

The weak absorption around 3750 $cm^{-1}$ could be attributed to-OH group. The imines ν (C=N) functional group absorbed strongly at 1605 $cm^{-1}$. The presence of the benzene ring conjugated to imines group in the ligand was noticed at 1551 $cm^{-1}$ [22]. In previous published literature it is reported that benzene ring conjugated to imines group shows absorption peak within the 1500-1600 $cm^{-1}$.

#### *3.1.3. $^{1}$H NMR Studies*

$^{1}$H NMR data from figure 3 shows the tautomeric equilibrium favours the phenol amine in $CDCl_3$. δ=9.825ppm, singlet for (-CH=N-), δ=6.92-7.4 ppm for aromatic ring.

### 3.2. Characterization of silver nanoparticle

#### *3.2.1. UV-VIS Spectral studies*

The specific colour of colloidal silver nanoparticle solution is due to Plasmon absorbance and produces peak at 405 nm ($\lambda_{max}$) wavelength in Fig. 4. Incident light produces oscillations in conductive electrons on the surface of nanoparticles and electromagnetic radiation is absorbed so peak is observed.

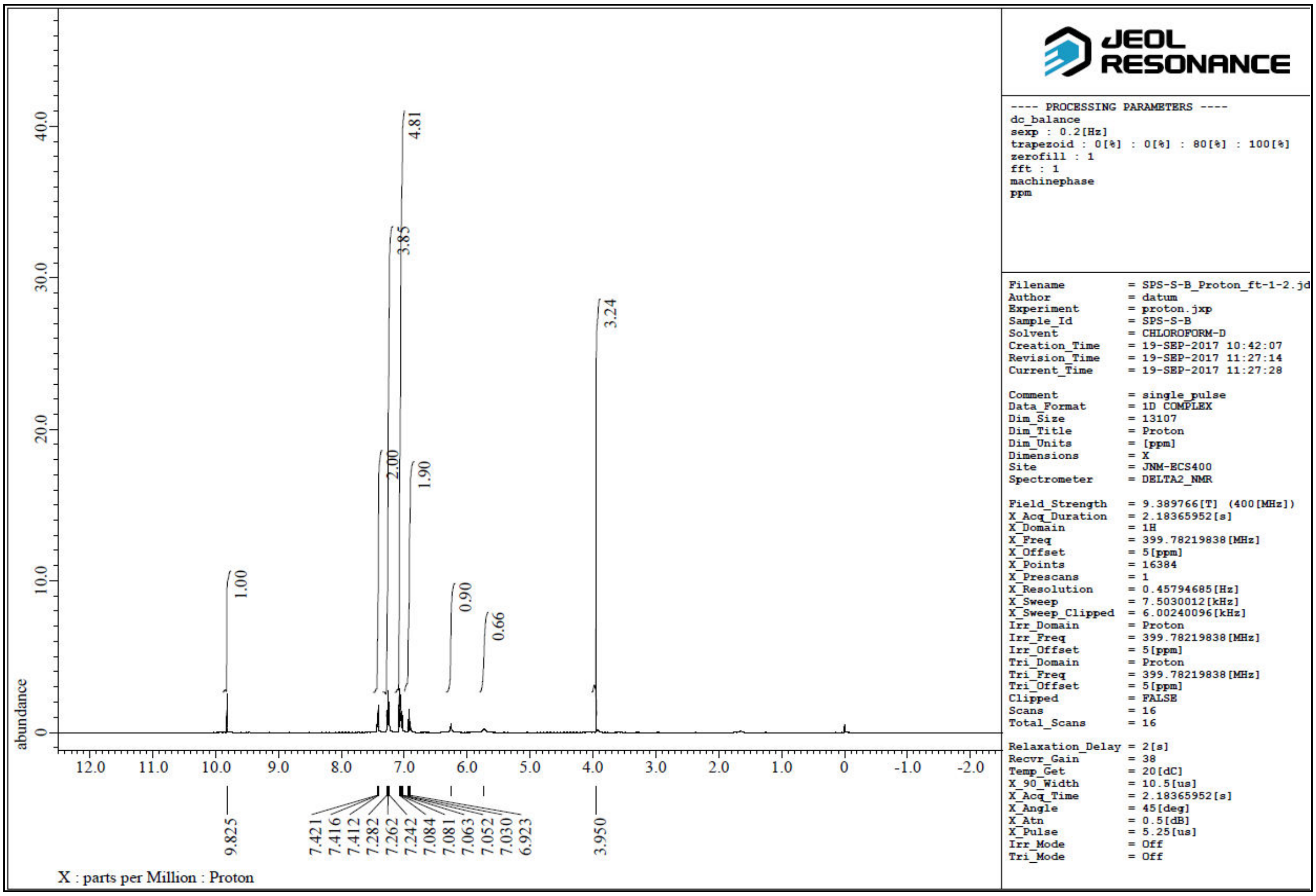



**Fig. 3: $^{1}$H NMR Spectra of Schiff base**

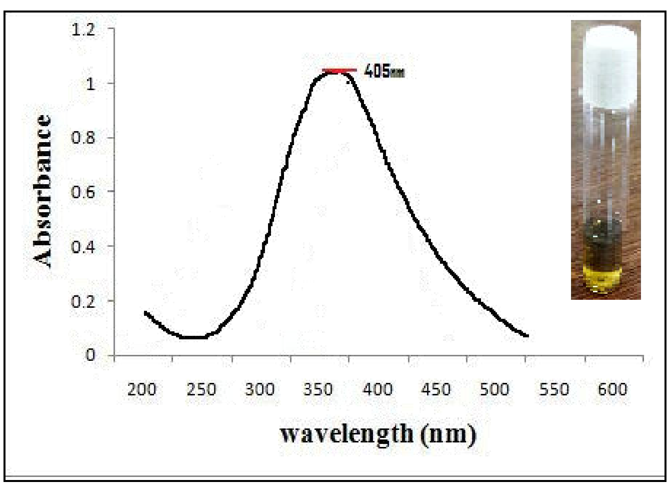



**Fig. 4: UV-Vis Spectra of Ag Nanoparticles**

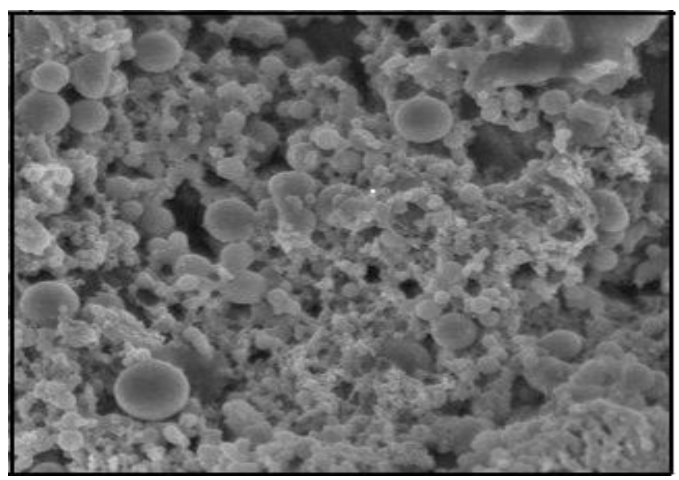


**Fig. 5: SEM image of Silver Nanoparticles**

### *3.2.2. SEM Studies*

The scanning electron microscope (SEM) image is shown in fig. 5. The synthesized silver nanoparticle shows the particle size is 50nm which confirms nanostructure.

## 3.3. Characterization of silver nanoparticle anchored with schiff base

### *3.3.1. UV-VIS Spectral studies*

In previous fig. 4 the UV-Vis spectra of silver nanoparticles (AgNps) shows $\lambda_{max}$ at 405 nm and in fig.

6 of Ag nanoparticle Schiff base shows λmax at 450 nm. So, a spectrum shows shifting at higher wavelength or red shift. The other bands at 207nm, 268nm and 305 nm were same as in Schiff base.

### *3.3.2. FTIR Studies*

Fig. 7 shows the strong absorption at 571 $cm^{-1}$ in the AgNps anchored with Schiff base which is absent in the Schiff base ligand is a strong indication of presence of silver metal.

### *3.3.3. SEM Studies*

Fig. 8 shows the SEM image of Ag nano trapped with Schiff base. The fig. 8 shows that the obtained material has a nearly porous structure which is suitable for adsorption studies.

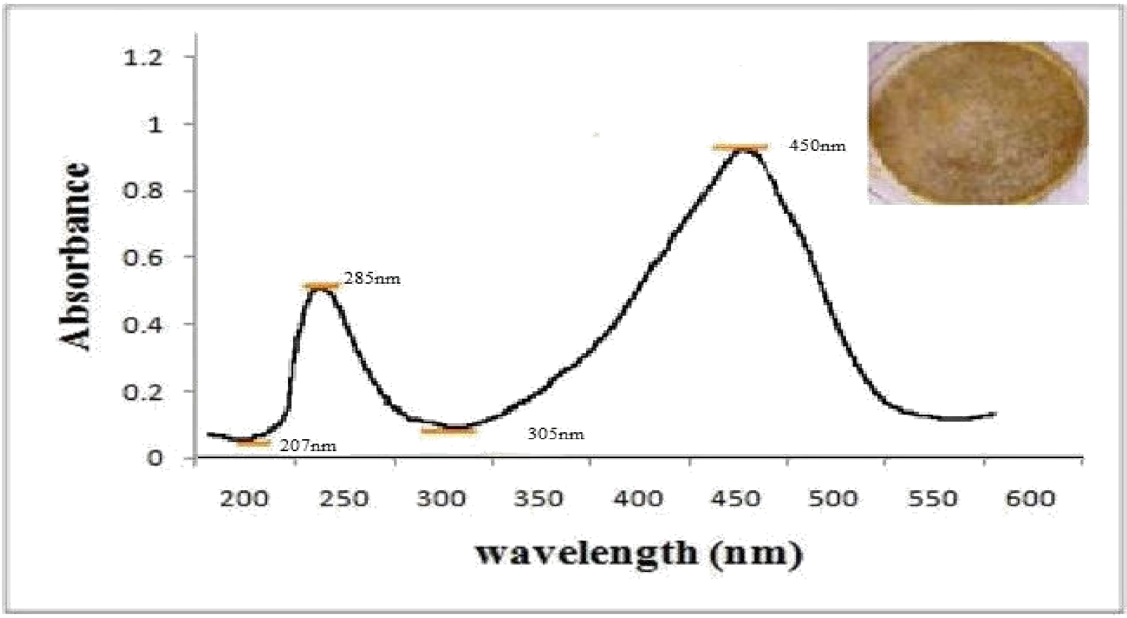



**Fig. 6: UV-Vis Spectra of Ag nanoparticle Schiff base**

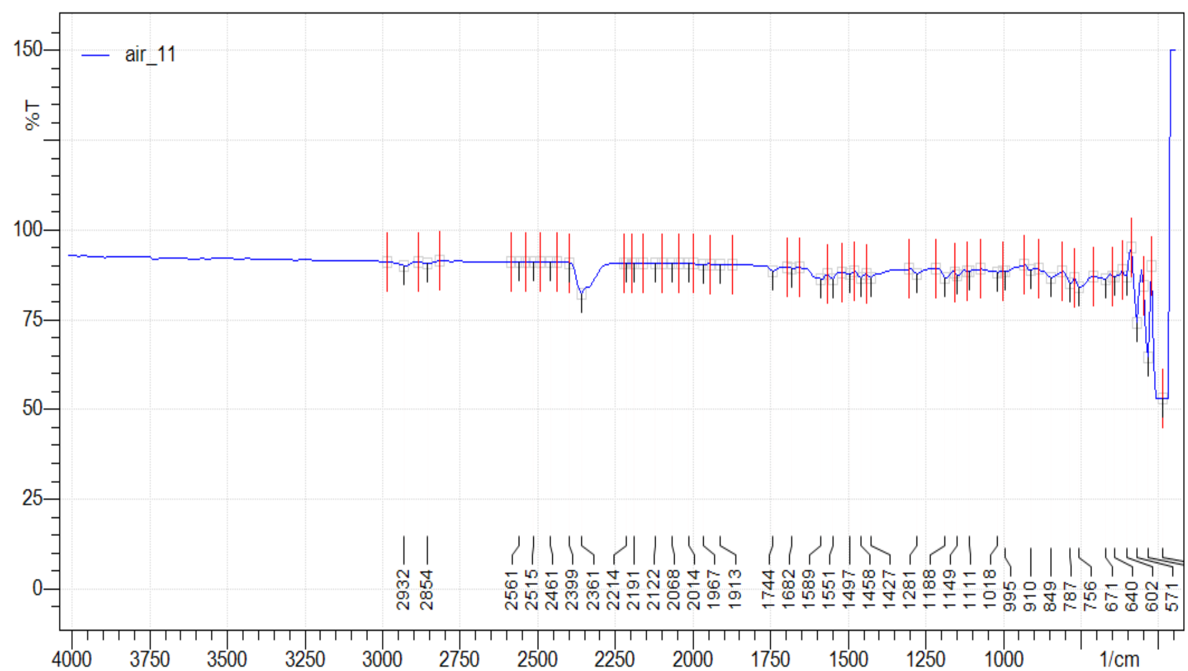



**Fig. 7: FTIR Spectra of AgNps Anchored with Schiff base**

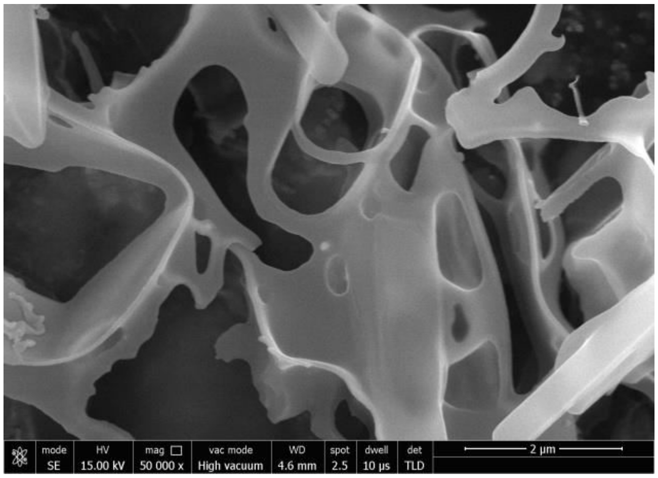



**Fig. 8: SEM image of AgNps Anchored with Schiff base**

### 3.4. Antifungal Studies

Evaluation of antifungal activity of all compounds *in vitro* was carried out by paper disc method against fungus such as *Aspergillus niger, Aspergillus nidulans* and *Candida albicans* by paper disc method and results were compared with the standard drug clotrimazole. The Disc Diffusion method [23, 24] was used to determine the antimicrobial activities of the Schiff bases using standard procedure of 6 mm disc were prepared from whatman's filter paper no. 1. Silver nanoparticles coated Schiff base solutions of varying concentrations ranging from 100, 500, 1000 ppm was prepared. In experiment inoculation medium was prepared by dissolving definite volumes of peptones, yeast extract, sodium chloride, potassium dihydrogen phosphate, methylene blue and glucose were dissolved in distilled water at desirable pH and Followed by preparation of base layer medium by dissolving potato dextrose agar in distilled water on magnetic stirrer. Base layer sterilized by autoclaving and maintained to appropriate pH. After base layer preparation overnight grown subculture of microbes were mixed with seed layer medium and immediately poured into petridishes containing the base layer and then allowed to attain room temperature. Antifungal disc having diameter of 6 mm (what man no.1), soaked in test solution, were dispensed on to the surface of this inoculated agar plate and incubated at 37 °C for 36 hours. The zone of inhibition was measured in mm for the particular compound. Clotrimazole was used as positive control. The data represent the values of three replicates and are evaluated as mean ± SEM values were determined and are shown in table 2.

**Table-2: Antifungal activity of AgNps anchored with Schiff base:**

| Conc.(ppm) | *Aspergillus niger* | *Candida albicans* | *Aspergillus nidulans* |
|---|---|---|---|
| 100 | 21(±0.264) | 19 (±0.411) | 18 (±0.206) |
| 500 | 29(±0.320) | 30(±0.259) | 28(±0.265) |
| 1000 | 39 (±0.100) | 36(±0.487) | 36(±0.265) |

Figs. 9- 11 are the graphical representation of antifungal activity of silver nanoparticle anchored with Schiff base at 100 ppm, 500 ppm, 1000ppm respectively. The significance level of all compounds were (P<.001), (*P<.01). The antifungal activity was evaluated by tube dilution method which depends on the inhibition of growth of a microbial culture in a uniform solution of antibiotic in a fluid medium that is favourable to its rapid growth in the absence of the antibiotic. In this method minimum inhibitory concentration MIC of the test compounds was determined. Their MIC values are presented in Table 3.

**Table 3: MIC value (in mg/ml) of Schiff base trapped with silver nanoparticle**

| *Aspergillus niger* | *Candida albicans* | *Aspergillus nidulans* |
|---|---|---|
| 0.16 | 0.21 | 0.34 |

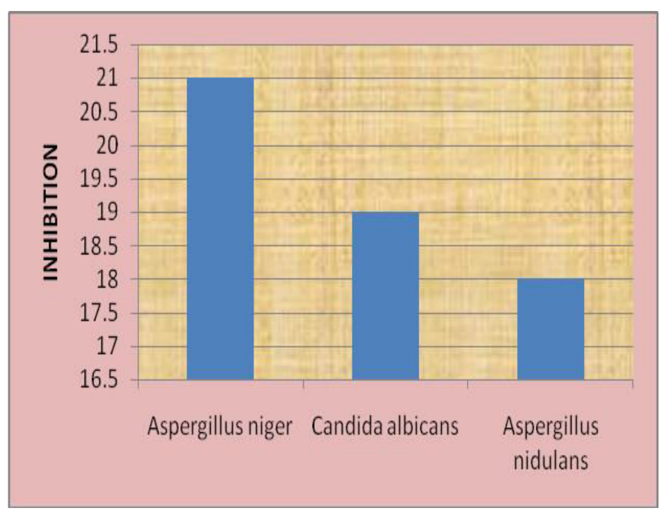



**Fig. 9: Graphical representation of antifungal activity of silver nanoparticle anchored with Schiff base at 100 ppm**

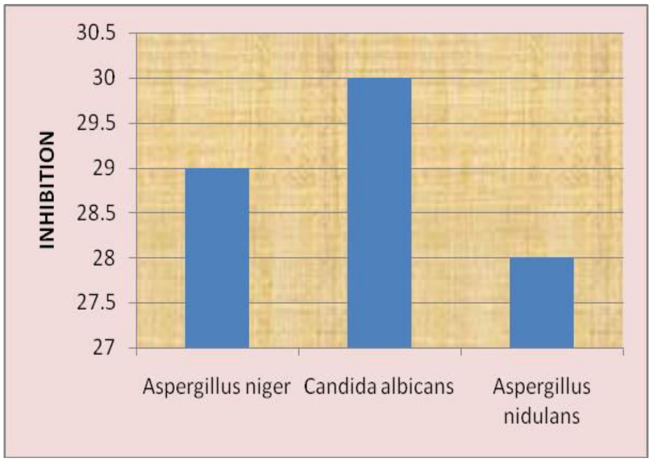



**Fig. 10: Graphical representation of antifungal activity of silver nanoparticle anchored with Schiff base at 500 ppm**

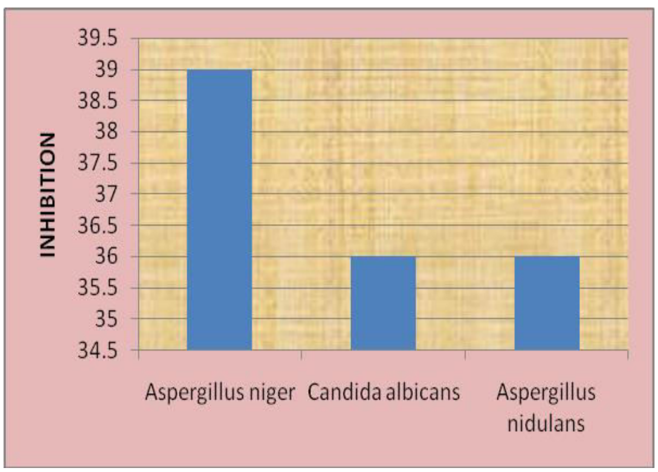



**Fig.11: Graphical representation of antifungal activity of silver nanoparticle anchored with Schiff base at 1000 ppm**

### 3.5. Extraction of Heavy metal Pb (II)

Silver nanoparticle trapped with Schiff base is attached on solid phase (sugarcane baggase) by previous method. Metal ion is extracted from industrial water samples by adsorption method. Column method is used for determination of the effect of various parameters on % removal of Pb (II) metal ion from industrial effluents. A glass column of 30 cm length and 2 cm internal diameter was used. Sugarcane baggase was put between two layers of glass wool, the first at the bottom to avoid loss of sorbent when the sample solution passes through the column and the second at the top to retain it. Then, water samples containing silver nanoparticle trapped with Schiff base passes through the column with flow rate 1.0 ml/ min.

Heavy metal Pb (II) is detected by flame AAS (Atomic absorption spectroscopy).The percentage of metal ion removal was calculated from following equation:

$$\% \text{ Removal} = (C_0 - Ce) \backslash C_0 \times 100,$$

Where $C_0$ is the initial concentration of metal ion and $C_e$ is the metal ion concentration at equilibrium.

#### 3.5.1. *Effect of initial concentration of the metal ion*

Effect of initial metal ion concentration on the percentage removal of Pb (II) metal ion by Ag nano@ Schiff base from industrial effluent was investigated. Table 4 shows the effect of initial metal ion concentration on the percentage removal of heavy metal in water sample. Effect of metal ion concentration is calculated at constant pH, biomass and weight of silver nanoparticle trapped with Schiff base. Maximum percentage removal of heavy metal takes place at concentration 10 ppm of Pb (II) in industrial waste water sample. As concentration of metal ion increases in water sample, the percentage removal of Pb (II) increases due to increasing binding site and at specific concentration of metal ion all the binding sites becomes saturated. But after reaching at saturation point as the concentration increases, percentage removal decreases as shown in fig.12.

**Table 4: Effect of Initial Concentration**

| Initial Pb(II) Concentration(PPM) | % Removal±SEM |
|---|---|
| 5 | 71%±1.154 |
| 10 | 90.3%±0.881 |
| 15 | 80%±0.577 |
| 20 | 78.66%±1.201 |
| 25 | 68.33%±1.452 |

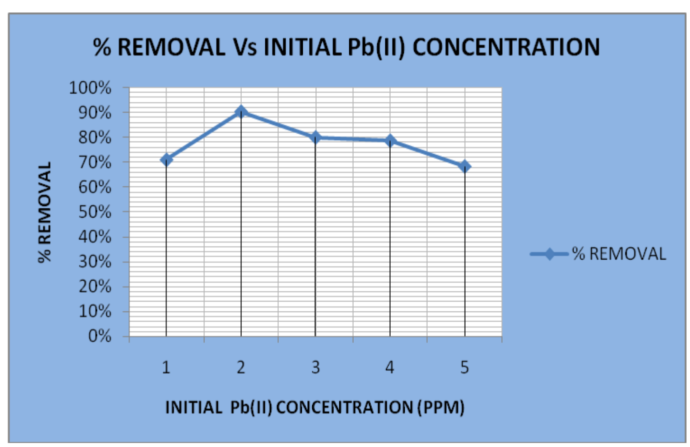



**Fig. 12: Effect of Initial Concentration on the Percentage Removal of Heavy metal ion**

### 3.5.2. *Effect of pH*

The pH was varied from 2 to 10 for Pb (II) and the pH of the solutions was adjusted to different values using suitable buffers. The removal efficiency was calculated at constant Schiff base weight, biomass and concentration of the metal ion. The Table-5 shows that maximum absorption takes place at pH 2 for Pb (II) in water sample. A figure 13 shows the uptake of Pb (II) which is depends greatly on pH .The metal ions are nearly stable at pH 2 in Pb (II) respectively due to stability of complexation of the metal ion with silver coated Schiff base.

**Table 5: Effect of pH**

| pH | % Removal±SEM |
|---|---|
| 2 | 91%±0.577 |
| 4 | 75.6%±0.881 |
| 6 | 69.66%±0.881 |
| 8 | 68.16%±1.098 |
| 10 | 68.13%±1.041 |

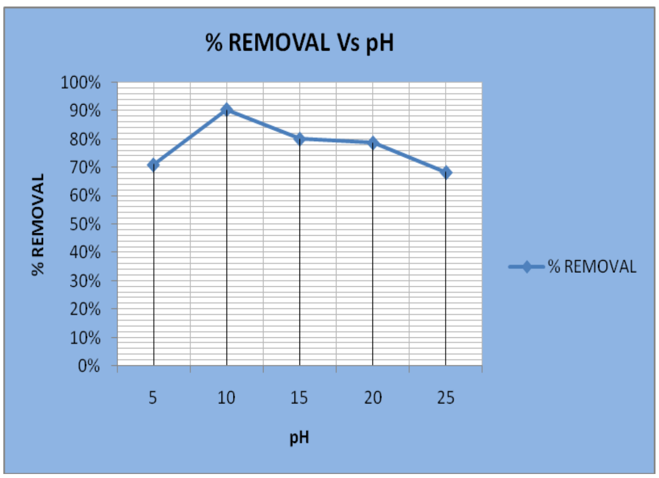



**Fig. 13: Effect of pH on the Percentage Removal of Heavy metal ion**

### 3.5.3. *Effect of Schiff base weight*

Different amount of Schiff base was added in industrial water samples at constant pH, constant concentration of the metal ion and at constant biomass. According to Table 6 and fig.14 maximum percentage removal of Pb (II) in water samples takes place at 5 mg weights. Metals adsorption efficiency was increased with increase in an adsorbent dose. This revealed that the adsorption sites remained unsaturated during the adsorption reaction whereas the number of sites available for adsorption site increases by increasing the adsorbent dose.

**Table 6: Effect of Weight of Schiff base**

| Weight of schiff base(mg) | % removal±SEM |
|---|---|
| 1 | 76.9%±0.881 |
| 2 | 87.3%±0.726 |
| 3 | 91.23%±0.577 |
| 4 | 93.5%±0.881 |
| 5 | 95.41%±0.898 |

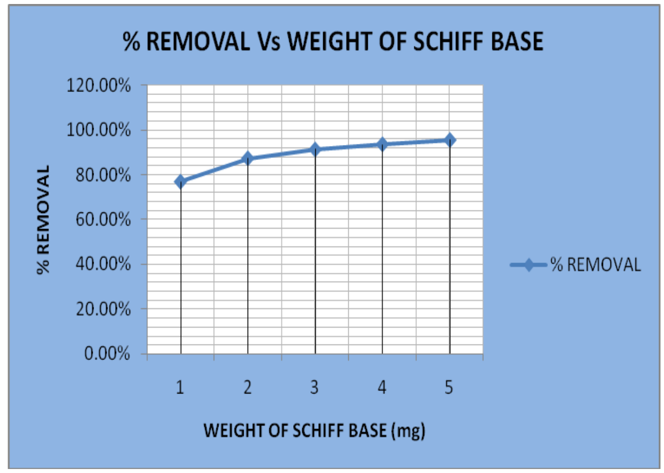



**Fig. 14: Effect of Weight of Schiff base on the Percentage Removal of Heavy metal ion**

### 3.5.4. *Effect of contact time*

The effect of contact time on the percentage removal of Pb (II) metal ion by Ag nano@ Schiff base from industrial effluent was investigated and result obtained in Table 7. Effect of contact time is calculated at constant pH, biomass, and weight of silver nanocomplex of Schiff base and constant initial metal ion concentration.

**Table 7: Effect of Contact Time (min)**

| Contact Time(Min) | % Removal±SEM |
|---|---|
| 5 | 75.2%±0.635 |
| 10 | 83.03%±0.92 |
| 15 | 90.86%±0.825 |
| 20 | 86.9%±0.793 |
| 25 | 86.83%±0.876 |

Fig. 15 shows maximum percentage removal of heavy metal in 15 minutes. Adsorption rate initially increased rapidly, and the optimal removal efficiency was reached within about 15 min. Further increase in contact time did not show the significant change in equilibrium concentration; that is, the adsorption phase reached equilibrium.

Figure shows the value of the percentage removal of Pb(II) increased steadily and after 20 minutes the adsorption quantity of the metal ion showed nearly no change. It describes the adsorption sites affected with contact time.

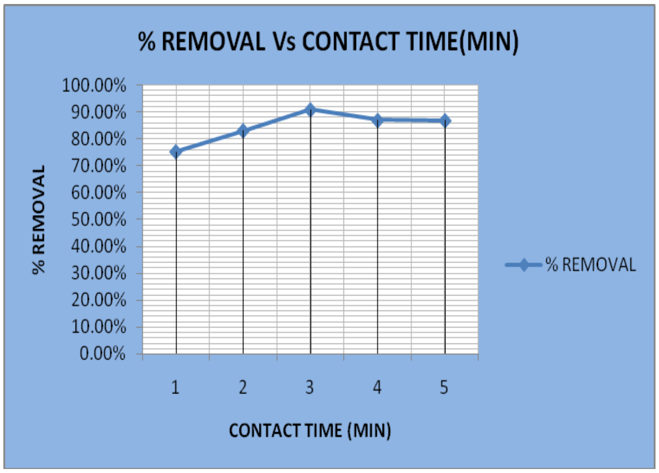



**Fig.15: Effect of Contact Time on the Percentage Removal of Heavy metal ion**

## 4. CONCLUSION

A novel silver nanoparticle anchored with Schiff bases was synthesized. The structure of synthesized compounds was confirmed on the basis of UV-VIS, IR, NMR and SEM spectra. The Antifungal activity of the synthesized compounds was studied using disc diffusion method and the concentration was fixed using Minimum inhibitory concentration (MIC) method. The antifungal study revealed that all compounds showed little to excellent activity as compared to standard drug clotrimazole. The silver nanoparticle trapped with Schiff base and attached with solid phase has the high surface area in low volume, and effectively and rapidly removes Pb (II) metal ion from waste water.

## 5. ACKNOWLEDGEMENTS

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