doi.org/10.36721/PJPS.2022.35.3.SP.923-930.1

Synthesis of dihydropyrimidine stabilized silver nanoparticles with significant anti urease and catalytic applications

Rafia Usman Khan[1]*, Nuzhat Arshad[1], Razia Sultana[2], Jamshed Hashim[3], Haniya Sheikh[1], Waqas Zaidi[1], Zain Ahmed Shah[1] and Samar Khan[1]
[1]Department of Chemistry, NED University of Engineering and Technology, Karachi, Pakistan
[2]Applied Chemistry Research Center PCSIR Laboratories Complex, Karachi. Pakistan
[3]Department of Chemical Sciences, University of Lakki Marwat, Lakki Marwat, KPK, Pakistan

Abstract: We synthesized and explored biological and environmental applications of novel silver nanoparticles (AgNps) stabilized by short chain heterocyclic thiol namely Ethyl 6-methyl-4-phenyl-2-thioxo1,2,3,4-dihydropyrim-idine-5-carboxylate (DHPM). Dihydropyrimidines (DHPM), a biological active class of compounds that contain a single thiol group at the focal point which strongly stabilized the nascent AgNps. The short alkyl chain of (DHPM) effectively controlled the growth kinetics and surface morphology of AgNps. The synthesized Dihydropyrimidine stabilized silver nanoparticles (DHPM-AgNps) were investigated using Ultraviolet– visible spectroscopy (UV-Vis), Atomic force Microscopy (AFM) and Fourier-transform infrared spectroscopy (FTIR). AFM exhibited the size and shape of the DHPM-AgNps with an average diameter of 10 $\underline{+}$ 1 nm. Our prepared DHPM-AgNps were examined for urease enzyme inhibition activity. The synthesized DHPM-AgNps showed significant level of urease inhibition activity (% of inhibition 40.3±0.28%) when compared with standard thiourea inhibition activity (% of inhibition value 79.6± 0.47%.). Moreover prepared DHPM-AgNps system successfully applied for the reduction of para-nitrophenol (p-Nip). It reduces the para-nitrophenol (p-Nip) to para-aminophenol (p-Amp) within one second in the presence of $NaBH_4$ under ambient temperature and pressure conditions, which followed the pseudo-first-order rate kinetics. This study will provide useful guidelines for designing efficient catalysts and stabilizing agents for Silver Nanoparticles.

Keywords: Aminophenol, Catalysis, Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-dihydropyrim-idine5-carboxylate stabilizer, Silver nanoparticles, Urease inhibition, 4-nitrophenol.

INTRODUCTION

Since thousands of years silver and its compounds have been used for therapeutic and other biological applications (Ahmad *et al.,* 2021). In the early 19th century, silver preparations were developed for wound infection and burn care that gave way to the silver based clinical introduction of antibiotics (Ahn *et al.,* 2019). Increasing use of antibiotics, bacterial resistance has become a greatest problem and silver began to receive attention again especially with the development of nanotechnology. Over the past few decades' silver nanocomposites (1-100 nm materials) have been attracting much attention in the fields such as biomedicine, catalysis, energy storage, and sensors etc., due to their unique physicochemical properties as compared to their bulk counter parts (Aisida *et al.,* 2019). AgNps have been also explored for promoting bone healing and wound repair, increasing the immunogenicity of vaccines (Alahmad *et al.,* 2021) and anti-diabetic effects (Badmus *et al.,* 2020). Compared to other noble metallic nano systems, AgNps is a more promising candidate as industrial heterogeneous catalyst because of its lower cost and higher efficiency in biological and environmental fields (W Gong, Q *et al* 2019).

In 1893, Binginelli first synthesized dihydropyrimidines (DHPM) (Hashim *et al.,* 2014). Dihydropyrimidines (DHPM) possess a wide range of biological activities. Various compounds containing dihydropyrimidine backbone like monastrol (Kaur *et al.,* 2017), have strong anticancer and HIV inhibition activities. Trimethoprim (Wróbel *et al.,* 2020) is commonly used as an antibacterial drug which contain a pyrimidine core. Nitrendipine in 1,4-dihydropyridine class (DHPs), is the most widely used drug for the treatment of cardiovascular diseases, it work as a calcium channel modulators (Elena González *et al,* 2019). Furthermore, dihydropyrimidines have neuropeptide c-antagonists, a-1a-antagonists properties (Ann Riya Ninan *et al.,* 2021) antihypertensive, antimalarial and anti-inflammatory activities, as well (Patil *et al.,* 2020).

There are numerous reports for the use of organic thiols as stabilizing agents for AgNps including the pioneering works by Brust and co-workers (Paul *et al.,* 2020). Recently heterocyclic thiol has been of particular interest for the stabilization of nanoparticles (Sidoryk *et al.,* 2020).

There are two different ways through which organic sulfur containing compounds including thiols (RSH) and

---

**Corresponding author:* e-mail: rkhan@neduet.edu.pk

its derivatives react with metal nanoparticles. The first approach is the RSH group physisorption on the metal nanoparticles surface while the second probability is the RSH or disulfides groups chemisorption on metal surfaces which generate SR groups (Beaupre *et al.,* 2021). The short energy gap between highest occupied molecular orbital and Lowest unoccupied molecular orbital (HUMO-LUMO) of aromatic thiols derivatives are promising candidate in serval molecular electrical applications. In short chain aromatic systems including thiols and disulfide compounds, electrons are delocalized throughout the system due to the presence of delocalization and shorter distances, the movement of electrons are much faster (Restrepo *et al.,* 2021). Additionally, alkanes are flexible in nature while aromatic compounds with their conjugation provide strong packing around the nanoparticles (Hühn *et al.,* 2017). The molecular interactions in short chain aromatic thiol include interaction between benzene ring and its surface, among different functional groups attached with the chain and with the ring structure.

Compounds from the class of DHPM of Biginelli type inherently possess a thiol and a short alkyl chain in its structure that offers the above-mentioned benefits and became the rational of our designed DHMP-AgNps. The presence of conjugation and high chemical reactivity of aromatic thiols structure may provide rigid covering on DHMP-AgNps surface (Ghomi SJ *et al.,* 2018).

Voluminous research articles were produced regarding the formation of stable and different shapes of (AgNps) through chemical reduction methods in aqueous medium by using various reducing agents including trisodium citrate (TSC), sodium borohydride ($NaBH_4$), hydrazine (N_2H_4), ascorbic acid ($C_6H_8O_6$), and others. Among them the use of trisodium citrate (TSC) as reducing agent for the reduction of Ag^+ into Ag^0, and the subsequent addition of short chain aromatic thiol compounds are reported to yielded highly stable AgNps (Demchenko *et al.,* 2020). Achieving another milestone in nano silver chemistry, we synthesized the AgNps stabilized by using very short chain hertocyclic compound namely Ethyl 6-methyl4-phenyl-2-thioxo-1,2,3,4-dihydropyrim-idine-5-carboxylate (DHPM) as stabilizer. This Biginelli adducts (3,4-dihydropyrimidin-2(1H)-thiones) stabilizer are an integral part of nitrogen containing heterocyclic compounds containing thiol (SH) group at the focal point and represents significantly important class of pyrimidine having diversified applications in biological fields. DHPM structure with a rigidity of the backbone chain stabilized the zero valent Ag atoms by strong chemisorption of terminal thiol (SH) group.

Our prepared DHPM-AgNps were successfully evaluated for urease inhibitory activity. Urease enzyme is responsible for many types of infections in humans for example, gastric ulcers, cancers (Hameed *et al.,* 2019). Urolithiasis, hepatic encephalopathy, hepatic coma and urinary catheter encrustation (Svane *et al.,* 2020).Hence, the discovery and application of potent and secure urease inhibitors are key area in the field of pharmaceuticals.

The prepared nano-composites DHPM-AgNps system revealed a promising potential to act as an efficient heterogeneous catalyst for environmental applications (Kaur *et al.,* 2019). Para-nitrophenol (p-Nip) a common pollutant in soils and surface and ground waters. It has a severe environmental impact and health risks due to its toxicity and mutagenic potential in humans and other living organisms (Samuela *et al.,* 2019). Moreover, the degradation rate of para-nitrophenol (p-Nip) in soil and water is slow. This causes it to accumulate in the environment (Serra, A *et al.,* 2019). Indeed, this is the reason for the United States Environmental Protection Agency (US EPA) including 4-NP and its derivatives to the list of top priority pollutants and its need immediate removal. Herein, we report the first synthesis of Ethyl 6-methyl-4-phenyl-2-thioxo1,2,3,4-dihydropyrim-idine-5-carboxylate stabilized silver nanoparticles (DHPM-AgNps) along with significant urease inhibition and excellent catalytic reduction (para-nitrophenol (p-Nip) to para-aminophenol (p-Amp) activity.

MATERIALS AND METHODS

General experimental details

All chemicals and solvents were commercially procured, from Sigma Aldrich Co. and Fanara Chemicals. All chemicals were used as received. Nitrogen environment was used for all atmospheric-sensitive reactions. Unless mentioned all experimental solutions were prepared by using distilled water. HNMR spectra were carried out using Bruker (AVIII), Bruker (AV500) and Bruker (AV600). UV visible Absorption and FTIR spectra were taken from (FTIR8900) Shimadzu and Bruker (Vector22) respectively. Atomic Force Microscope (AFM) 5500 from Agilent Technologies was used to measure the size of as prepared AgNps.

Procedure for the Synthesis of Ethyl 6-methyl-4-phenyl-2-thioxo-1, 2, 3, 4-dihydropyrim-idine-5carboxylate (DHPM)

Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-dihydropyrim-idine-5carboxylate (DHPM) was prepared as shown in scheme 1 in excellent yield (78%) on 10 (mmol) scale. By utilizing Biginelli reaction given in the literature (Sahota *et al.,* 2019). In a round bottom flask fitted with water condenser containing the sample mixture of 10mmol of the benzaldehyde, 15 mmol of ethyl acetoacetate, 761 mg (10 mmol, 1.0 equiv) of thiourea, 1.27mL (10mmol, 1.0 equiv) of tri methyl silyl chloride (TMSCl) and 7.0mL of anhydrous acetonitrile under nitrogen cover, was stirred for 1 minute at 25°C for homogenization, followed by 24 hours of reflux in oil bath. Rotary evaporator was used under reduced pressure to evaporate the solvent after cooling the reaction mixture. After that 500mL of ice-cold water was added into the reaction mixture and stirred it for an hour to obtained maximum precipitation as product. Filtration was carried out to get the pure DHPM. The physical and spectroscopic results of DHPM were found in agreement with the literature (Maskrey *et al.,* 2018).

Preparation of DHPM stabilized AgNps

In a conical flask, under vigorous stirring and heating at 80° C fresh solution of $AgNO_3$ (23.2mg) in 200mL of distilled water was prepared. To this $AgNO_3$ solution, fresh solution of trisodium citrate (TSC, 22.7mg) in 1mL of distilled water was added. After heating for 20 minutes, the colorless solution changed to yellow that indicated the formation of AgNps. The prepared AgNps solution was cooled till room temperature and transferred in a 30mL vial and 5µL (2.5 mM in 10mL of DMSO) of Ethyl 6-methyl-4-phenyl-2-thioxo-1, 2, 3, 4dihydropyrim-idine-5-carboxylate (DHPM) was added. The persistent yellow coloration of the solution indicated the stabilization of the formed AgNps. The DHPM stabilized AgNps were confirmed by UV-visible spectroscopy, Atomic force spectroscopy, and Fourier transform infrared spectroscopy techniques.

UV- Visible Analysis of DHPM-AgNps

Confirmation of Dihydropyrimidine stabilized silver nano particle (DHPM-AgNps) formation and surface plasmon resonance (SPR) was done by analysis of the absorbance data of UV-VIs spectroscopy. The UV-Vis absorption spectra clearly demonstrated signature SPR peak of DHPM-AgNps peak at 405 nm. The UV-Vis absorption spectra of (DHPM) capped AgNps were recorded at the concentration 2.5mM of DHPM, 0.7mM of $AgNO_3$ and 0.08M TSC fig. 3 A.

FTIR Analysis of DHPM-AgNps

Fourier Transform-Infrared Spectroscopy (FTIR) was performed to confirm the formation of DHMP-AgNps system. The spectrum was drawn in the wave number region of 500 to 4000cm^{-1}.

Surface Morphology of DHPM-AgNps

Atomic electron microscope (AFM) was used to examine the surface morphology of the synthesized DHPM-AgNps. The sample was mounted on the stub with double side adhesive tape, which was previously lyophilized and scattered.

Urease inhibition protocol of AgNps- DHPM

Reaction mixtures containing 25µL of enzyme (Jack bean Urease) solution and 55µL buffers and 100mM urea were incubated with 5µL of (AgNps- DHPM) test compounds (1mM concentration) at 30°C for 15min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method by Weatherburn. In this method, 45µL each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200µL. The results (change in absorbance per min) were carried out by using SoftMax Pro software (Molecular Device, USA). All the assays were at pH 8.2 (0.01M $K_2HPO_4.3H_2O$, 1mM EDTA and 0.01M $LiCl_2$). Performed Thiourea was used as the standard inhibitor of urease. Percentage inhibitions were calculated from the formula:

$$\%\,\text{inhibition} = 100 - \left(\frac{\text{mean OD of Test Well}}{\text{mean OD of control}} \times 100 \right)$$

Protocol for Para-nitrophenol (p-Nip) reduction

DHPM-AgNps catalytic reduction of para-nitrophenol (p-Nip) in aqueous medium was Performed in a standard absorption cuvette made up of quartz with path length of one cm. In a quartz cell, under vigorous stirring para-nitrophenol (p-Nip) (0.125mM, 2mL aqueous), freshly prepared $NaBH_4$ (0.5M, 1mL aqueous) was added, respectively. To this, catalyst DHPM-AgNps (2.5mM, 100µL aqueous) was immediately added. The sample solution within 1 sec turned to colorless from dark yellow. UV-Vis absorption spectra of the reaction progress of the sample was tracked with a time difference of one second in a wavelength range of 200-1000 nm at room temperature.

STATISTICAL ANALYSIS

All values in tables were expressed as standard error of mean (± SEM) and three (03) replicate tests were performed. The result (change in absorbance per min) was carried out by using SoftMax Pro software (Molecular Device, USA).

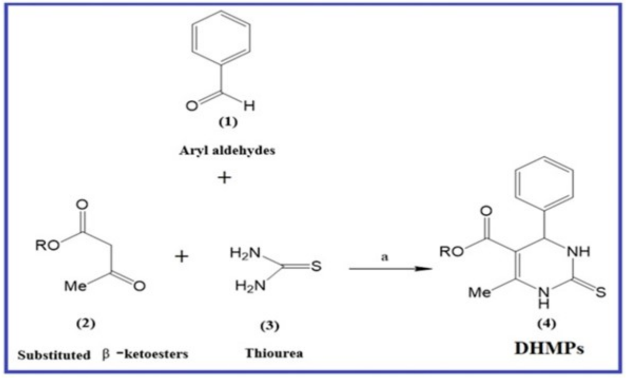
RESULTS

Synthesis of Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-dihydropyrim-idine-5carboxylate (DHPM)

Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-dihydropyrim-idine-5carboxylate (DHPM) was prepared as shown in Scheme 1 in excellent yield (78%) at 10 (mmol) scale. The physical and spectroscopic results of DHPM were found in agreement with the literature. (de Fátima, Â *et al.,* 2020).

Confirmation of Surface Plasmon resonance by UV-Visible spectroscopy

The surface plasmon resonance (SPR) as the evidence of the formation of (DHPM) capped AgNps was originated by UV visible absorption spectra. The transparent solution of $AgNO_3$ and TSC turned yellow after heating at 80°C for 20 minutes without turbidity fig. 1 (A).Upon subsequent addition of 5uL solution of (2.5mM of DHPM solution in DMSO), the yellow coloration of the solution remained persistent that gave the indication of stabilization of formed AgNps. This was further confirmed by signature surface plasmons resonance (SPR) absorption maxima of AgNps at 405 nm fig. 1 (B).



Scheme 1: Synthesis of Ethyl-6-Methyl-4-Phenyl-2-Thioxo-1,2,3,4-Tetrahydropyrimidine-5-Carboxylate (DHMP) Reagents and conditions: 1) benzaldehyde (10 mmol), 2) ethyl acetoacetate (15 mmol), 3) thiourea (10 mmo), TMSCl (10 mmol.), MeCN (7.0 mL), N_2, reflux, 24 hours.

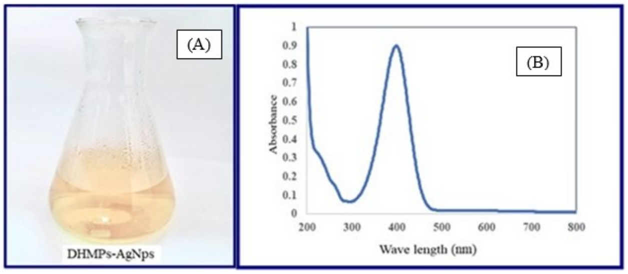


Fig. 1: (A) UV-visible absorption spectra of DHPM-AgNps systems. $\lambda max = 405$ nm. (B) Color of the colloidal DHPM-AgNps systems.

Surface Morphology DHMP-AgNps by AFM

AFM images (fig. 2 (A) further confirmed the growth, size and shape of (DHPM) capped AgNps. The AFM photomicrographs were corresponding to the time dependent UV-vis spectroscopic observations. AFM images of DHPM-AgNps systems exhibited mono dispersion and spherical shape of DHMP-AgNps. The average particle sizes of AgNps- DHPM system was found to be 10 +1 nm (fig. 2 (B).

FTIR analysis of DHPM-AgNps systems

FTIR spectroscopy was performed to further reconfirm the process of chemisorption of DHPM with AgNps. The comparison of the spectrum of pure DHPM (fig. 3 A) and DHPM stabilized AgNps (fig. 3 B) reviled the interactions of the DHPM with AgNps. The thiol stretching frequency at 2559 cm^{-1}as completely disappeared in the spectra of DHPM-AgNps which shows the involvement of SH group for chemisorption with AgNps (fig. 3 B).

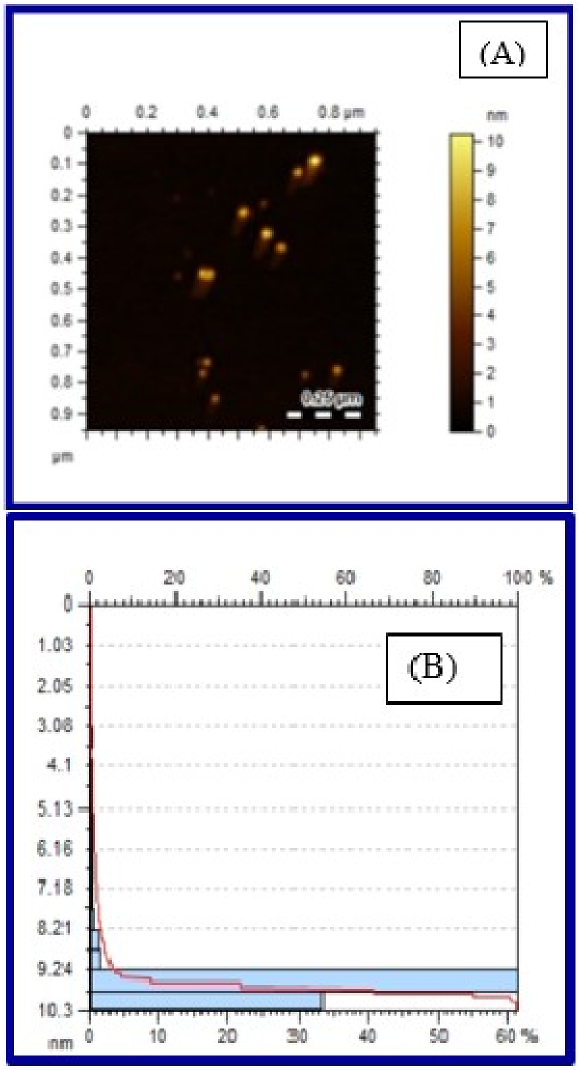
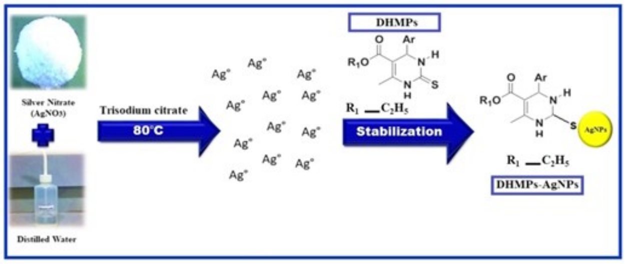


Fig. 2: (A) AFM images of DHPM-AgNps systems. (B) Histogram of corresponding size distribution of AgNps - DHPM.



Scheme 2: Schematic representation model of DHPM stabilized AgNps

Anti-Urease Activity DHPM-AgNps

Our prepared DHPM-AgNps exhibited significant urease enzyme inhibitory activity (% of inhibition 40.3±0.28%)

as compared to control Thiourea providing (% of inhibition =79.6±0.47%).

Table 1: Urease inhibition activity of DHPM-AgNps % inhibition values

Entry	Compound	% Inhibition
1.	DHPM-AgNps	40.3± 0.28
2.	Thiourea	79.6± 0.47

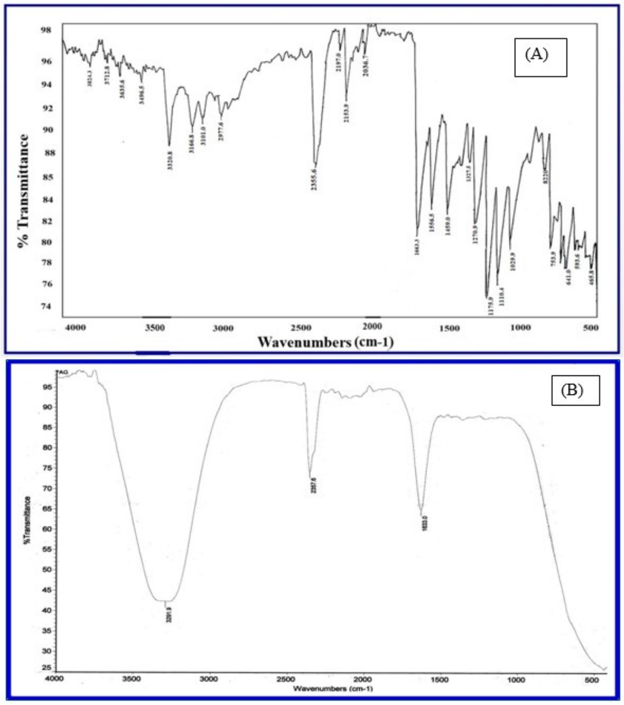


Fig. 3: (A) FTIR spectra of pure DHPMs. (B) FTIR spectra of DHPMs-AgNps system.

Heterogeneous Catalytic activity of DHPM-AgNps

To evaluate the catalytic activity of DHPM-AgNps systems we have chosen the reduction of para-nitrophenol (p-Nip) to para-aminophenol (p-Amp) as an effective model reaction. The advantage of this reaction is its easy monitoring using UV-Vis spectroscopy (Cheng *et al.,* 2022). The UV-Vis absorption spectra of para-nitrophenol (p-Nip) at 318 nm was accordingly red shifted to 400 nm upon the addition of $NaBH_4$ into the reaction mixture exhibited the formation of para-nitrophenolate ion fig.4 A. UV Visible absorption profile of the reaction mixture was unaltered even after 48 hours monitoring. This clearly revealed the stability of para-nitrophenolate ion. To accomplish reduction of this ion into para-aminophenol (p-Amp) a catalytic system is necessary to pass the required high kinetic negative barrier between two similarly repelling BH_4 and para- nitrophenolate ions. Upon addition of our prepared heterogeneous catalyst (DHPM-AgNps). Successfully the complete reduction of para-nitrophenolate ion, was achieved only in one second as monitored by UV-Visible spectroscopy (fig. 4 B) that showed the complete disappearance of the absorption peak at 400nm for para-nitrophenolate ion and appearance of another absorption maxima at 290 nm for para-aminophenol (p-Amp).

DISCUSSION

Trisodium citrate (TSC) was used to reduce silver ion (Ag^+) into zero valent silver (Ag^0). The transparent solution of $AgNO_3$ and TSC turned yellow after heating at 80°C for 20 minutes. Upon subsequent addition of 5uL solution of (2.5 mM of DHPM solution in DMSO), the yellow coloration of the solution remained persistent that gave the indication of stabilization of formed AgNps through DHPM. This was confirmed by signature surface plasmons resonance (SPR) absorption maxima at 405 nm fig. 1 (B). The signature narrow UV-Vis absorption SPR band of AgNps revealed the formation of spherical shape, mono disperse, stable and small DHMP-AgNps which was also observed in AFM images fig. 2 (A). The average particle sizes of DHMP-AgNps was found to be 10 + 1 nm. fig. 2 (B). The formation of DHPM-AgNps was accomplished by the reduction of the focal thiol (S-H to S-Ag) group of Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-dihydropyrim-idine-5-carboxylate (DHPM) as shown in scheme 2.

The FTIR spectra of pure DHPM fig. 3 (A). clearly shown the thiol stretching frequency at 2559cm^{-1} was completely disappeared in the spectra of DHPM-AgNps fig. 3 (B). which shows the chemisorption of SH group with AgNps. The FTIR spectrum not only supported the interactions of the DHPM with AgNps but also showed of characteristic functional groups of DHPM for example the N-H (amine) symmetric stretching frequency at 3291cm^{-1}, Sp^{2}C-H stretches of aromatics at 2357cm^{-1} and in-plane vibrations of aromatic ring at 1633cm$^{-1.}$ fig. 3 (B).

To evaluate the biological applications of DHPM-AgNps, urease inhibition activity was performed against Jack bean Urease enzyme which was found significant (% of inhibition 40.3±0.28%) when compared with standard thiourea inhibition activity (% of inhibition value 79.6±0.47%).

To evaluate the catalytic potential of DHPM-AgNps systems we have chosen the reduction of para-nitrophenol (p-Nip) to para-aminophenol (p-Amp) as an effective model reaction.

Fig. 4 (A) illustrates UV-Vis absorption spectra for the reduction of para-nitrophenol (Nip), in the presence of our prepared heterogeneous catalyst (DHPM-AgNps). Para-nitrophenol(Nip) was reduced in the presence of DHPM-AgNps and $NaBH_4$. The intensity of the absorption peak at 400 nm disappeared within one second and a new absorption peak appeared at 299 nm appeared. This new

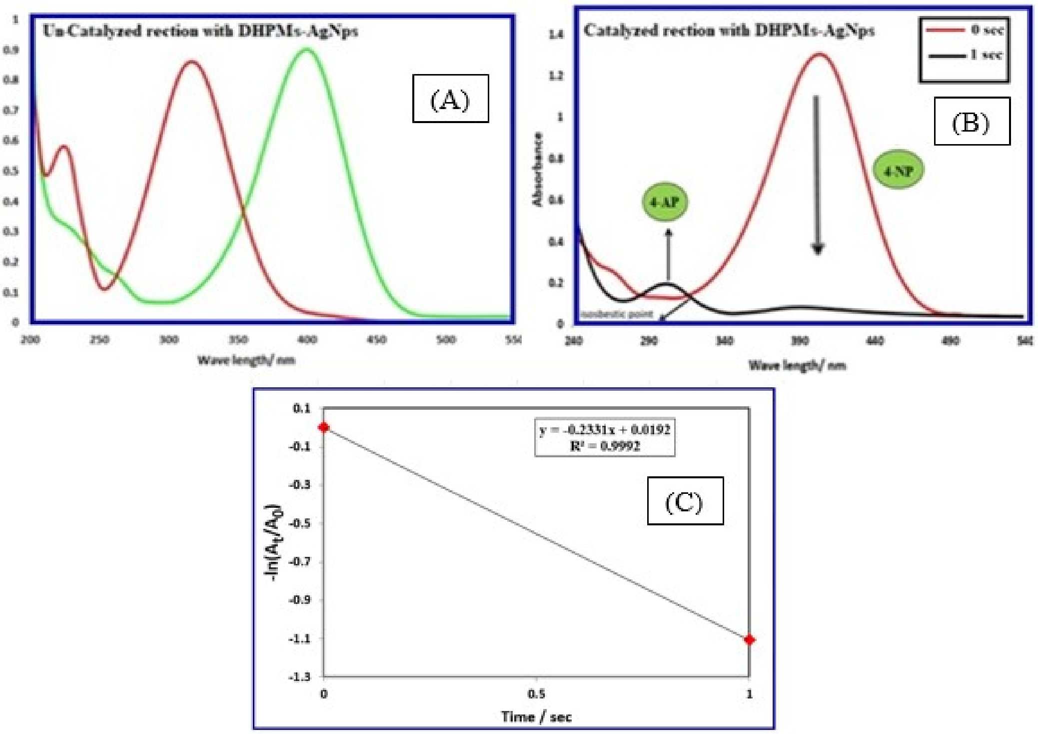


Fig. 4: (A) UV- vis absorption spectra of un-catalyzed reduction of para-nitrophenol(p-Nip) to para-aminophenol (p-Amp). (B) UV- vis absorption spectra of reduction of para- nitrophenol (p-Nip) to para-aminophenol (p-Amp) in the presence of catalyst [DHPM-AgNps] system. (C) Plot of ln(A_t/A_0) against the reaction time for pseudo- first-order reduction kinetics of para-nitrophenol (p-Nip) to para-aminophenol (p-Amp)in the presence of catalyst [DHPM-AgNps] system

peak is attributed to the typical absorption of para-aminophenol (p-Amp). The dark yellow color due to the formation of nitrophenolate changed to transparent after the addition of (AgNps- DHPM) as heterogeneous catalyst within one second. The presence of transparent color of para-aminopheonol (p-Amp) indicated the completion of the reduction process of p-Nip to p-Amp.

The progress of the reduction reaction of the Para-nitrophenol (Nip) solution was determined by monitoring the quick disappearance of absorption maxima at 400 nm within one second. In this reaction, the initial concentration of $NaBH_4$ was high 0.5M and it remained constant throughout the reaction and p-nitrophenol concentration was 0.125 mM. In order to ascertain the heterogeneous catalytic activity of DHPM-AgNps by considering the higher $NaBH_4$ concentration as compared with that of p-nitrophenol, the pseudo- first-order kinetics could be applied with respect to p-nitrophenol. The absorbance of p-nitrophenol is proportional to its concentration in solution, the absorbance at time t (A_t) and time t = 0 (A_0) are equivalent to the concentration at time t (C_t) and time t = 0 (C_0). The rate constant (*k*) was determined from the linear plot of -ln(A_t/A_0) versus reduction time in seconds, and the rate constant (fig. 4 C) was estimated to be 0.2331 sec^{-1}.

CONCLUSIONS

The present study provides a comprehensive study regarding the synthesis of stable AgNps by using short chain Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-dihydropyrim-idine-5-carboxylate (DHPMs). DHPM stabilized AgNps rigidly under an ambient temperature and pressure conditions. AFM showed the Ag-NPs-DHPMs were in spherical shape with a mean average diameter of 10+1nm. Our prepared DHPM-AgNps system worked very well as urease inhibitor. DHPM-AgNps system exhibited excellent catalytic activity as heterogeneous catalyst in the reduction of 4-NP to reduce into nontoxic aromatic compound 4-AP within one second. The facile method used for the formation of DHPM-AgNps has several benefits such as biocompatibility, reproducibility, cost, and time effective which make them excellent candidate in commercial and environmental applications.

REFERENCES

Ahmad F, Taj MB, Ramzan M, Ali H, Ali A, Adeel M and Imran M (2021). One-pot synthesis and characterization of in-house engineered silver nanoparticles from *Flacourtia jangomas* fruit extract with effective antibacterial profiles. *J. Nanostr. Chem.,* **11**(1): 131-141.

Ahn EY, Jin H and Park Y (2019). Green synthesis and biological activities of silver nanoparticles prepared by *Carpesium cernuum* extract. *Arc. Pharmacal Res.,* **42**(10): 926-934.

Aisida SO, Ugwu K, Akpa PA, Nwanya AC, Ejikeme PM, Botha S, Ahmad I, Maaza M, Ezema FI (2019). Biogenic synthesis and antibacterial activity of controlled silver nanoparticles using an extract of *Gongronema latifolium*. *Mater. Chem. Phys.,* **237**(11): 1-8.

Alahmad A, Feldhoff A, Bigall NC, Rusch P, Scheper T, Walter JG (2021). *Hypericum perforatum* L.-Mediated green synthesis of silver nanoparticles exhibiting antioxidant and anticancer activities. *Nanomat*., **11**(2): 487-492.

Ann Riya Ninan RA, Babbar R, Dhiman S, Singh TG and Kaur K (2022). Dhiwan Y. A Systematic Review on 1, 4-Dihydropyridines and its Analogues: An Elite Scaffold. 2022. *Biointerface Res. Appl. Chem.*, **12**(3): 3117-3134.

Badmus JA, Oyemomi SA, Adedosu OT, Yekeen TA, Azeez MA, Adebayo EA, Lateef A, Badeggi UM, Botha S, Hussein AA and Marnewick JL (2020). Photo-assisted bio-fabrication of silver nanoparticles using Annona muricata leaf extract: exploring the antioxidant, anti-diabetic, antimicrobial, and cytotoxic activities. *Heliyon.,* **6**(11): e05413.

Beaupre DM, Weiss GR (2021). Thiol- and disulfide-based stimulus-responsive soft materials and self-assembling systems. *Molecules.,* **26**(9): 3332.

Cheng H, Hempenius AM, Sui X and Vancso JG (2022). Catalytic performance of Pd nanoparticles obtained by direct reduction in cellulose-poly (ferrocenylsilane) hybrid sponges. *Adv. Mater. Interfaces.,* **11**(3): 1-8.

De Fátima A, Braga TC, da S. Neto L, Terra BS, Oliveira BG and Da Silva DL (2015). A mini-review on biginelli adducts with notable pharmacological properties. *J. Adv. Res.* **6**(3): 363-673.

Demchenko V, Riabov S, Kobylinskyi S, Goncharenko. L, Rybalchenko N, Kruk A, Moskalenko O and Shut O (2020). Efect of the type of reducing agents of silver ions in interpolyelectrolyte-metal complexes on the structure, morphology and properties of silver-containing nanocomposites. *Sci. Rep.,* **10(1)**: 7126.

Elena González-Hernández EG, Aparicio R, Garayoa M, Montero MJ, Sevilla MA, Concepcion Perez-Melero PC (2019). Dihydropyrimidine-2-thiones as Eg5 inhibitors and L-type calcium channel blockers: Potential antitumour dual agents. *Medchemcomm.,* **10**(9): 1589-1598.

Ghomi SJ, Tavazo M, Mahdavinia HG (2018). Ultrasound promoted one-pot synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones/thiones using dendrimer-attached phosphotungstic acid nanoparticles immobilized on nanosilica. *Ultrason Sonochem.,* **40**(1): 230-237.

Gong W, Wu Q, Jiang G and Li G (2019). Ultrafine silver nanoparticles supported on a covalent carbazole framework as high-efficiency nanocatalysts for nitrophenol reduction. *J. Mater. Chem. A.,* 7(1): 13449-13454.

Hameed A, Al-Rashida M and Uroos M (2019). A patent update on therapeutic applications of urease inhibitors (2012-2018). *Expert Opin. Ther. Pat.,* **29**(3): 181-189.

Hashim J, Arshad N, Khan I, Nisar B, Ali B, Choudhary I (2014). Preparation of dihydrotetrazolo[1,5-a] pyrimidine derivatives from Biginelli 3,4-dihydropyrimidine-2-thiones. *Tetrahedron,* **70**(1): 8582-8587.

Huhn J, Carrillo-Carrion C, Soliman MG, Pfeiffer C, Valdeperez D, Masood A, Chakraborty I, Zhu L and Gallego M (2017). Selected standard protocols for the synthesis, phase transfer and characterization of inorganic colloidal nanoparticles. *Chem. Mater. Chemical Reviews.,* **29**(1): 399-461.

Kaur J, Singh J and Rawat M (2019). An efficient and blistering reduction of 4-nitrophenol by green synthesized silver nanoparticles. *SN Appl. Sci.* **1060**(1): 101-103.

Kaur R, Chaudhary S, Kumar K, Gupta KM, Rawal RK (2017). Recent synthetic and medicinal perspectives of dihydropyrimidinones: A review. *Eur. J. Med. Chem.*, **132**(2): 108-134.

Maskrey ST, Frischling CM, Rice LM and Wipf P (2018). A five-component biginelli-diels-alder cascade *Reaction. Front. Chem.* **6**: 376-385.

Patil VR, Chavan UJ, Dalal SD, Shinde SV and Beldar GA (2019). Biginelli reaction: Polymer supported catalytic approaches. *ACS Comb. Sci.,* **21**(3): 105-148.

Paul S, Chakraborty BB, Anwar S, Paul BS and Choudhury S (2020). Self-assembly of silver nanoparticles through functionalization with coumarin-thiazole fused-ring thiol. *Heliyon.,* **6**(4): e03674.

Restrepo CV and Villa CC (2021). Synthesis of silver nanoparticles, influence of capping agents, and dependence on size and shape: A review. *Environ. Nanotechnol. Monit. Manag.,* **15**(1): 100428-39.

Sahota N, AbuSalim ID, Wang ML, Brown JC, Zhang Z, El-Baba JT, Silas P. Cook PS and Clemmer ED (2019). A microdroplet-accelerated Biginelli reaction: mechanisms and separation of isomers using IMS-MS. *Chem. Sci.,* **10**(9): 4822-4827.

Samuela SM, Jose S, Selvarajanc E, Mathimani T, Pagazhendhie A (2020). Biosynthesized silver nanoparticles using Bacillus amyloliquefaciens; Application for cytotoxicity effect on A549 cell line and photocatalytic degradation of p-nitrophenol. *J. Photochem. Photobiol. B: Biol.* **202**(1): 111642.

Serra A, Artal R, Pozo M, Amoros J and Gomez E (2020). Simple environmentally-friendly reduction of 4-nitrophenol. *Catalysts.,* **10**(1): 458-462.

Sidoryk K, Michalak O, Kubiszewski M, Les Andrzej, Cybulski M, Stolarczyk UE and Doubsky J (2020).

Synthesis of thiol derivatives of biological active compounds for nanotechnology application. *Molecules,* **25**(15): 3470.

Svane S, Sigurdarson JJ, Finkenwirth F, Eitinger T, Karring H (2020). Inhibition of urease activity by different compounds provides insight into the modulation and association of bacterial nickel import and ureolysis. *Sci. Rep.,* **10**(12): 8503-8517.

Wrobel A, Maliszewski D, Baradyn M, Drozdowska D (2020). Trimethoprim: An old antibacterial drug as a template to search for new targets. Synthesis, biological activity and molecular modeling study of novel trimethoprim analogs. *Molecules,* **25**(1): 116.