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Research Article

NANOPARTICLE BASED DRUG DELIVERY SYSTEM FOR SULFADIAZINE DRUG ALONG WITH HERBAL EXTRACT

Dr. Shaik Shabbeer [*1], Korepaka Anjaneyulu [2], Santhosh Illendula [3], Dr. K.N.V. Rao[4].

1. Head of the Department, Department of Pharmaceutics, Nalanda College of Pharmacy, Cherlapally (v), Nalgonda (Dt), Telangana (St), India, 508001

2. Post Graduate Scholar, Department of Pharmaceutics, Nalanda College of Pharmacy, Cherlapally (v), Nalgonda (Dt), Telangana (St), India, 508001

3. Associate Professor, Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, (v), Cherlapally Nalgonda (Dt), Telangana (St), India, 508001

4. Professor & Principal, Nalanda College of Pharmacy, Cherlapally (v), Nalgonda (Dt), Telangana (St), India, 508001

Corresponding Author- Dr. Shaik Shabbeer

ABSTRACT

Silver sulfadiazine has been used in the treatment of wounds associated with burns since ages due to its strong antiseptic properties. However, the penetration of drug from the conventional gel and cream formulations was not so high. With advent of nanotechnology, it has become possible to size down the metallic silver for better wound penetration and hence better efficacy. This study aims at functionalization of Silver nanoparticles synthesised by green synthesis employing aqueous leaf extract of *Aegle marmelos* and silver nitrate with sulfadiazine. So that the drug becomes ecologically safer as no or minimal drug has been used in the whole process. Silver nanoparticles has been prepared by reducing 1mM of Silver nitrate solution with Bael extract in the ratio of 1:10 and conforming the formation of silver nanoparticles using Surface plasmon resonance(SPR) from UV spectroscopy. The conjugation of silver sulfadiazine with silver nanoparticles was done in the ratio of 1:1. The particle size of Silver nanoparticles and silver sulfadiazine was found to be 149 nm and 132 nm respectively, however TEM images showed particle size within 100nm for Silver nanoparticles and within 70nm of Silver sulfadiazine. Zone of inhibition was calculated in order to find the efficacy of prepared conjugate against *Bacillus subtillis*, *Escherichia coli* and *Aspergillus niger*. Further characterisations PXRD, DSC, FTIR, Zeta Potential, ^{1}H and ^{13}C NMR was done in order to confirm the conjugation, stability, and to determine their physicochemical properties. It was found from all the observations that Silver Sulfadiazine complex was formed and could be used in future through its administration from multiple dosage forms.

Keywords: Sulfadiazine, nanoparticles, Surface Plasmon Resonance, Zone of inhibition

INTRODUCTION

Traditionally, the silver sulfadiazine is an agent of choice for the outpatient treatment of minor and partial-thickness burns. However, some published reports state that there are superior treatment options available. Used primarily as a cream formulation. It has remarkable antiseptic property which reduces the count of bacterial colonization in a burn wound for a long time. When compared to the effect of 0.5% silver nitrate solution, dermazin (1% silver sulfadiazine containing cream) showed following advantages:

- Better penetration in the wound
- More convenient local application
- Better patient compliance
- Better tolerability in patients
- Does not stain the skin and clothes
- Does not cause any electrolytic disturbance
- Possesses strong bactericidal effect along both, gram positive and gram negative bacteria

The advances in the field of nanotechnology have led to the possibility of converting metallic silver into finer nanoparticles. These nanoparticles are more effective than the original form against microbes. These nano-sized particles have given promising results which allow the making of topical silver treatment more effective and safer. The current nano-scale strategies (for drug related & scaffold and carrier) have shown to possess a great potential for augmenting therapeutic ability of biological and synthetic molecules.

History of Silver

Back in the 18th century the usage of silver in the management of wounds came into the light, during which Silver Nitrate (AgNO3) was used in the treatment of ulcers. The antimicrobial activity of the silver ions were first identified in 19th century, and the colloidal silver was accepted by the US Food and Drug administration (FDA) as being effective for wound management in 1920s.. However, after introductions of antibiotics such as penicillin in the year 1940s, antibiotics became the standard treatment for bacterial infections and use of silver diminished. Silver began to be used again for the management of burn cases in the year 1960s, during which it was used as 0.5% AgNO3 solution.. Later in the year 1968 a broad spectrum silver based-antimicrobial was prepared when AgNO3 was combined with sulphonamide antibiotic to produce Silver- Sulfadiazine (SDZ) Cream which continued to be prescribed mostly for the management of burns..

Synthesis of Silver nano-particles using *Aegle marmelos*

Indian plants are considered as a vast source of several pharmacologically active principles and compounds which are commonly used in home remedies .

Similarly, *Aegle marmelos* is a species of tree native to India and it belongs to family Rutaceae. It is commonly known as Bael, Golden Apple and Bengal quince. The fragrant leaves and fruits carry medicinal values and are used in treatment of various diseases.

Chemical Constituents

A.marmelos contains alkaloids of aegelin (N-[2-hydroxy-2(4-methoxyphenyl)ethyl]-3- phenyl-2-propenamide) is a known constituent and is consumed as a dietary supplement. Other chemical present in the plant are several bioactive compounds such as marmelosin, luvangetin, auraptene, psoralen, marmelide and tannin. (**Krupa and Raghavan, 2014**).

Various proved therapeutic values of *Aegle marmelos*: (Sharma et al., 2011)

- Anti-Diabetic activity
- Hepatoprotective activity
- Antimicrobial activity
- Analgesic, Antipyretic & Anti-Inflammatory activity
- Anti-fungal activity
- Anti-spermatogenic activity
- Anti-cancer activity
- Antiulcer activity

***Aegle Marmelos* plant profile**

Fig. : A: Leaves of *Aegle marmelos*; B: Flowering Bud; C: Bael fruit

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Description of plants physicochemical properties

Botanical Name	**Aegle marmelos**
Common Name	Bael
Classification	**Kingdom:** Plantae **Subkingdom:** Tracheobionta **Division:** Magnoliophyta **Class:** Magnoliopsida **Subclass:** Rosidae

Drug Profile of Sulfadiazine

Chemical Names	Sulfadiazine,Sulfadiazine,Sulfapyrimidine,68-35-9,Sulfadiazin,Sulfazine,Sulfonsol
IUPAC Name	4-amino-N-pyrimidin-2-ylbenzenesulfonamide
Molecular Formula	$C_{10}H_{10}N_4O_2S$
Molecular Weight	250.277g/mol

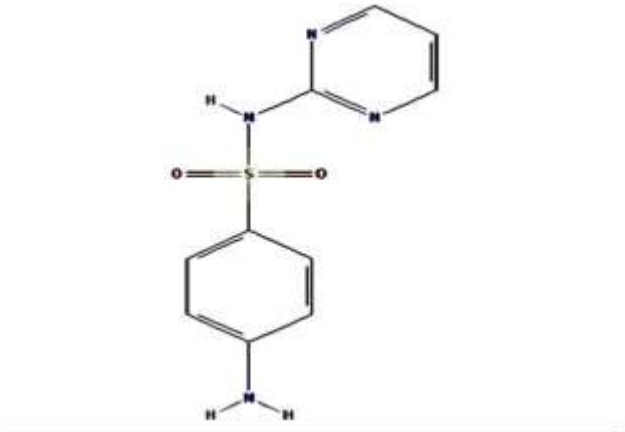

Fig. 2D structure of Sulfadiazine

Fig. 3D conformer of Sulfadiazine

AIM AND OBJECTIVE OF STUDY

Aim of study

Nanoparticle based drug delivery system for sulfadiazine drug along with herbal extract

Objectives of the study

- Designing and development of silver nano-particles using biogenic Synthesis.
- Characterization of prepared nanoparticles
- Conjugating it with sulfa group antibiotic sulfadiazine to produce silver sulfadiazine (SDZ).
- Characterization of silver sulfadiazine

MATERIALS AND RESEARCH METHODOLOGY

Chemicals used

Table : List of chemicals used

Chemicals	Manufacturers
Ethanol	Chong Yu Hi-Tech chemicals, china
Ferric Chloride	Burgoyne Burbidges and Co., India
Muller-Hilton agar media	Himedia laboratories, India
Potassium Bromide	Loba chemicals (P) Ltd
Saboraud agar media	Himedia laboratories, India
Silver Nitrate	Thomas Baker (Chemicals) Pvt. Ltd
Sodium Hydroxide pellets	Loba chemicals (P) Ltd
Sulfadiazine	Sigma-aldrich , India

Equipment used

Table :List of equipment used

Name	Source
Digital Melting Point Apparatus	Popular Traders, Ambala Cantt, India
Digital pH Meter	Systronics, pH system, India
Double Beam UV Spectrophotometer	Shimadzu Co. Ltd., Japan
DSC	DSC Q20 (TA Instruments, U.S.A)
Electronic Balance	Shimadzu Co. Ltd., Japan
FTIR Spectrophotometer	Shimadzu Co. Ltd., Japan
Hot Air Oven	Navyug, Mumbai, India
Hot Plate	Popular Traders, Ambala Cantt, India
Magnetic Stirrer	Remi, Pvt. Ltd. Mumbai, India
NMR analyzer	JEOL JNM ECS-400 (400MHz) spectrometer ,JEOL, Japan
Particle Size	Zetasizer ,Malvern Instruments Ltd
Spray dryer	Spray Mate, JISL, Maharashtra
TEM analyzer	FEI Tecnai G^2 F20 model, The Netherlands
XRD analyzer	PANanalytical X'$pert^3$ Pro, The Netherlands
Zeta Sizer	Beckman Coulter Delsa™Nano

RESULTS AND DISCUSSION

Characterization of Sulfadiazine

Physical appearance test

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Result of physical characterization of sulfadiazine is listed in Table. variations were found in its specification in Certificate of Analysis (COA) and observations recorded at the time of experimentation.

Table : Physical characterization of sulfadiazine

Sr.No.	Parameter	Observation
1.	Odour	Odourless
2.	Colour	White
3.	Appearance	Powder

Melting point

Experimentally observed melting point (Table) complies with reported melting point in COA, Sigma-Aldrich, India.

Table Melting point of Sulfadiazine

Sr.No.	Parameter	Specification in literature	Melting point
1.	Melting point	253ºC	253ºC-255ºC

FTIR spectra analysis

The FT-IR spectra of procured sample show comparable principle absorption bands with that of FT-IR spectra of working standard of sulfadiazine obtained from industry . Compliance between the values of characteristic peaks indicates the purity of drug

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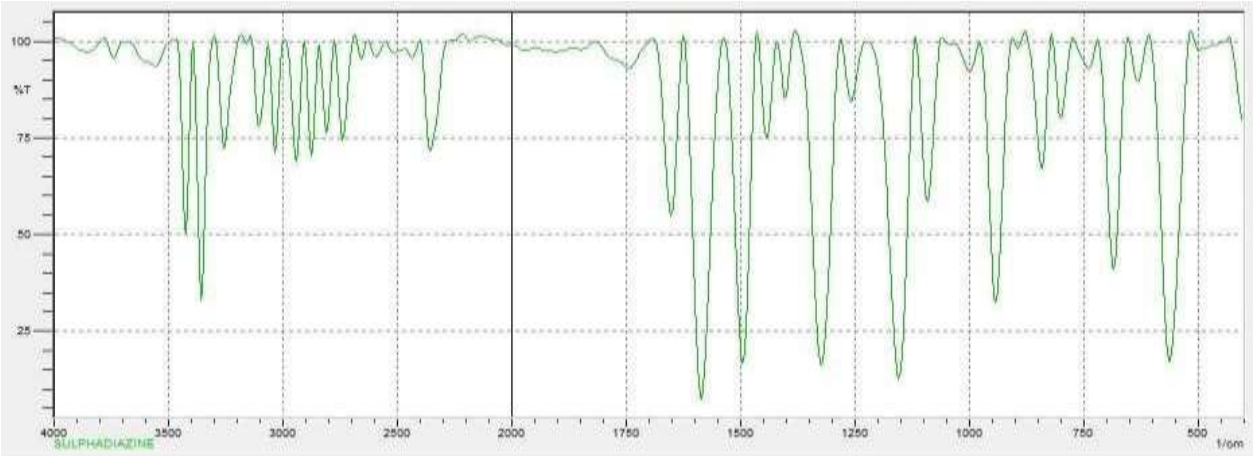


Fig. IR spectra of Sulfadiazine Table FTIR

spectra analysis of sulfadiazine

Sr.No.	Standard value range (cm^{-1})	Observed value	Interpretation
1.	3300-3500	3400	N-H stretch (secondary amine)
2.	3300-3000	3300	C-H stretch
3.	1080-1360	1150	C-N stretch
4.	1400-1600	1580	C=C stretch
5.	1550-1640	1560	N-H bending

The IR spectra of the given sample show comparable principle absorption band. This matching for characteristic peak of drug with that of standard confirms the purity of drug.

Analytical method development

Determination of absorption maxima

Irrespective to the nature of media the λmax of sulfadiazine was found to be 254nm (USP Monograph)

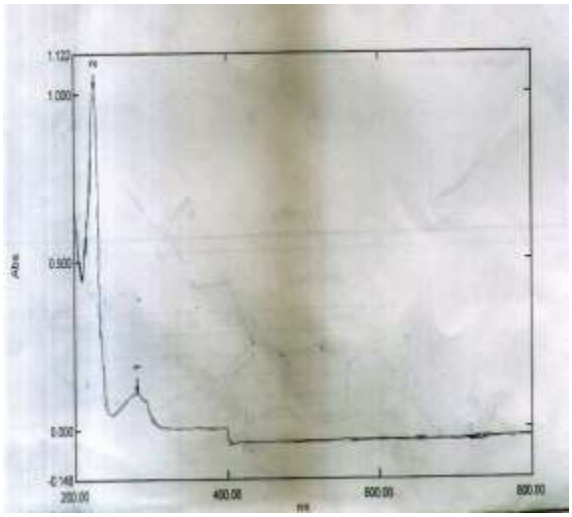


Fig. Determination of λmax of Sulfadiazine in 0.05N NaOH solution

Construction of calibration plots for sulfadiazine

Calibration plots of sulfadiazine was developed in 0.05N NaOH solution. Reason behind selecting above mentioned solution is its solubility criteria and its wide acceptance in USP monographs.

Table : Calibration curve of Sulfadiazine in 0.05N NaOH solution

Sr.No.	Conc.	Abs.
1	2	0.188±0.00051
2	4	0.376 ±0.0048
3	6	0.546 ± 0.00128
4	8	0.677 ± 0.00698
5	10	0.866 ±0.00746

Data represented as mean ± S.D (n=3)

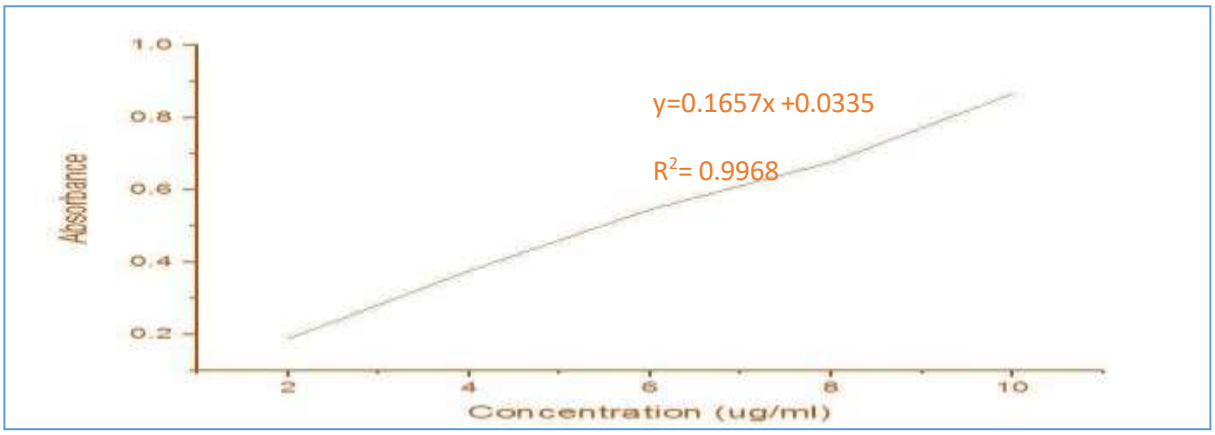


Fig. Calibration curve of Sulfadiazine in 0.05N NaOH solution

Preparation of *Aegle marmelos* leaf extract

Aegle marmelos leaf extract was prepared as per previously stated process. The colour of the leaf extract was found to be dark green

Detection test for phenols in leaf extract

Ferric chloride test was done in order to detect the presence of phenols and tannins in the Bael leaf extract. Plant extract was brought to boiling in a test tube and 5% Ferric chloride solution was added, the colour of the extract changed to bluish black colour which indicated presence of tannins.

Fig.Test for presence of phenols

Synthesis of Silver nanoparticles

Synthesis of silver nanoparticles was done using bael leaf extract. The concentration of $AgNO_3$ solution was varied from 1 mM- 5 mM and bael leaf extract in the ratio of 1:10. The confirmation of formation of silver nanoparticles was done by change in colour from light green solution to dark orange solution and its further characterisation was performed.

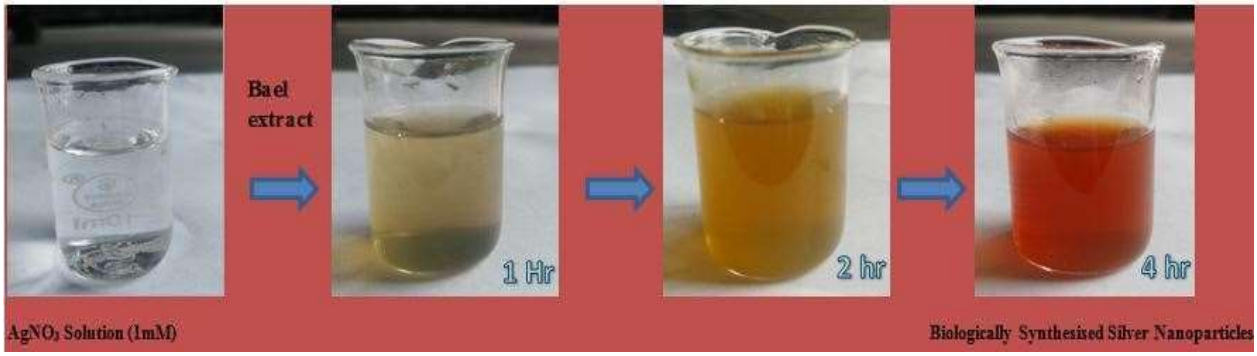
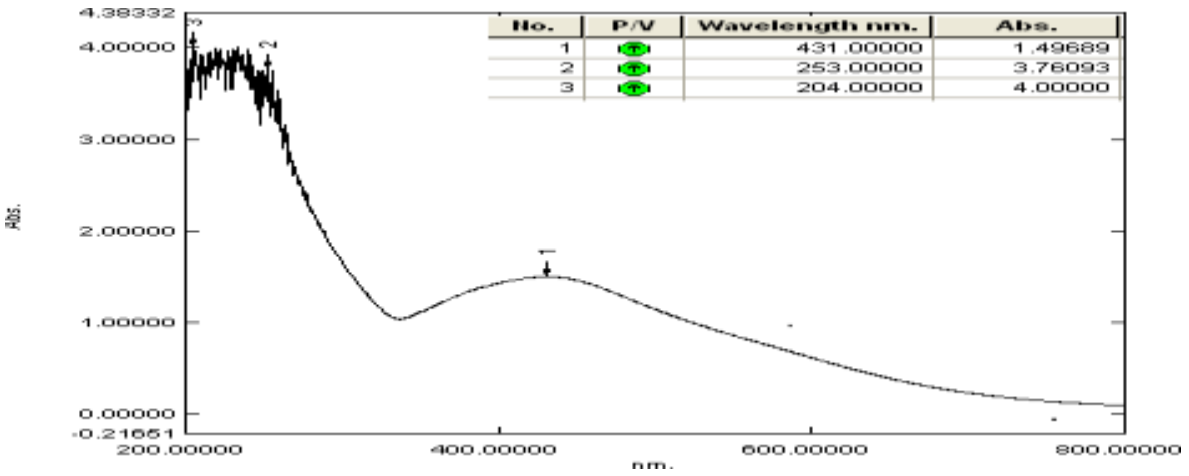


Fig. Synthesis of Silver nanoparticles at various time intervals

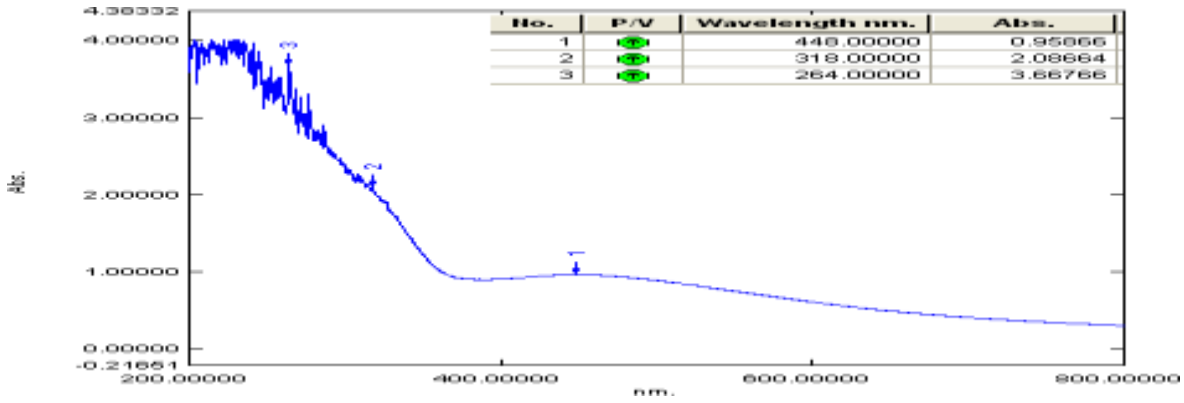
UV Spectrophotometric Analysis

UV analysis was done in scanning mode of various concentrations of $AgNO_3$ solution and bael extract at the

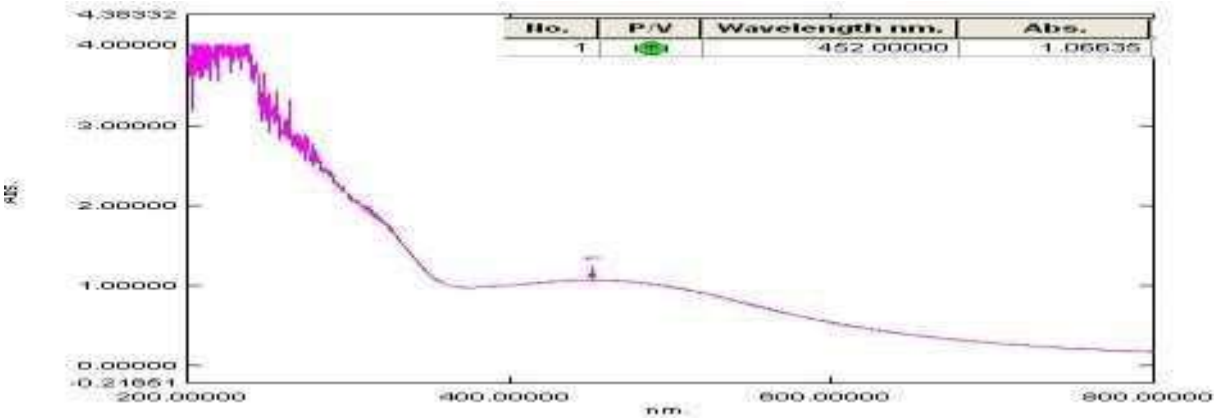
range of 200-800 nm. The ratio of bael extract was kept constant i.e. 1:10 and concentration of $AgNO_3$ (20ml) was varied from 1 mM -5 mM. Due to formation of silver nanoparticles we observed peaks at 370-460 nm which is known as Surface Plasmon Resonance.It also came into the notice that upon increasing the concentration of $AgNO_3$ there was aggregation of nanoparticles which resulted in broad peaks. Hence, minimal concentration was chosen i.e. 1mM $AgNO_3$ solution + Bael extract in the ratio of 10:1 for further studies.



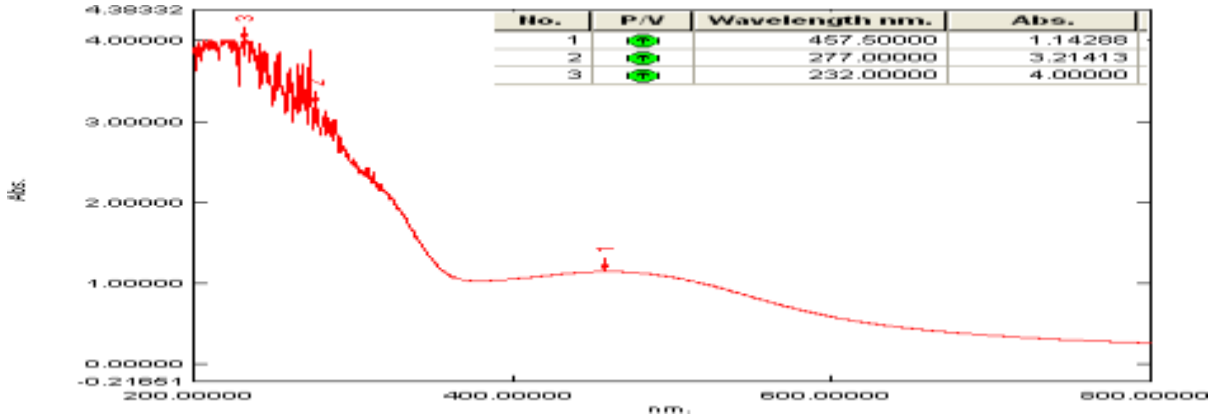
(A)



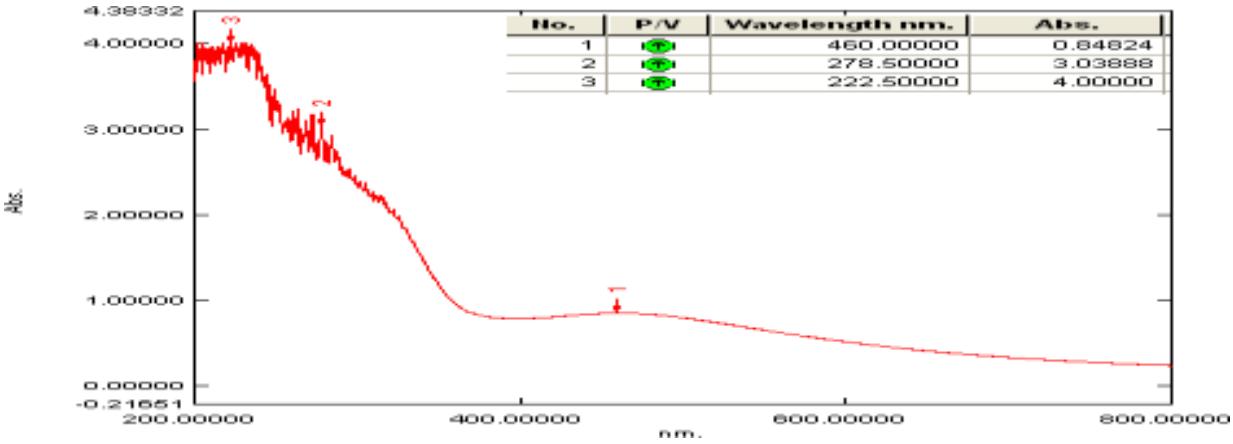
(B)



(C)



(D)



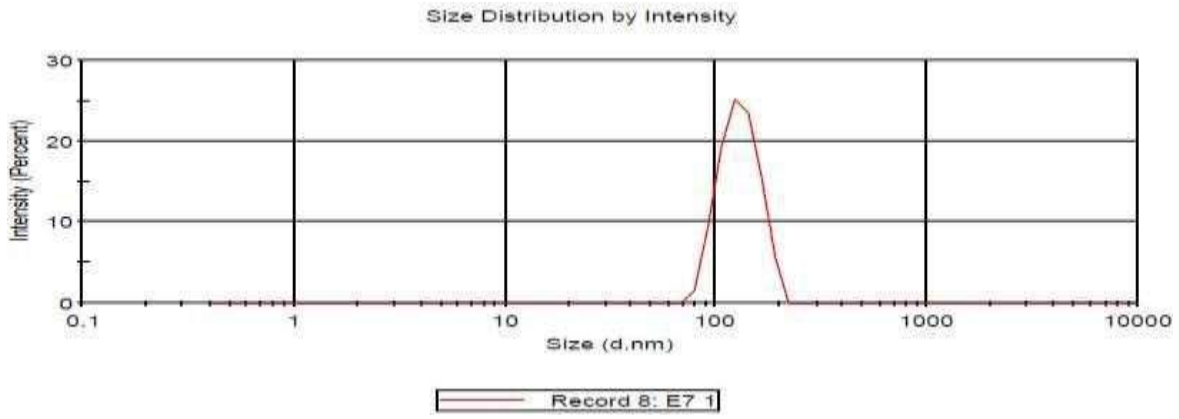
(E)

Fig. UV peaks showing SPR region (A) $AgNO_3$ [1mM] (B) $AgNO_3$ [2mM] (C) $AgNO_3$ [3mM] (D) $AgNO_3$ [4mM] (E) $AgNO_3$ [5mM]

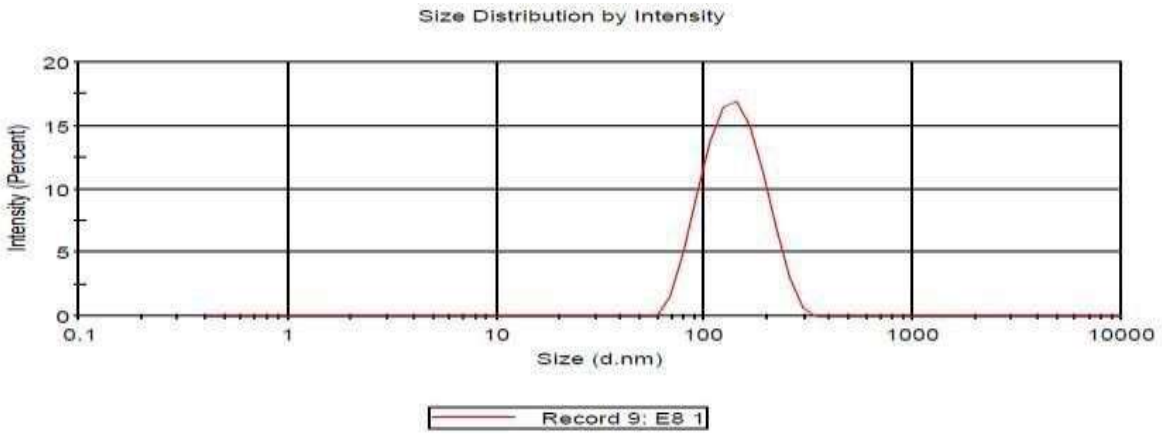
Particle Size and Zeta Potential Determination

Particle size of biologically prepared silver nanoparticles (BSN) and spray dried silver nanoparticles (SSN) was measured using Zetasizer, Malvern Instruments Ltd. The average particle size of biologically prepared silver nanoparticles was found to be 149nm with Polydispersity index (P.I) value of 0.305 and average particles size of reconstituted spray dried silver nanoparticles was found to be 138nm with polydispersity index (0.178).

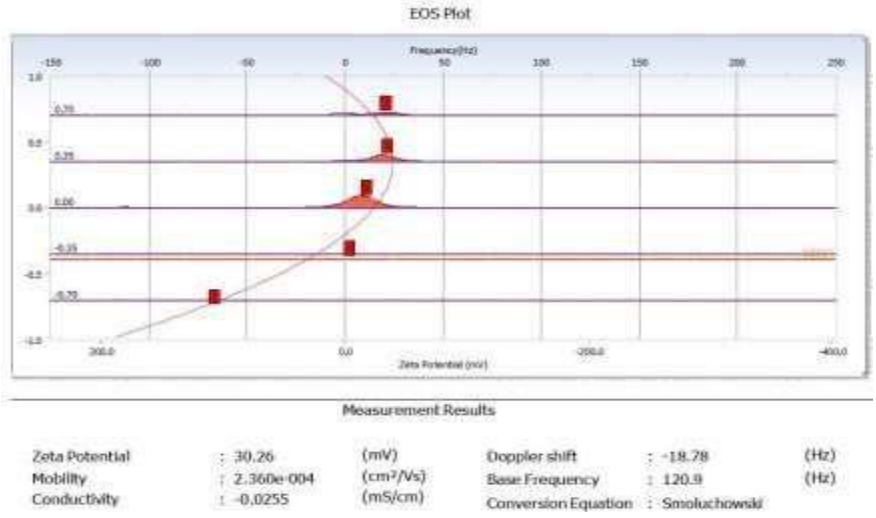
The Zeta potential of BSN was measured using Beckman Coulter Delsa™Nano and it was found to be 30.26 mV from which we can consider it to be moderately stable under provided conditions whereas Zeta potential of reconstituted SSN measured under same conditions was found to be 51.53 mV from which we considered it to be Stable.



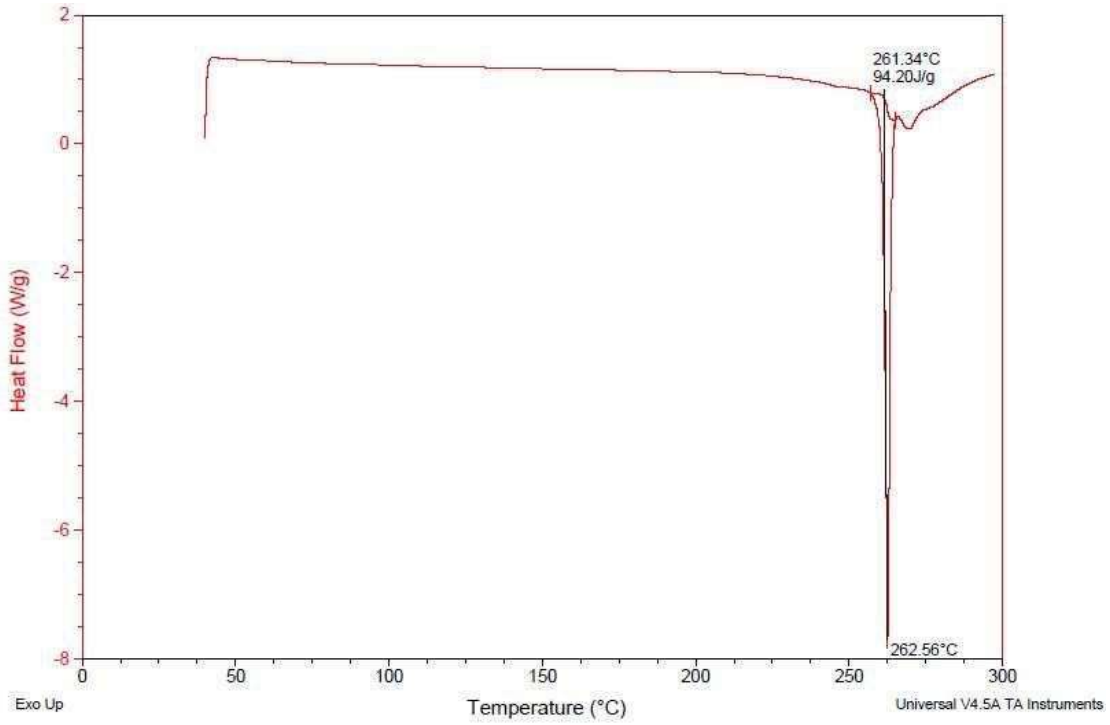
(A)



(B)



(C)



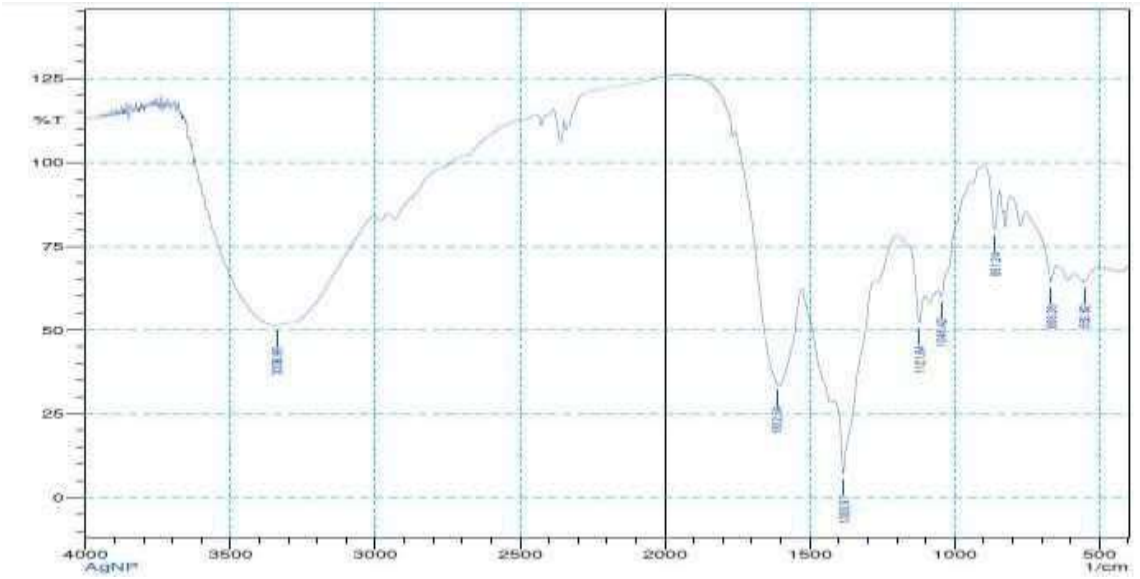
(D)

Fig.(A) Particle size distribution of biologically prepared Silver nanoparticles (B) particle size distribution of Spray dried silver nanoparticles(C) Zeta Potential of biologically synthesised silver nanoparticle (D) Zeta potential of spray dried silver nanoparticles.

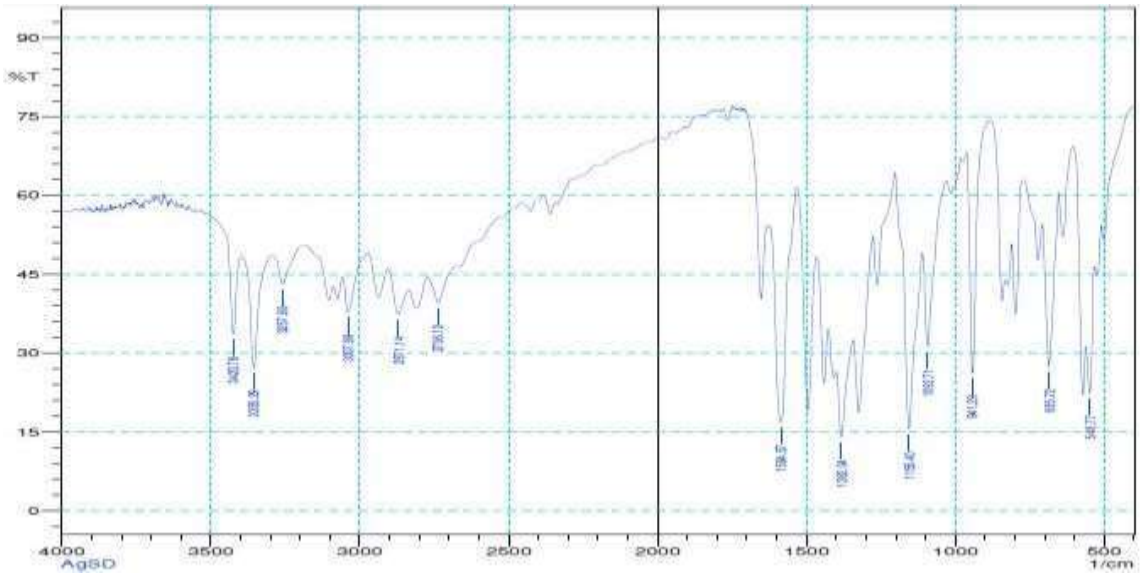
FTIR Spectra analysis

The FT-IR spectra of synthesised sample show comparable principle absorption bands with that of FT-IR

spectra of working standard of silver sulfadiazine and silver nanoparticles (Fig. 8.13 and Table 8.5 and 8.6).



(A)



(B)

Fig.FTIR spectra of (A) Silver nanoparticles (B) Silver sulfadiazine

Table :FTIR spectra analysis of Silver nanoparticles

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Sr.No.	Standard value range (cm^{-1})	Observed value	Interpretation
1.	3200-3600	3336.96	O-H Stretch (H Bonded)
2.	1550-1640	1612.54	N-H Bending
3.	1350-1480	1383.97	-C-H Bending
4.	1050-1150	1064.42	C-O Stretch
5.	675-1000	861.24	=C-H Bending

The table shows peaks at various ranges, this may be due to the presence of functional groups from the aqueous Bael leaf extract which may have conjugated during the reduction of $AgNO_3$.

The Spectra of Silver sulfadiazine resembles quiet to that of sulfadiazine depicted above. The loss of peak for –NH bending resembles the conjugation of Silver ion to the $–N^{(-)}$

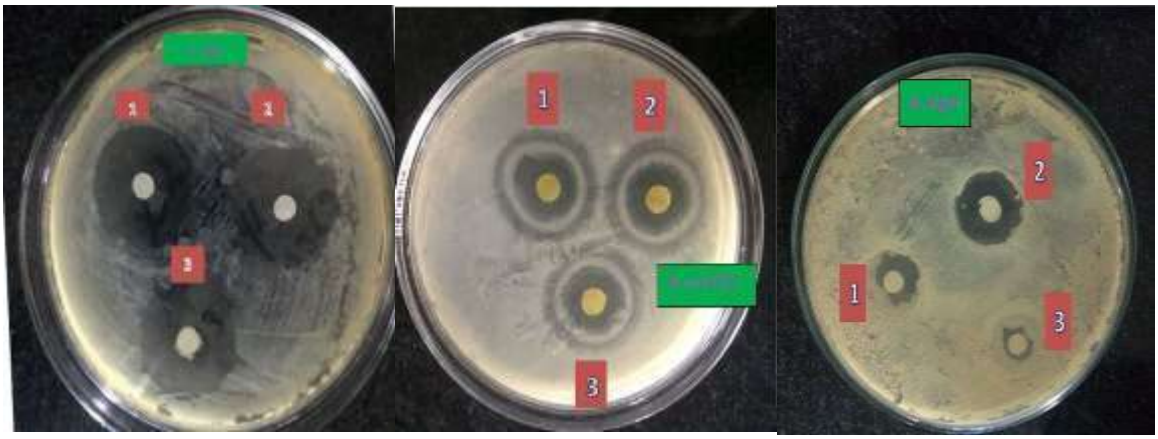
Table :FTIR Spectra of Silver sulfadiazine

Sr.No.	Standard value range (cm^{-1})	Observed value	Interpretation
1.	3200-3600	3423.76	O-H Stretch
2.	3010-3100	3037.99	=C-H Stretch
3.	3000-3100	3037.99	C-H Stretch (Aromatic)
4.	2850-3000	2871.14	C-H Stretch
5.	2720-2750	2736.12	=C-H Stretch

Disc diffusion assay and Minimum inhibitory concentration (MIC) determination

Disc diffusion assay was performed for assessing the antibacterial and antifungal efficacy of AgNPs and Silver sulfadiazine against E.coli, B.subtillis and A.niger. Results for disc diffusion assay are shown in Fig.8.14, which represents zone of inhibitions (ZOIs) around individual discs, inoculated with (A) 10 % AgNPs solution (B) 1% SDZ solution and (C) aqueous bael leaf extract (AEM). As assessed from the results the significant antibacterial and antifungal effects of AgNPs and Silver sulfadiazine against both gram classes of bacteria and fungi. It is also clear from the result that AgNPs and SDZ are more effective towards E.coli (Gram negative) as compared to B.subtillis (Gram positive) and A.niger (Fungi). It may be because gram negative bacteria bears weaker cell wall due to less peptidoglycan content as compared to gram positive bacteria .Minor zone of inhibitions were also observed around the discs containing AEM. Herbal extracts poses antimicrobial activity due to their phytochemicals. AgNPs exhibit their antimicrobial and antifungal

activity due to their capacity to disrupt cell wall; produce Reactive oxygen species (ROS) mediated toxicity and interfering activity with DNA replication.



(A) **(B)** **(C)**

Fig. Disc (A) E.coli (B) B.subtillis (C) A.niger and (1) ZOI of AgNPs (2) ZOI of SDZ (3) ZOI of AEM

Table :Zone of inhibition of various test compounds

S.no	Culture	AgNPs	SDZ	AEM
1.	***Escherichia coli*** **(Gram '-'Ve bacteria)**	9mm	12mm	10mm
2.	***Bacillus subtillis*** **(Gram '+'Ve Bacteria)**	5mm	6mm	5mm
3.	***Aspergillus niger*** **(Fungi)**	3mm	6mm	2mm

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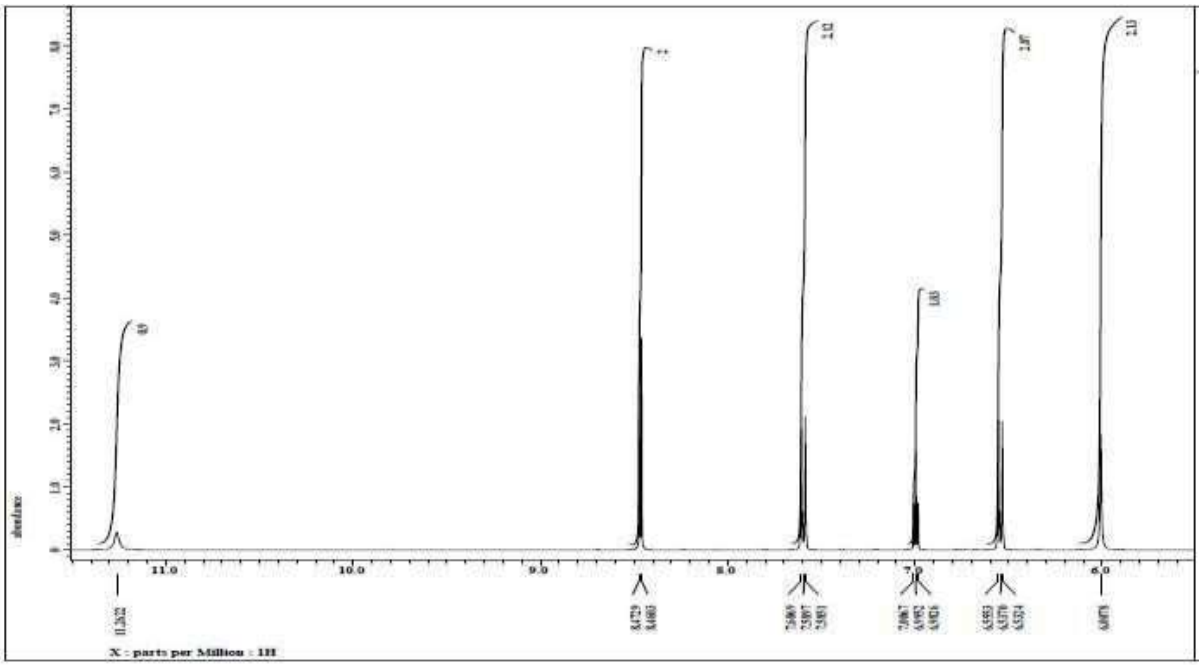

Fig. ^{1}H NMR of Sulfadiazine

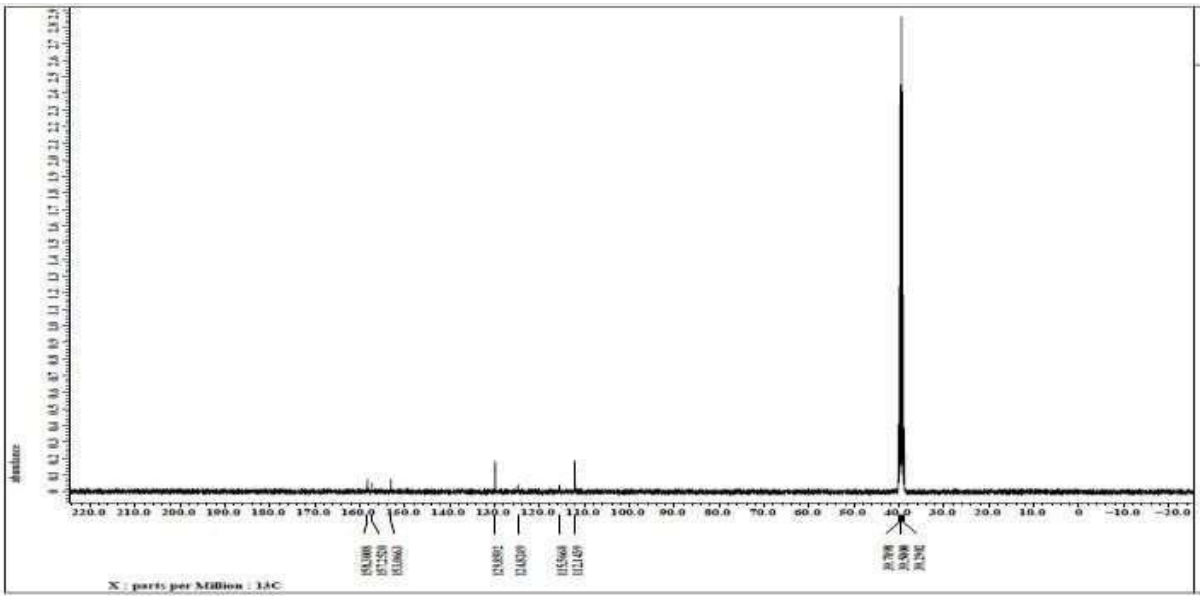

Fig. ^{13}C NMR of Sulfadiazine

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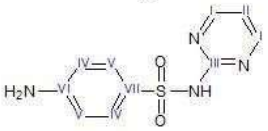
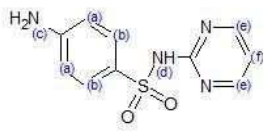
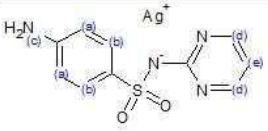
Table : NMR Spectra of sulfadiazine Table depicting results of ^{13}C and ^{1}H NMR of Sulfadiazine and Silver Sulfadiazine complex.

Structure and arrangements of Carbon and protons	^{13}C NMR							^{1}H					
	I	II	III	IV	V	VI	VII	(a)	(b)	(c)	(d)	(e)	(f)
^{13}C 	112.143 s	115.56 s	157.252 d	129.85 d	158.300 s	124.824 d	153.066 s	6.555 t	7.606 t	6.007 s	11.262 s	8.472 d	7.006 t
^{1}H 													
	Complete ^{13}C NMR and ^{1}H NMR of Silver sulfadiazine could not be obtained due to poor solubility of SDZ in DMSO-d6. Though the Proton NMR of Silver sulfadiazine gave some roughly similar peaks as compared to sulfadiazine but they were not considered to be in relation with its structure due to presence of unknown number and type of chemicals in the leaf extract of *Aegle marmelos* which was used during their conjugation.												

SUMMARY AND CONCLUSION

In this present study, Silver nanoparticles were prepared by using aqueous leaf extract of plant *Aegle marmelos* (green synthesis) to increase its ecological safety and it was conjugated with sulphonamide group containing antibiotic Sulfadiazine to produce sulfadiazine loaded silver nanoparticles. The prepared silver sulfadiazine complex was found to be having the average particles size of 132nm which was less in comparison to silver nanoparticles. The confirmation of silver nanoparticle was done firstly by visual observation as silver nanoparticles gives orange colour and Surface Plasmon Resonance (SPR) peaks at 380- 450nm was observed using UV Spectrophotometer. Silver nanoparticles gave a steady peak at 430nm. FTIR spectra of both AgNPs and SDZ resembled to their reported values. The XRD peaks of silver sulfadiazine showed a decreased crystallinity in comparison to sulfadiazine (Pure) and synthesised silver nanoparticles which may be due to conjugation between silver nanoparticles and sulfadiazine. The results of Differential Scanning Calorimetry (DSC) confirmed their conjugation as they gave a sharp and broad exotherm and endotherm peaks for Sulfadiazine and silver nanoparticles respectively but no peak was found for Silver sulfadiazine due to the conjugation between the Silver nanoparticles and sulfadiazine. Transmission electron microscopy results showed that, most of the particles were roughly spherical in shape with particle size in the range of 10-100nm and 10-70nm in case of Silver nanoparticles and Silver sulfadiazine respectively. Antimicrobial study and zone of inhibition study using disc diffusion assay was done and the results showed positive results for Gram '-ve' bacteria *E.coli*, Gram '+ve' bacteria *B. subtillis* and Fungi *A.niger*, Silver sulfadiazine was most effective against E.coli as compared two strains. The supplementary data of NMR (^{1}H and ^{13}C) of sulfadiazine resembled to its structure but NMR of Silver sulfadiazine could not be obtained due

to its poor solubility in DMSO- d6.Hence, it can be concluded rom the results and observations that sulfadiazine loaded silver nanoparticles were obtained which was ecologically more safer and less toxic as compared to other formulation present in market. Though it has many possibilities to be used through various dosage forms such as vesicular gels and Nano systems, creams, medicated patches etc. Though the *in vitro* and *in vivo* experiments needs to be conducted in order to prove their efficacy and release.

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