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Research

Formulation And Evaluation Of Sulfadiazine Loaded Silver Nanoparticle

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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: Nanotechnology, Green Synthesis, Surface Plasmon Resonance, Zone of inhibition

INTRODUCTION

WHO defines burns as an injury to the skin or other organic tissue primarily caused by heat or due to radiation, radioactivity, electricity, friction or contact with chemicals. Also, respiratory damage from inhalation of smoke is also considered as burn. Approximately 2,65,000 deaths occur annually from fires alone. Over 96 percent of lethal fire associated burns occur in low and middle income countries (World Health Organization, 2017). NHS lays down certain first aid measures for burns (Fig.1) (NHS Choices, 2015).

The earliest documented treatment for burns included dressings impregnated with milk from lactating mothers of newborn male children. Usage of honey and grease was documented for treating burns in the Edwin Smith papyrus (1500 BC). On the other hand, mud, oil, excreta, and plant extracts were employed for treatment of burns as per the Ebers Papyrus (1500 BC). They also made use of aloe, tannins and honey. For analgesia, belladonna, opium and thyme were employed. Characa and Sushruta have also mentioned the usage of honey to treat burns, in the Ayurvedic texts (Pećanac et al., 2013; Zbuchea, 2014).

Traditionally, it has been taught and practiced that 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 (Chung and Herbert, 2001). 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 (Vŭglenova, 1991).

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 (Adhya et al., 2014). 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 (Tocco et al., 2012; Wang and Uludag, 2008).

An *in vitro* release study has reported that the release of SSD was better from solutions and nanosuspensions with respect to gel formulation. The bacterial inhibitory activity of SSD nanosuspension was found to be just as good as that of the solution against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa.* The *in vivo* studies reported that a nanogel comprising 0.5% SSD was more effective in healing the burn wounds as compared to 0.5% and 1% marketed cream (Venkataraman and Nagarsenker, 2013) .

The present study aims at synthesising a biologically and ecologically safe drug which could be the key towards the management of burn. The way for achieving this will be use of minimum number of chemicals which may or not catalyse the in-advert reactions in the sensitive cases of burns and its consequences.

RESULTS AND DISCUSSION

Characterization of Sulfadiazine
Physical appearance test

Result of physical characterization of sulfadiazine is listed in Table 8.1. No variations were found in its specification in Certificate of Analysis (COA) and observations recorded at the time of experimentation.

Table 1: Physical characterization of sulfadiazine

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

Melting point

Experimentally observed melting point (Table 8.2) complies with reported melting point in COA, Sigma-Aldrich, India.

Table 2: Melting point of Sulfadiazine

S.No.	Parameter	Specification in literature	Melting point
	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 (Fig. 1, Table 3). Compliance between the values of characteristic peaks indicates the purity of drug.

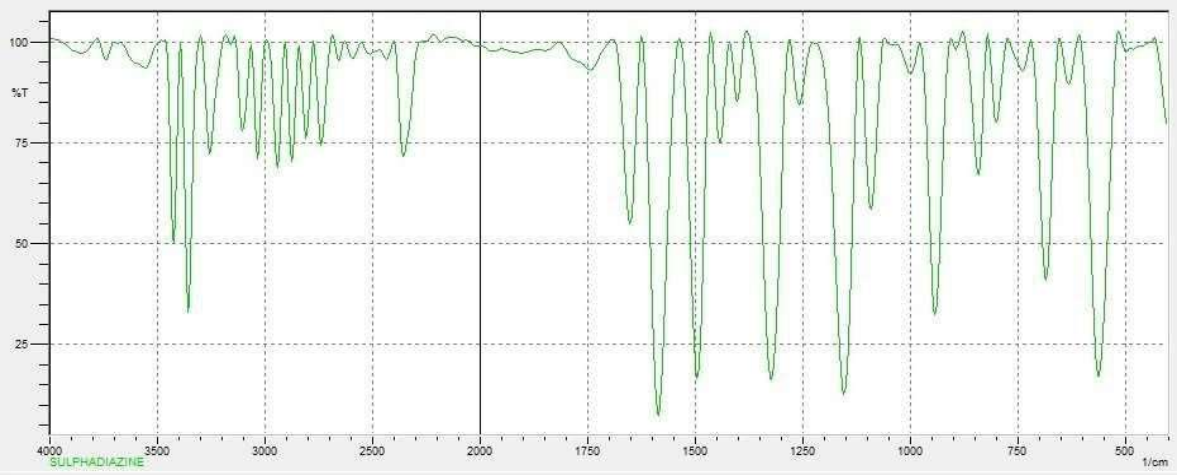


Fig 1: IR spectra of Sulfadiazine Table 8.3

Table 3: 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 2).

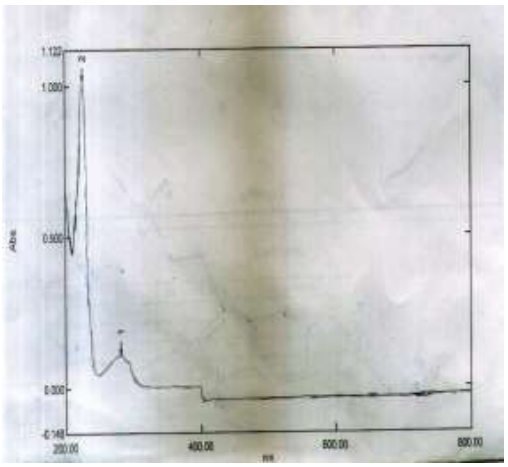


Fig 2: Determination of λmax of Sulfadiazine in 0.05N NaOH solution

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 4: 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)

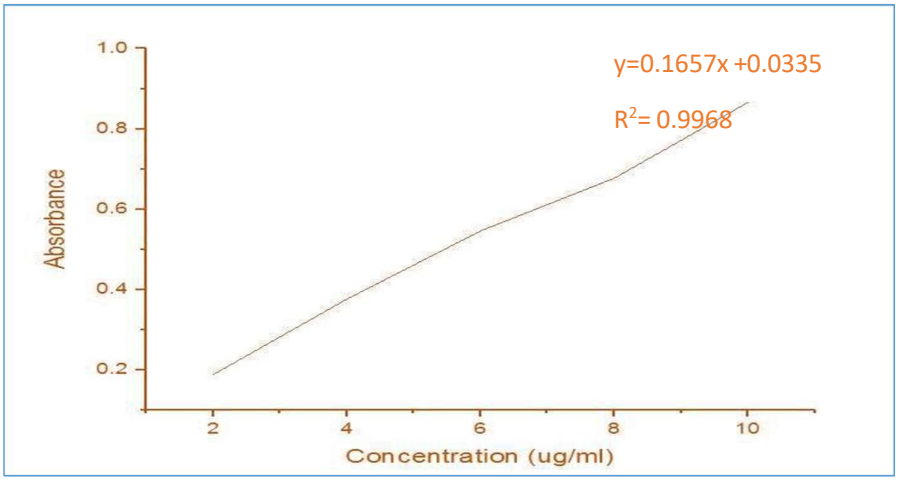
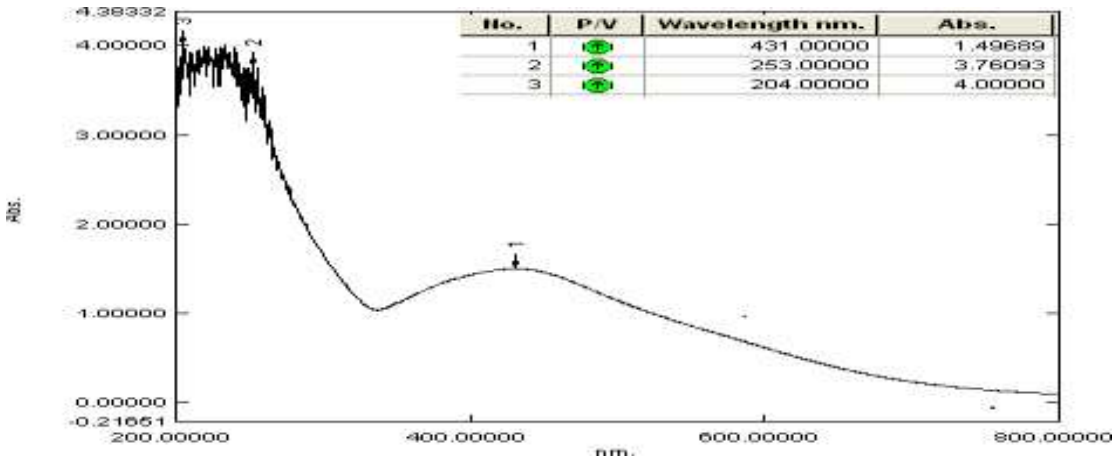


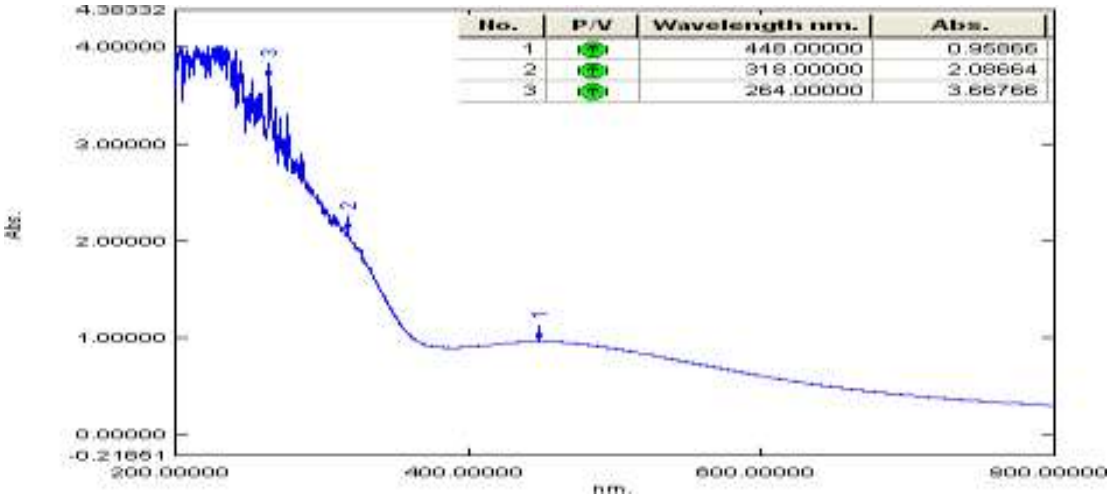
Fig 3: Calibration curve of Sulfadiazine in 0.05N NaOH solution

UV Spectrophotometric Analysis

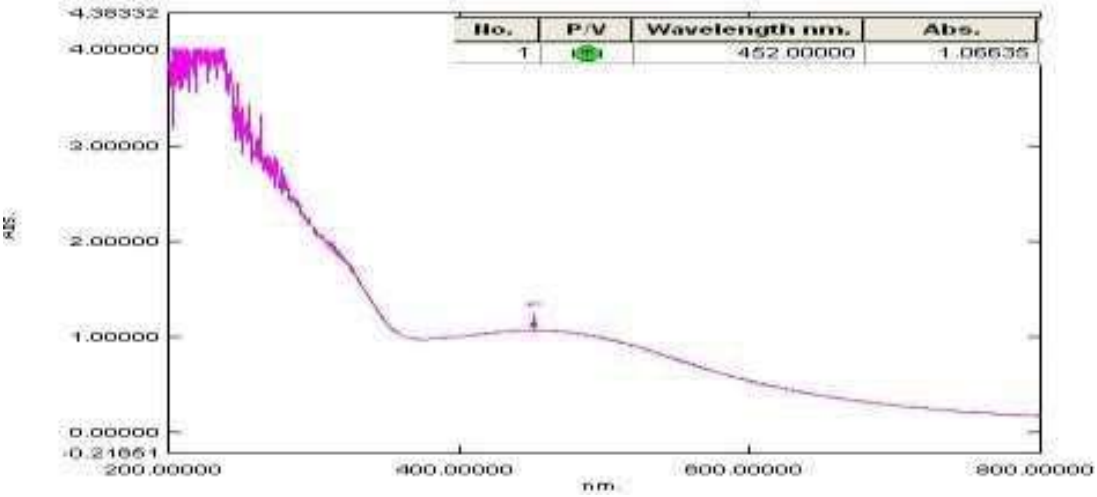
UV analysis was done in scanning mode of various concentrations of $AgNO_3$ solution and bael extract at the range of 200-800 nm. The ratio of bael extract was kept constant i.e. 1:10 and concentration of $AgNO_3$ (20ml) was varied from 1 mM -5 mM. Due to formation of silver nanoparticles we observed peaks at 370-460 nm which is known as Surface Plasmon Resonance. (Fig) 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.


No. P/V Wavelength nm. Abs.
1 431.00000 1.49689
2 253.00000 3.76093
3 204.00000 4.00000
Abs.
nm.

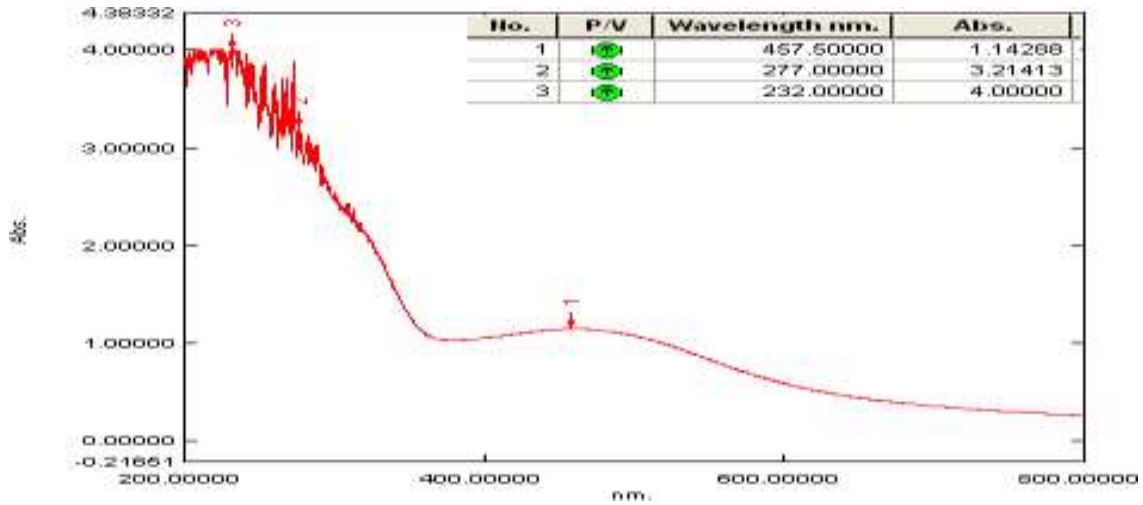
(A)


No. P/V Wavelength nm. Abs.
1 448.00000 0.95866
2 318.00000 2.08664
3 264.00000 3.66766
Abs.
nm.

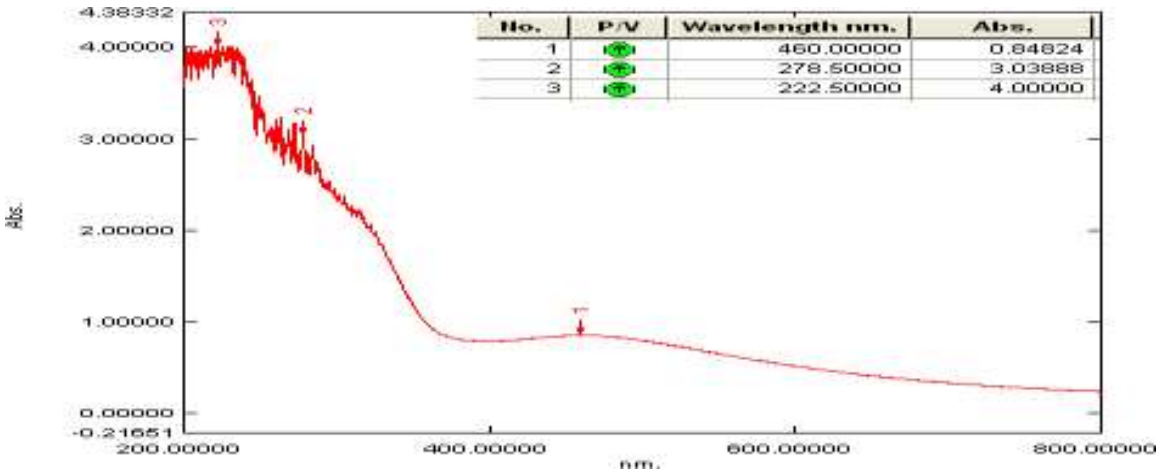
(B)


No. P/V Wavelength nm. Abs.
1 452.00000 1.06635
Abs.
nm.

(C)



(D)



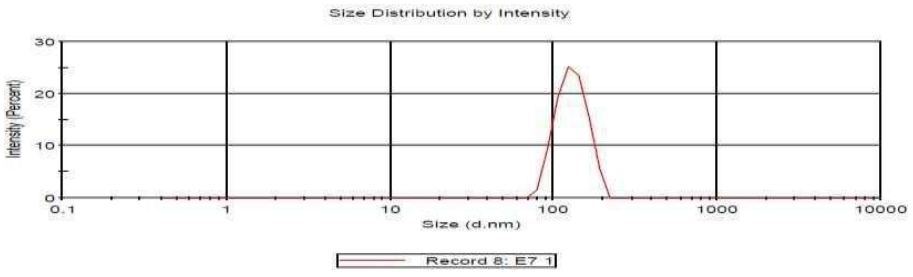
(E)

Fig 4: 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]

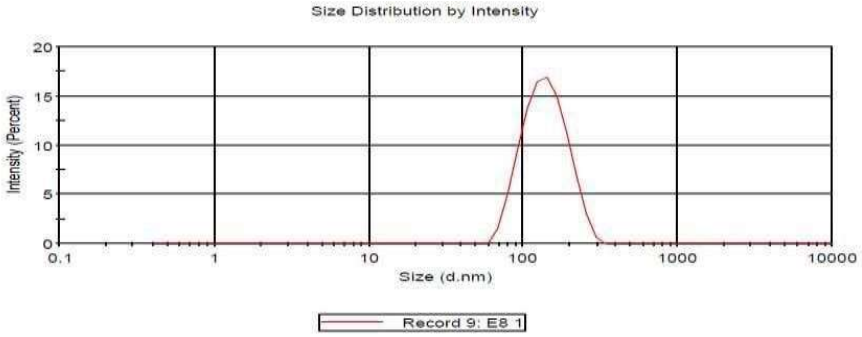
Particle Size and Zeta Potential Determination

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).

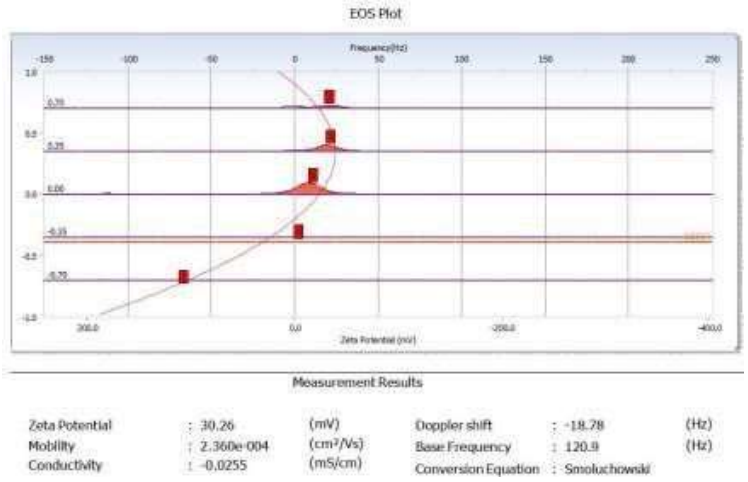
The Zeta potential of BSN was measured using Beckman Coulter Delsa™Nano and it was found to be 30.26 mV from which we can consider it to be moderately stable under provided conditions whereas Zeta potential of reconstituted SSN measured under same conditions was found to be 51.53 mV from which we considered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



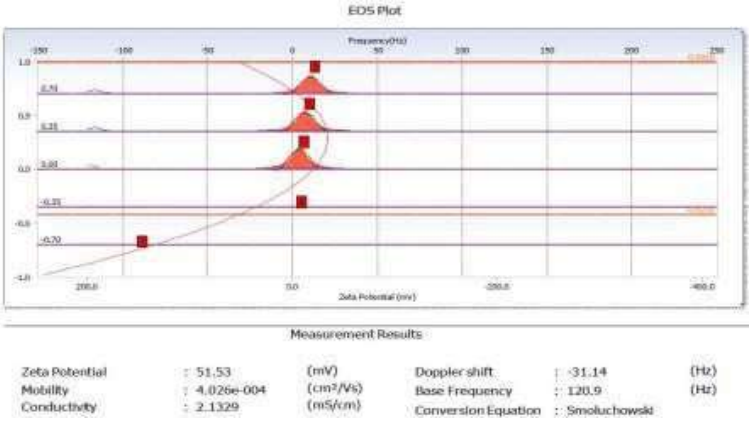
(A)



(B)



(C)



(D)

Fig 5: (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.

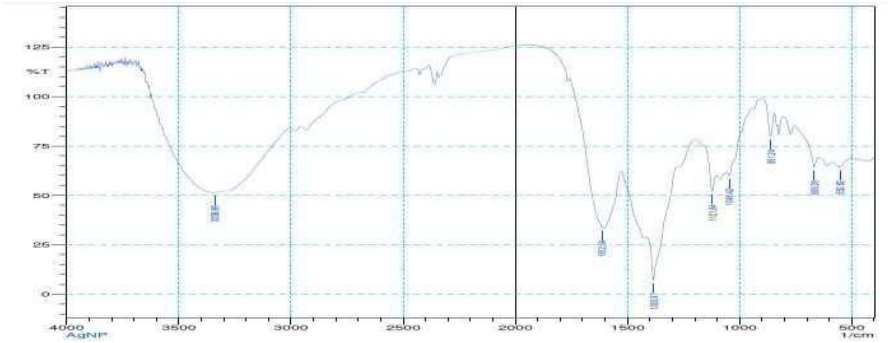
Differential scanning calorimeter

With DSC, it is possible to measure the small energy changes that occurs as matter undergo thermotropic transitions with heating from initially in solid to liquid phase. In this study, DSC thermogram for sulfadiazine was determined with DSC Q20 (TA Instruments, U.S.A) and sample size was 1.00 mg. DSC curve of sulfadiazine

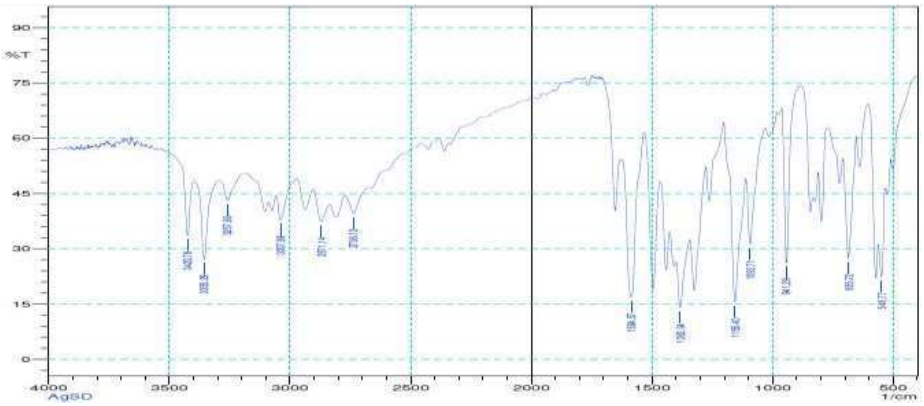
provides a sharp exotherm peak at 261.34ºC indicating its microcrystalline nature. The thermogram of silver nanoparticles exhibits a broad endotherm ranging from 37.5ºC to 284.41ºC. It could be due to the aqueous leaf extract used for its synthesis and it could be explained by conjugation plot of Silver sulfadiazine, as in DSC thermogram of sulfadiazine there were no peaks present which confirms the conjugation between silver nanoparticles and sulfadiazine as the crystallinity of both the powders have decreased and may be converted into amorphous form.

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 6: FTIR spectra of (A) Silver nanoparticles (B) Silver sulfadiazine

Table 5: FTIR spectra analysis of Silver nanoparticles

Sr.No.	Standard value range (cm^{-1})	Observed value	Interpretation
1.	3200-3600	3336.96	O-H Stretch
2.	1550-1640	1612.54	(H Bonded) 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

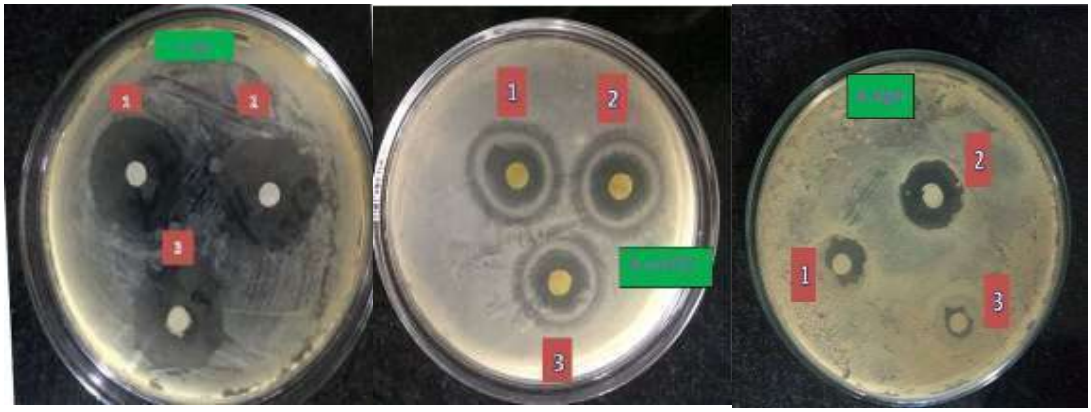
The table shows peaks at various ranges, this may be due to the presence of functional groups from the aqueous Bael leaf extract which may have conjugated during the reduction of $AgNO_3$.

The Spectra of Silver sulfadiazine (Table 8.6) 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 6: 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
4.	2850-3000	2871.14	(Aromatic) C-H Stretch
5.	2720-2750	2736.12	=C-H Stretch



(A) (B) (C)

Fig 7: Disc (A) E.coli (B) B.subtillis (C) A.niger and (1) ZOI of AgNPs (2) ZOI of SDZ(3) ZOI of AEM

Table 7: 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

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.

REFERENCES

1. Abbasi, E., Milani, M., Fekri Aval, S., Kouhi, M., Akbarzadeh, A., Tayefi Nasrabadi, H., Nikasa, P., Joo, S.W., Hanifehpour, Y., Nejati-Koshki, K., Samiei, M., Silver nanoparticles: Synthesis methods, bio-applications and properties. Crit. Rev. Microbiol. 2014.7828, 1–8.
2. Abou El-Nour, K.M.M., Eftaiha, A., Al-Warthan, A., Ammar, R.A.A., Synthesis and applications of silver nanoparticles. Arab. J. Chem. 2010.
3. Adhya, A., Bain, J., Ray, O., Hazra, A., Adhikari, S., Dutta, G., Ray, S., Majumdar, B.K., Healing of burn wounds by topical treatment: A randomized controlled comparison between silver sulfadiazine and nano-crystalline silver. J. basic Clin. Pharm. 2014. 6, 29–34.
4. Ahmad, N., Sharma, S., 2012. Green Synthesis of Silver Nanoparticles Using Extracts of Ananas comosus. Green Sustain. Chem. 2, 141–147.
5. Ahmad, N., Sharma, S., 2012. Green Synthesis of Silver Nanoparticles Using Extracts of Ananas comosus. Green Sustain. Chem. 2, 141–147.
6. Ahmed, S., Ikram, S., 2015. Silver Nanoparticles: One Pot Green Synthesis Using Terminalia arjuna Extract for Biological Application 6.
7. Ainbinder, D., Touitou, E., 2005. Testosterone ethosomes for enhanced transdermal delivery. Drug Deliv 12, 297–303.
8. Anitha.P., Delivery, T.D., Drug, C., View, D., 2016. Ethosomes -A noninvasive vesicular carrier for transdermal drug delivery.
9. Attama, A., Reichl, S., Müller-Goymann, C., 2009. Sustained release and permeation of timolol from surface-modified solid lipid nanoparticles through bioengineered human cornea. Curr. Eye Res. 34, 698–705.
10. Bangham, A.D., Standish, M.M., Watkins, J.C., Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 1965. 13, 238–IN27.
11. Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., Pyne, S., Misra, A., Green synthesis of silver nanoparticles using seed extract of Jatropha curcas. Colloids Surfaces A Physicochem. Eng. Asp. 2009. 348, 212–216.
12. Bragg, P., Rainnie, D., The effect of silver ions on the respiratory chain of Escherichia coli. Can. J. Microbiol. 1974. 20, 883–889.
13. Brown, T., D.S., The effects of silver nitrate on t`he growth and ultrastructure of the yeast Cryptococcus albidus. Microbios Lett. 1976. 3, 155 – 162.
14. C. Caro, P. M. Castillo, R. Klippstein, D.Pozo, A.P.Z., Silver Nanoparticles, Nanotechnology and Nanomaterials. 2010.
15. Cevc, G., Transdermal drug delivery of insulin with ultradeformable carriers. Clin Pharmacokinet. 2003; 42, 461–474.
16. Cevc, G., Blume, G., Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. BBA - Biomembr. 1992; 1104, 226–232.
17. Cevc, G., Vierl, U., Spatial distribution of cutaneous microvasculature and local drug clearance after drug application on the skin. J. Control. Release. 2007; 118, 18–26.
18. Chandel, A., Patil, V., Goyal, R., Dhamija, H., Parashar, B., Ethosomes: A Novel Approach towards Transdermal Drug Delivery. 2012; 1, 563–569.
19. Chaud, M. V, Tubino, M., Jodar, K.S.P., Ao, V.M.B., Yoshida, I.M.H., Development and Characterization of a Hydrogel Containing Silver Sulfadiazine for Antimicrobial Topical Applications. 2015; 2241–54.
20. Chopra, I., The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern. 2007; 587–590.
21. Chung, J.Y., Herbert, M.E., Myth: silver sulfadiazine is the best treatment for minor burns. West. J. Med. 2001; 175, 205–6.
22. Delivery, T.D., Drug, C., View, D., Ethosomes -A noninvasive vesicular carrier for transdermal drug delivery. 2016.
23. Demling, R.H., Leslie DeSanti, M.D., The rate of re-epithelialization across meshed skin grafts is increased with exposure to silver. Burns. 2002; 28, 264–266.