FORMULATION AND EVALUATION OF SULFADIAZINE LOADED SILVER NANOPARTICLES

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT

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MASTER OF PHARMACY

IN

PHARMACEUTICS

By

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MAY, 2017

DEDICATED TO MY LOVING PARENTS, CARING FRIENDS AND INSPIRATIONAL FACULTY MEMBERS WITHOUT WHOM THIS WORK COULD NOT HAVE BEEN POSSIBLE.

PAC APPROVAL FORM

TOPIC APPROVAL PERFORMA

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Sr.No.	**Parameter**	**Rating (out of 10)**
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This is to submit that this written submission in thesis entitled **"*Formulation and evaluation of Sulfadiazine loaded silver Nanoparticles*"** represents original ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the school and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

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The work described in this project report entitled **"*Formulation and evaluation of Sulfadiazine loaded Silver Nanoparticles*"** has been carried out by **Chandra Prakash Jha, Reg.No.11501879** under my supervision. I certify that this is his bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

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Chandra Prakash Jha

LIST OF ABBREVIATIONS

S.No	Abbreviation	:	Full form
1.	AEM	:	Aqueous extract of *Aegle marmelos*
2.	AgNPs	:	Silver nanoparticles
3.	COA	:	Certificate of analysis
4.	DNA	:	Deoxy Ribo Nucleic acid
5.	DSC	:	Differential scanning calorimetry
6.	E.g.	:	Example
7.	F	:	Fahrenheit
8.	fcc	:	Face centered cubic
9.	FTIR	:	Fourier transmission infrared
10.	g	:	Grams
11.	GIT	:	Gastrointestinal tract
12.	hr	:	Hour
13.	IP	:	Indian pharmacopoeia
14.	Kg	:	Kilogram
15.	L	:	Litre
16.	M	:	Molar
17.	mg	:	Milligram
18.	MIC	:	Minimum inhibitory concentration
19.	ml	:	Millilitre
20.	mm	:	Millimetre
21.	mM	:	Millimolar
22.	MS	:	Mass spectroscopy
23.	N	:	Normality
24.	nm	:	Nanometre
25.	NMR	:	Nuclear magnetic resonance spectroscopy

26.	NPs	:	Nanoparticles
27.	SAED	:	Selected area electron diffraction pattern
28.	SDZ	:	Silver Sulfadiazine
29.	SPR	:	Surface Plasmon Resonance
30.	TEM	:	Transmission Electron Microscopy
31.	ZOI	:	Zone of Inhibition

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

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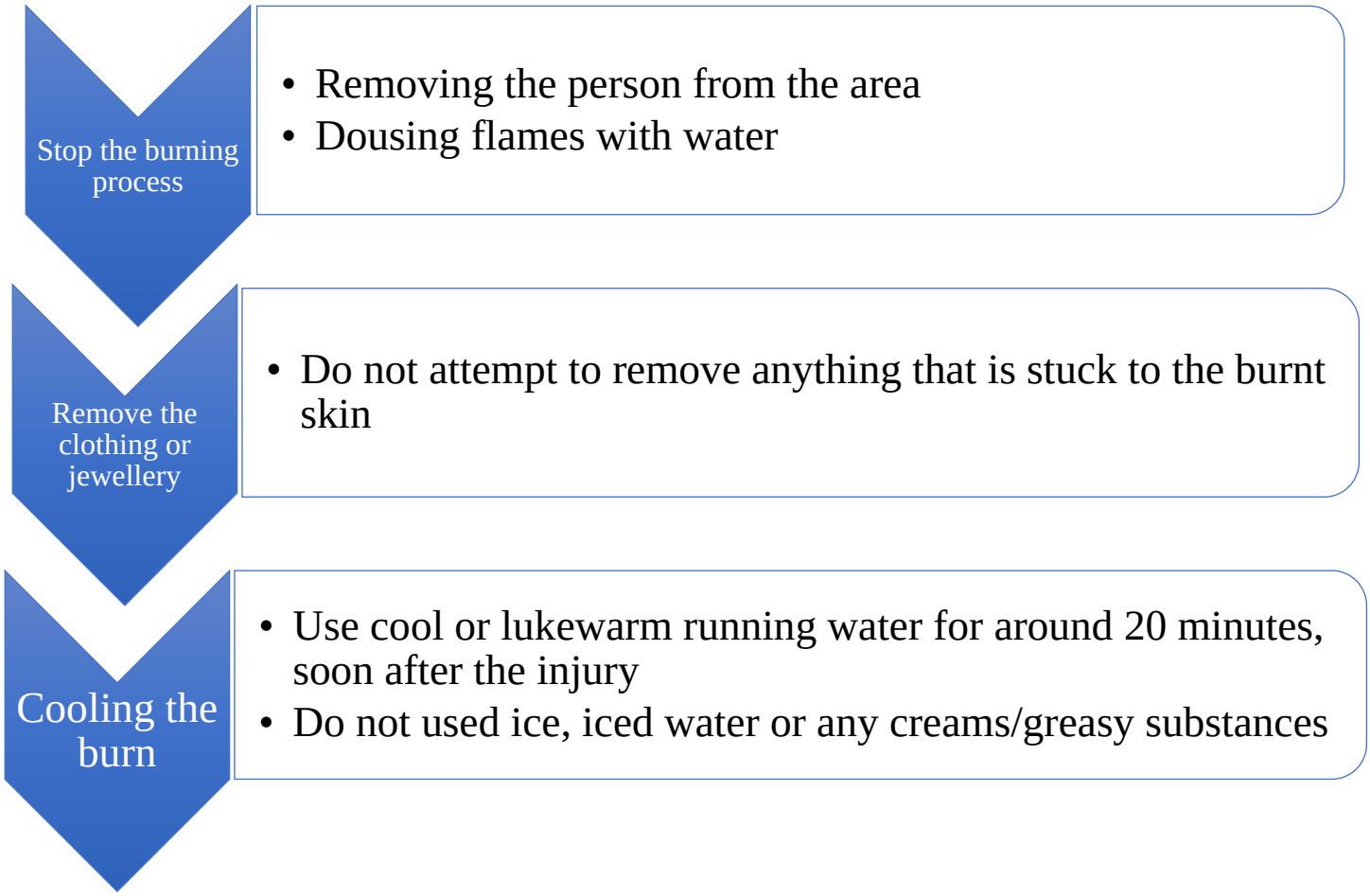
CHAPTER 1

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)**.



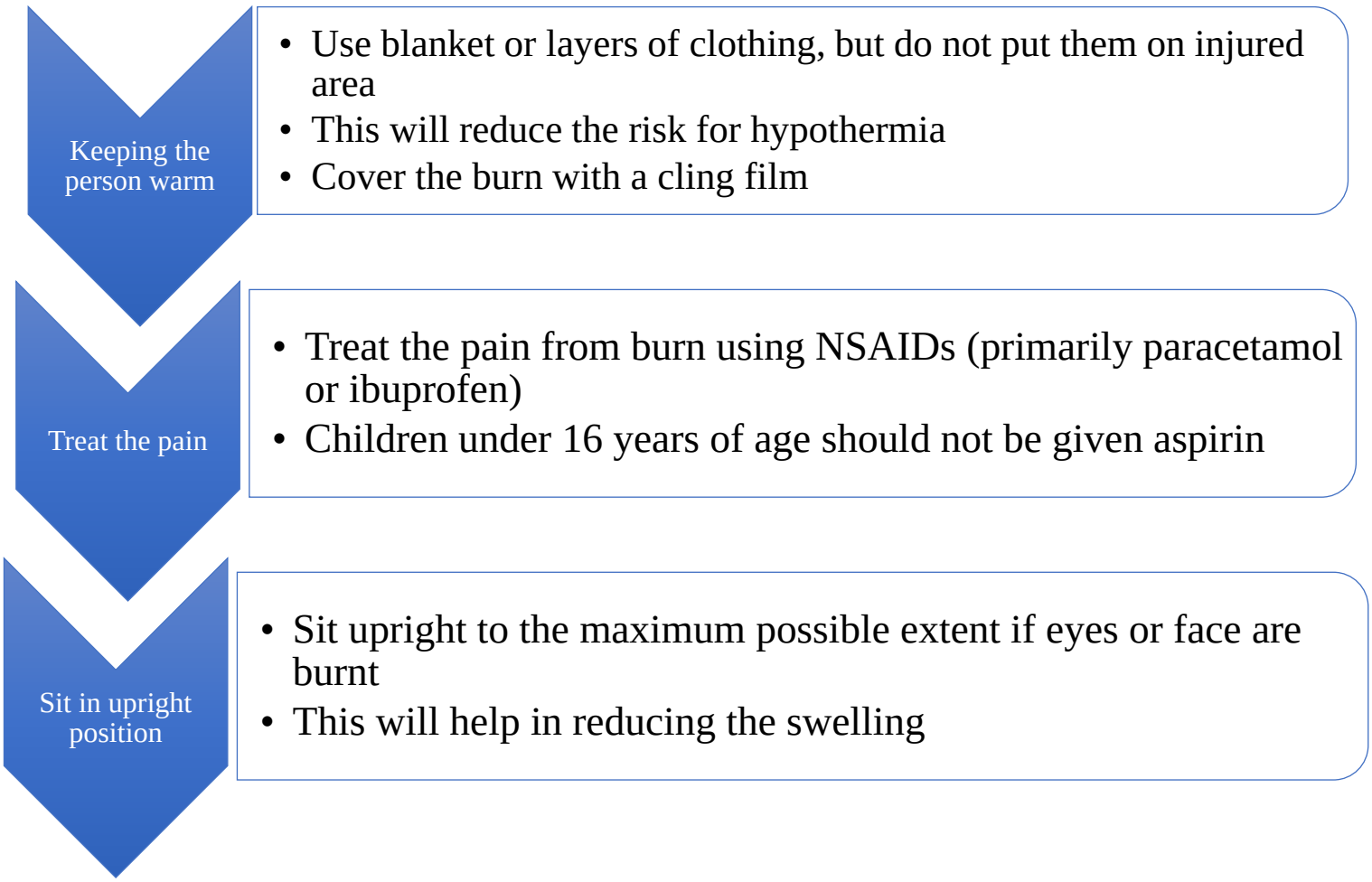


Fig.1.1. Management of Burn

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.

TERMINOLOGY

CHAPTER 2

TERMINOLOGY

1) Calibration

The set of operations that establish, under specified conditions, the relationship between values indicated by an instrument or system for measuring (for example, weight, temperature and pH), recording, and controlling, or the values represented by a material measure, and the corresponding known values of a reference standard.

2) Surface Plasmon resonance

Surface Plasmon Resonance (SPR) is a physical process that can occur when plane-polarized light hits a thin metal film under total internal reflection conditions.

3) Nanoparticles

A nanoparticle (or Nano powder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm.

4) Parts per million

Ppm is an abbreviation of parts per million. Ppm is a value that represents the part of a whole number in units of 1/1000000. Ppm is dimensionless quantity, a ratio of 2 quantities of the same unit.

5) Antimicrobial

An antimicrobial is any substance of natural, semisynthetic or synthetic origin that kills or inhibits the growth of microorganisms but causes little or no damage to the host.

6) Green Chemistry

Green chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products

LITERATURE REVIEW

CHAPTER 3

LITERATURE REVIEW

3.1 Introduction to silver and its nanoparticles

3.1.1 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.**(Chopra**, **2007**) 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.(**Demling and Leslie DeSanti, 2002; Fraise et al., 2008**). 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.(**Chopra, 2007**) 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. **(Chopra, 2007; Moyer et al., 1965**) . 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.(**George and Faoagali, 1997; Modak and Fox, 1981)**.

3.1.2 Mechanism of action of silver

The mechanism behind the antimicrobial action of silver ions is closely related to their interaction with thiol (Sulfahydryl) groups.**(Jung et al., 2008,Furr et al., 1994,Bragg and Rainnie, 1974)** Although the other target sites remain a possibility **(Thurman et al., 1989,Richards et al., 1984)**. Amino acids and other compounds containing thiol group such as cysteine and sodium thioglycolate neutralised the activity of silver against bacteria **(Liau et al., 1997**). In contrast, Amino acids containing disulphide bonds, non –sulphur containing amino acids, and sulphur containing compounds such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfate, were all unable to neutralize. All this findings implied that the interaction of silver ions with thiol groups in enzymes and proteins play an essential role in its antimicrobial action.(**Furr et al., 1994**). Silver is also proposed to be acting by

binding to the key functional groups of enzymes. Silver ions cause the release of potassium ions from the bacteria; thus bacterial plasma or their cytoplasmic membrane which is associated with many enzymes is an important target for silver ions **(Jung et al., 2008; Schreurs and Rosenberg, 1982**).

Silver ions showed marked inhibition of bacterial growth and were deposited in the vacuole and cell wall as granules in addition to their effects on bacterial enzymes.**(Brown, T., 1976**). They inhibited cell division and damaged the cell envelope and contents of bacteria (**Richards et al., 1984)**.Finally, silver ions interact with nucleic acids; they interact preferentially with the bases in DNA rather than with the phosphate groups, although the significance if this in terms of their lethal action is unclear**(Rahn and Landry, 1973; Thurman et al., 1989; Zavriev et al., 1979)**.

3.1.3 Silver Nanoparticles

Nanoscience has been established recently as a new interdisciplinary science. It is considered as a defined knowledge on basic fundamental properties of nano-size objects **(Sergeev and Shabatina, 2008; Sergeev, 2003**). The prefix "nano" indicates one billionth or 10^{-9} units. The nature of this unit being determined by the word that follows. It is widely accepted that nanoparticles are clusters of atoms in the size range of 1-100nm (**Williams, 2008**). They habitually show unique and considerably changed or modified physical, chemical and biological properties compared to their macro scaled counterparts. (**Li et al., 2001**). Gold, silver and copper have been mostly used for the synthesis of stable dispersions of nanoparticles, which are useful in multiple fields such as photography, catalysis, antimicrobial applications etc.(**Abbasi et al., 2014; Smith et al., 2006)** .

Silver nanoparticles (AgNPs) have been known to have inhibitory and bactericidal effects. Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is major health problem. (**Nanda and Saravanan, 2009)**. Several physical and chemical methods have been used for synthesising and stabilising silver nanoparticles.

Though the synthesis of silver nanoparticles has been carried out by number of methods such those based on chemical reduction in solution, chemical and photochemical reactions **(Henglein, 1998**), and decomposition of silver compounds by thermal method

(**Muthukrishnan and Muthukumar, 2016; Quang et al., 2013**), and microwave assisted process (**Jiang et al., 2006)**. Synthesis of silver-bionanoparticles using green synthesis i.e. by using plant extracts have been shown to be more advantageous due to their simple methodology and eco-friendly nature (**Kanipandian et al., 2014; Muthukrishnan et al., 2015; Singh et al., 2013)** moreover they minimises the risk of environmental toxicity as compared to those of conventional chemical synthesis methods. (**Verma et al., 2015**)

3.2 Green synthesis of silver-bionanoparticles

The process development of rapid and eco-friendly methods for the synthesis of silver nanoparticles is of great importance in the field of nanotechnology. Green synthesis is advantageous over chemical and physical methods because it is cost effective, environment friendly , easily scaled up and no use of high pressure, temperature, energy and toxic chemicals(**Ahmed and Ikram, 2015; Jain et al., 2009; Lokina, 2011**). Green chemistry utilises non-toxic plant extracts and environment friendly solvent water for synthesising nanoparticles.(**Lokina, 2011**)

3.2.1 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(**Sharma et al., 2011**) .

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.

3.2.2 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**).

3.2.3 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

3.3 *Aegle Marmelos* plant profile

Fig.3.1 : A: Leaves of *Aegle marmelos*; B: Flowering Bud; C: Bael fruit

Table 3.1 Description of plants physicochemical properties

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

	Order: Sapindales **Family:** Rutaceae **Genus:** Aegle **Species:** marmelos
Part used	Fruit, root, bark, seeds, leaves, flowers
Medicinal Properties	Antibilious, antiparasitical, antipyretic, aphrodisiac, aromatic, alternative, astringent, digestive stimulant, febrifuge, hemostatic, laxative, nutritive, stomachic, stimulant, tonic. Fruits: cooling and laxative.
Cultivation	NA
Regional Habitat	Bael is suitable for dry forests on hills and plains of Rajasthan, though it is found throughout the country.
Description	A spinous, deciduous, aromatic tree, spines, straight, strong, axillary. It grows up to 18 meters tall and bears long thorns. Leaves: usually 3-foliolate, sometimes 5-foliolate; leaflets ovate-lanceolate, lateral sessile, terminal long-petioled. Flowers: borne in few-flowered, axillary panicles, and greenish-white, sweet-scented. Fruits: large, upto 15 cm diameter, globose, ovoid or pyriform, 8-15 celled, rind grey or greyish-yellow, woody, pulp orange, sweet. Seeds: numerous in aromatic pulp, oblong, compressed, testa woolly and mucilaginous.

3.4 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

2D Structure

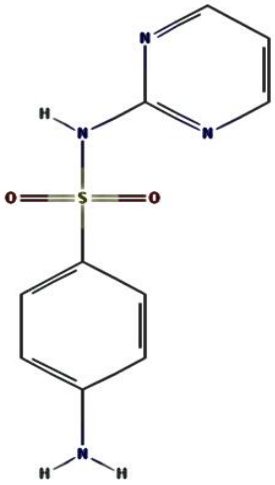


Fig.3.2. 2D structure of Sulfadiazine

3D Conformer

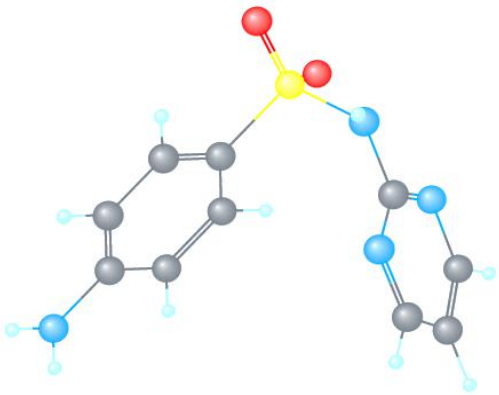


Fig.3.3. 3D conformer of Sulfadiazine

3.4.1 Pharmacology of Sulfadiazine

Sulfadiazine is a sulfonamide antibiotic. The sulfonamides are synthetic bacteriostatic antibiotics with a wide spectrum against most gram-positive and many gram-negative organisms. However, many strains of an individual species may be resistant. Sulfonamides inhibit multiplication of bacteria by acting as competitive inhibitors of *p*-aminobenzoic acid in the folic acid metabolism cycle. Bacterial sensitivity is the same for the various

sulfonamides, and resistance to one sulfonamide indicates resistance to all. Most sulfonamides are readily absorbed orally. However, parenteral administration is difficult, since the soluble sulfonamide salts are highly alkaline and irritating to the tissues. The sulfonamides are widely distributed throughout all tissues. High levels are achieved in pleural, peritoneal, synovial, and ocular fluids. Although these drugs are no longer used to treat meningitis, CSF levels are high in meningeal infections. Their antibacterial action is inhibited by pus.

3.4.2 Physical Description

Property Name	Property Value
Physical Description	Solid
Melting Point	255.5 ºC

3.4.3 Physical Property

Property Name	Property Value
Solubilty	Water Solubilty – 77mg/L (at 25ºC)
LogP	-0.009 (HANSCH,C ET AL. (1995)
LogS	-3.51 (ADME Research, USCD)

3.4.4 Chemical and Physical Properties

Computed Properties

Property Name	Property Value
Molecular Weight	250.276 g/mol
IUPAC Name	4-amino-N-pyrimidin-2-ylbenzenesulfonamide
Hydrogen Bond Donor Count	2
Hydrogen Bond Acceptor Count	6

Rotatable Bond Count	3
Monoisotopic Mass	250.052 g/mol
Exact Mass	250.052 g/mol
Molecular Formula	C10H10N4O2S

3.4.5 Table 3.2 Clinical Trials on sulfadiazine and their Status

Update date	Cid	Ctid	Title	Phase	Status
2016-09-28	5215	NCT01189448	Multicenter, Randomized Clinical Trial to Compare the Efficacy and Tolerance of Prenatal Therapy With Pyrimethamine + Sulfadiazine vs Spiramycine to Reduce Vertical Transmission of Toxoplasma Gondii Following Primary Infection in Pregnancy	3	Completed
2016-08-22	5215	NCT02124512	Dietary Fat, Lipoprotein and Lipopolysaccharide: Role in Insulin Resistance	2	Recruiting
2016-03-02	5215	NCT01904409	A Randomized, Double-blind, Placebo-controlled, Dose-ranging, Multicenter Study to Assess the Efficacy and Safety of Rifaximin Soluble Solid Dispersion (SDZ) Tablets for the Prevention of Complications in Subjects With Early Decompensated Liver Cirrhosis	2	Completed
2016-01-25	5215	NCT02108535	Comparative Analysis of Cost-effectiveness of Silver Dressing in Burns	4	Completed

2015-07-23	5215	NCT02116010	Phase I/II Clinical Trial Randomized, Multicentric, Open Label, Standard of Care (Silver Sulfadiazine) Controlled Aiming at Assessing Tolerance and Efficacy of Local Bacteriophage Treatment of Wound Infections Due to E. Coli or P. Aeruginosa in Burned Patients Using Pherecydes Pharma Anti-Escherichia Coli and Anti-Pseudomonas Aeruginosa Bacteriophages GMP Produced Cocktails . This Project is a European Research & Development (R&D) Project Funded by the European Commission Under the 7th Framework Pro	2	Recruiting
2014-09-26	5215	NCT01439074	An Open, Randomized, Comparative, Multi-centre Investigation Evaluating the Efficacy and Tolerance of Mepilex Ag Versus Silver Sulfadiazine in the Treatment of Deep Partial Thickness Burn Injuries.	4	Completed
2014-04-09	5215	NCT02109718	A Randomized Controlled Clinical Trial Comparing The Efficacy and Safety of Open Dressing With Petrolatum Jelly vs. Standard Gauze Dressing With Silver Sulfadiazine in the Treatment of Filipino Adults Aged 18-45 Years Old With Superficial Partial Thickness Burns Less Than or Equal to 10% Total Surface Area Who	3	Completed

			Are Seen at the Philippine General Hospital Burn Outpatient Clinic		
2013-08-20	5215	NCT01926392	A Prospective Randomized Trial Comparing Silver Sulfadiazine Cream to a Water-Soluble Poly-Antimicrobial Gel in Partial Thickness Burn Wounds	4	Completed
2013-03-19	5215	NCT01553708	The Clinical Efficacy of Epidermal Growth Factor With Silver Sulfadiazine Cream Compared With Silver Zinc Sulfadiazine Cream on Acceleration of Partial Thickness Burn Wound Healing	3	Completed
2012-04-02	5215	NCT00000794	Phase II Randomized Open-Label Trial of Atovaquone Plus Pyrimethamine and Atovaquone Plus Sulfadiazine for the Treatment of Acute Toxoplasmic Encephalitis	2	Completed
2009-08-07	5215	NCT00156988	The Effect of Two Versus Ten Days Application of Flammacerium in Partial Thickness Burns	4	Completed

3.5 Literature review on Silver and Silver Nanoparticles

Liu et al., 2007 prepared silver nanoparticles using gas-solution reaction of silver nitrate solution and ammonia gas. The obtained average sizes of nanoparticles were about 10nm and spherical shape. Quenching of silver nanoparticles fluorescence was done by adding *ct*-DNA, which provided a simple and rapid spectro-fluorimetric method for the determination of *ct*-DNA

Chopra et al., 2007 reviewed that there is an increased need for silver MIC levels and breakpoints to be developed and standardised. Though silver resistance has been documented but current evidences shows that clinical threat is low. Genetic linkage of silver resistance genes and antibiotics resistance genes has been documented in the context of plasmid-mediated silver resistance. Silver-based dressings are effective alternative to antibiotics in the management of wound infection, though clinicians should choose dressings that release high levels of silver ions and that should demonstrate rapid bactericidal activity.

Lok et al., 2007 tested physicochemical properties of nano-silver synthesised by reduction using borohydride reduction methods. The nano-Ag is delicate to oxygen and just in part oxidized nano-Ag show antibacterial activities. The development of Ag^{+} on the surface of the nanoparticles was uncovered by trademark changes in SPR retention amid oxidation and lessening. The plausibility for a higher level of disintegration of nano-Ag to free Ag+ in a more unpredictable medium with adequate chelating properties can't be barred.

Jain et al., 2009 synthesised and evaluated plant mediated silver nanoparticles using papaya fruit extract for its anti-microbial activities. The reduction of metals through leaf extracts led to formation of well-defined dimensions. The advantages of using green synthesis are ease with which the process can be scaled up, economic viability, No toxicity, Wound healing etc.

Bar et al., 2009 developed a green method for synthesis of silver nanoparticles using the aqueous extract of *Jatropha curcas*. The seeds of Jatropha act as both reducing and stabilising agent. Obtained nanoparticles were spherical in shape. The prepared nanoparticles were quiet stable and remained intact for nearly two months if they were protected under light proof conditions.

Sadeghi et al., 2010 reviewed that AgNPs show strong antibacterial activity. They inhibit growth of wide variety of fungi and bacteria. Also the study showed that AgNPs require a lower concentration to inhibit the development and growth of bacterial strains which is probably due to increasing surface area in AgNPs.

Moulton et al., 2010 synthesised Silver nanoparticles using epicatechin or tea leaf extract as a reducing as well as capping agent which were not produced by benign process but that these particles were non-toxic. In several cases these nanoparticles created a good response, which is more likely due to presence of antioxidants on the surface of the nanoparticles. Though these methods needed *in vivo* studies before saying that they are biocompatible but *in vitro* studies showed to have promising effects.

Rastogi and Arunachalam, 2011 developed a completely green and fast method for synthesising silver nanoparticles using aqueous garlic extract and sunlight. As compared to other reported studies it has fast reduction kinetics. Light was being used as constructive agent for the synthesis of silver nanoparticles and they were found to be stable at room temperature for more than a year. AgNPs were found to have equal bactericidal effect against both gram positive and gram negative bacterial strains.

Krishnamoorthy and Jayalakshmi, 2012 synthesised and evaluated AgNPs using phyllanthusniruri. It was found that the leaf extract was suitable for the synthesis of silver nanoparticles as they resulted into the formation of stable nanoparticles with spherical and cubic morphologies which range from 32-53nm size. The physicochemical characterisation (UV-vis, SEM etc.) was useful in proving the formation of AgNPs.

Rao and Paria, 2012 synthesised Silver nanoparticles using *Aegle marmelos* leaf extracts. The formed nanoparticles were of approximately 60 nm. Kinetics of SNPs formation showed faster reduction reaction and subsequent particle growth. Furthermore the polyphenols present in the leaf extracts are crucial for nanoparticles formation and capping by adsorbing on the surface of the formed nanoparticles. The novelty of this study was its simplicity and instantaneous synthesis of capped AgNPs with uniform size without use of any capping agent.

Ahmad and Sharma, 2012 synthesised silver nanoparticles of average size approximately 10-12 nm using pineapple Juice. Probably the biomolecules responsible for the reduction and stabilisation of AgNPs are phenols. Therefore use of natural anti-oxidants for the synthesis of AgNps seems to be a good alternative.

Van Dong et al., 2012 investigated variety of chemical synthesis methods for the preparation of silver nanoparticle solutions. Special attention was paid to modification of nanoparticles average size as well as shape distribution. Zone inhibition test for antibacterial activity showed that triangular silver nanoprisms had greater antibacterial activity as compared with spherical silver nanoparticles due to their geometric structures and crystal planes. In fabrication of antibacterial materials Silver nanostructures are of great interest.

.**Umadevi et al., 2013** synthesised silver nanostructures using green nano chemistry methodology, avoiding the presence of hazardous and non-toxic solvents using low cost biological reducing agent *S.lycopersicums* fruit extract. Spherical shape silver nanoparticles were prepared which were predicted from UV spectrophotometer, SEM and TEM measurements.

Ghanbarzadeh and Arami, 2013 reviewed AgNPs as one of the most attractive nanomaterials for commercial applications. They have been broadly used for antimicrobial, electronic and biomedical products. The focus was on disinfectant ability of Ag-NPs with respect to environmental treatments. A new class of nanosilver-containing disinfectant nano products will be promising for advanced environmental treatments including air disinfection, water disinfection, surface disinfection and personal hygiene which will further help in preventing outbreak of diseases.

Isaac et al., 2013 demonstrated an approach which was low cost and used One-pot green synthesis for preparation of stable sold and silver nanoparticles. Synthesis was done using *Averrhoa bilimbi Linn*.fruit extract as a reducing agent. Average nanoparticles were having the diameter of 50-150nm. This method eluded the use of toxic chemicals for the synthesis of nanoparticles and has various biological applications. This approach is cost effective and carried unique physical properties, chemical reactivity and potential applications in catalysis,

biological labelling, drug delivery, biosensing antibacterial and antiviral activity, gene therapy, detection of genetic disorders and DNA sequencing.

Geetha et al.,2014 synthesised silver nanoparticles using *Azadirachta indica, Tridax procumbens* and *Aegle marmelos leaves* against wound infection causing bacterial pathogens. AgNPs obtained from them varied in their antibacterial activity against pathogens due to varied concentrations of Ag in the nanoparticles. Study showed that biological synthesised silver nanoparticles could be of immense use in the medical field for their efficient antimicrobial function.

Ghaffari-Moghaddam and Hadi-Dabanlou, 2014 synthesised AgNPs using *C.douglassi* fruit. Biosynthesis of AgNPs using green resources like *C.douglassi* was a good method over chemical synthesis as they are eco-friendly methods. Nanoparticles of nearly spherical shape were obtained. It also showed anti-bacterial activity on both Gram-Positive and Gram – negative bacteria and has vast applications in pharmaceutical industry.

Krupa and Raghavan, 2014 synthesised AgNPs from aqueous fruit extract of *Aegle marmelos*. Synthesised nanoparticles were observed for their ability to control/prevent the biofilm forming bacterial communities by conducting antimicrofouling studies. The formed NPs were found to control the growth and survival of biofilm forming bacteria. Thus, the present work gave a scope for the possible development of formulations containing AgNPs as effective antifouling agent thereby preventing marine biofouling.

Ahmed and Ikram, 2015 synthesised and evaluated One-pot green synthesis of AgNPs using *Terminalia arjuna* plant extract. He reported that AgNPs has been successfully synthesized using this simple, fast, cost effective and environment friendly method. The synthesised nanoparticles showed efficient antimicrobial activities and it has a potential us in medical applications.

Verma et al., 2015 showed a very simple green chemistry approach for the synthesis of AgNPs using *Tamarindus indica* leaf extract. This process was photocatalytic, swift, economic and eco-friendly in nature for large scale silver nanoparticles. Leaf extract was alone capable to provide both bioreductant and stabilizer required for nanoparticle synthesis. The formation of AgNPs was observed by change in colour of reaction mixture from light

green to dark reddish brown which was confirmed by UV-vis spectroscopy. Anti-microbial studies showed that AgNPs were more effective against Gram Negative bacteria as compared to Gram positive bacteria.

Muthukrishnan et al., 2015 synthesised silver nanoparticles using H.isora root extract. H.isora root contains more triterpenes that play major roles as reducing as well as capping agent for use in synthesis of AgNPs. The extract acts as both reducing and stabilising agent which was confirmed by the FTIR studies. The average particle size was found to be 30-40nm. Biosynthesised AgNPs were found to be having multifunctional activities with good antioxidant property. Thus this method is a good alternative for both chemical and other physical methods.

Rao and Paria, 2015 synthesised gold and silver nanoparticles using different plant surfactants and *Aegle marmelos* leaf extract as reducing agents. Surfactants helped in further reduction of size of nanoparticles. Taguchi method was used for the synthesis and particle size (~13nm) was obtained. AgNPs SPR mostly lied in the visible region.

3.6 Literature review on Sulfadiazine

Coward et al., 1973 studied the effect of silver sulfadiazine on growth and ultrastructure of *staphylococci* the reports showed that all the strains tested were readily inhibited by levels of silver sulfadiazine and this seems plausible that SDZ might be useful not only in the prevention and treatment of burn infections but also in the treatment of other staphylococcal manifestations.

Van et al., 1995 studied *In vitro* effects of sulfadiazine and its metabolites alone and in combination with pyrimethamine on *Toxoplasma godii* .He found that sulfadiazine inhibited *T.gondii* growth in vitro at high concentrations. The study also revealed that hydroxylated metabolites of sulfadiazine possess antimicrobial activity against *T.gondii*. Though the presence of high concentration of active metabolites may have consequences for the efficacy of treatment, therefore extrapolation from animal studies to human situation concerning the pharmacokinetic and efficacy of sulfadiazine should be done with care.

Gear et al., 1997 formulated a new water soluble silver sulfadiazine water soluble gel which had advantage over commercially available silver sulfadiazine (SDZ) creams. This new gel was easy to remove as the conventional SDZ creams formed pseudo-eschar which was difficult to remove from the wound surface. On the basis of this investigation it suggested clinical trials for SDZ gels.

George and Faoagali, 1997 examined the in-vitro efficacy of Silvazine^{TM} (Silver Sulfadiazine and chlorhexidine) against organisms commonly found in burn wounds. The test was conducted on 200 non-replicative sequential clinical isolates. All organisms showed zones of inhibition. There was no bacterial regrowth within the zones of inhibition. Thus *in vitro* testing of Sivazine^{TM} confirmed its efficacy towards treatment of burns.

Venkataraman and Nagarsenker, 2013 prepared stable silver sulfadiazine nano suspensions in a combination of hydrophilic and hydrophobic stabilisers, cremophor EL and Lauroglycol 90, using micro-precipitation homogenisation technique. SDZ nanosystems exhibited improved antibacterial activity against pathogens commonly invading burn wounds. The formulation also showed better results in comparison to that of SDZ marketed cream in hot water induced burn wounds in female Sprague dawley rats. It showed good potential for faster burn wound healing which reduced trauma of the patient. Thus, SDZ nano gels and

nanosuspensions proved to be promising systems to provide relief to patients suffering from large-surface area burns.

Dharashivkar et al., 2015 formulated a new SDZ niosomal gel using carbopol 934 as the gelling agent. The gel appeared to have distinct advantages over the commercially available SDZ cream. The SDZ niosomal gel showed improved antibacterial activity against *S.aureus*, which is commonly invading burn pathogens, thus will reduce the trauma of the patients suffering from large surface area burn wounds.

Dumitriu et al., 2015 synthesised Sulfadiazine-chitosan conjugates destined to the management of burn wounds. Chitosan was functionalized with Sulfadiazine, enhancing its bacteriostatic effect and subsequently the ability to promote wound healing. The prepared PECs demonstrated their antimicrobial efficiency against *E.coli, L.monocytogenes* and *S.thyphymurium.* The obtained results showed that PECs prepared from SDZ-modified chitosan represent an attractive alternative as efficient systems for prolonged drug delivery with enhanced bacteriostatic effect.

Pires et al., 2015 developed novel 5-FU-SUL-PLGA NPs that provided efficient delivery of 5-Flurouracil to Caco-2 and A431cancer cells. Moreover functionalisation of sulfadiazine did not cause morphological or size differences in the particles, nor did it change the 5-FU solubility in polymer core. The presence of SUL enhanced the cytotoxicity of 5-FU-SUL-PLGA NPs as compared to 5-FU-PLGA NPs and had no or minimal effect on normal cells.

Wen et al., 2015 investigated in vitro and in vivo effects of bacterial cellulose (BC) dressing containing uniform silver sulfadiazine nanoparticles for burn wound healing. Well dispersed SDZ nanoparticles with narrow size distribution were procured and impregnation of SDZ into the bacterial cellulose had no effect on the overall 3D nanofibril network of BC. Application of BC-SDZ composite through disc diffusion method showed effective antimicrobial activities against *S.aureus, P.aeruginosa* and *E.coli*. The in vivo analysis showed BC-SDZ composite membrane prevented bacterial infections. The wounds treated with BC-SDZ showed faster rate of healing and faster onset on re-epithelization compared to control according to photographic and histological observations.

Chaud et al., 2015 developed and optimised a hydrogel impregnated with silver sulfadiazine aimed for antimicrobial topical applications. The prepared hydrogel displayed good characteristics in relation to release of the active antimicrobial principle, verified using swelling tests, kinetics of release and antimicrobial activity. Since the release of SDZ occurred by erosion of the hydrogel's polymeric chain, it can be utilised in occlusive wound dressings for long period of time.

RATIONALE, SCOPE OF STUDY AND FUTURE SCOPES

CHAPTER 4

SCOPE OF THE STUDY

4.1 Scope and rationale

Burn wound sepsis are a major source of morbidity and mortality in burn patients.**(Gear et al., 1997)**. Burn injury disrupts both the normal skin barrier and many of the systemic host defense mechanisms that prevent infections. The burned skin remains vulnerable to invasive microbial infections of all kinds until complete epithelial repair has occurred. Eighty percent of these infections are autogenous in origin and 20 percent is as a result of cross infections(**George and Faoagali, 1997**). Topical antimicrobial therapy remains one of the most important methods of burn wound care. The motive of topical antimicrobial therapy is to control microbial colonization and subsequent proliferation.**(Gear et al., 1997)**

Thus the need for continuous re-evaluation of therapeutic modalities utilized in modern burn centers presents a challenge in response to the ever-increasing demands for cost containment without compromising the quality of center rendered to our patients.(**Gerding et al., 1988**). The use of silver in the wound management and treat infection is one of the earliest forms of wound care, documented as early as 69 BC (**Murphy and Evans, 2012**) as silver does have such a favorable broad –spectrum activity, especially is antibacterioresistant organisms.. In the year 1960s, AgNO3 solutions were used in effective burn management. **(Moyer et al., 1965)**.However in 1968 AgNO3 was combined with sulphonamide antibiotic sulphadiazine to produce Silver Sulfadiazine (SDZ) cream. SDZ serves as reservoir of silver in the wound and slowly liberates silver ions. All kinds of sulfa drugs have been tested in combination with silver but sulphadiazine was found to be most effective. **(C. Caro, P. M. Castillo, R. Klippstein, D. Pozo, 2010**).

The Complex prepared will be used as Anti-fungal, Anti-Microbial in cases Burn and Urinary tract infections as their will be mutual synergistic effect of both Silver and sulfadiazine and usage of bael extract will enhance activity towards the treatment and management of burn wound and also it will be advantageous as it is easy to prepare, is a non-invasive technique and better therapeutic outcome.

Future scope of prepared silver sulfadiazine complex

In the last on decade and half decades, the field of drug delivery has seen tremendous development marked by the appearance of new formulations and technologies. The two major bottlenecks for the successful drug delivery are sub-optimal physicochemical properties of drugs and the physiological barriers present in the body, like skin and membrane lining of various organs of body **(Mbah et al., 2014)**. It is well known that the nature of active pharmaceutical ingredient (API) and its bioavailability at the site of action could influence the therapeutic efficacy of the delivery systems. Drugs with poor solubility in biological fluid and poor permeability through physiological barriers are not able to exhibit their complete efficacious potential. In the past, several approaches have been reported to solve the problems related to solubility and permeation of the drugs. Among them, particle Size reduction, Formation of metastable polymorphs, Prodrugs, Solid Dispersions , Complexation of drug with hydrophilic carriers **(Garg et al., 2016)**, have proven to be useful. Emergence of nanotechnology has been able to solve some solubility related issues of drugs. Nanoparticles have been found to enable the drug to cross various biological barriers like those of eye, nose, urinary bladder and even brain.**(Attama et al., 2009)** Through the use of biodegradable polymers, successful controlled delivery of macromolecules like proteins, peptides, genes, antigens, growth factors and vaccines has also been reported **(Mundargi et al., 2008)**

Use of lipid vesicles for transdermal drug delivery is known to enhance the penetration of both hydrophilic as well as lipophilic drugs, mask the effect of lipophilicity or hydrophilicity of drug on skin permeation, and act as drug carrier to provide sustained as well as controlled drug delivery of drugs **(Honeywell-Nguyen and Bouwstra, 2005; Redziniak, 2003)**. These vesicles are made up of biodegradable phospholipids**(Garg et al., 2016)**. Bangham et al in 1965 first reported liposomes while working on phospholipids **(Bangham et al., 1965; Garg et al., 2016)**.

Both liposomes and niosomes are not able to completely penetrate deep into skin and a large fraction is generally retained in upper layers of stratum corneum on topical application **(Kajimoto et al., 2011)** This is attributed to the inherent rigidity of both these conventional vesicles **(Elsayed et al., 2007)**. Their vesicle size and other physicochemical properties affect their penetration into the skin.

The quest for bilayer vesicular drug delivery system which could surpass the membrane and provide transdermal drug delivery, led to the development of elastic nanovesicular (ENV) systems like transferosomes **(Cevc and Blume, 1992)**and ethosomes**(Verma et al., 2003)**. ENV systems combine the advantages of conventional vesicular system with elasticity. ENVs are able to penetrate deep skin layers through pores in the bio membranes which are even smaller than their own size.

They offer following advantages **(Cevc, 2003; Garg et al., 2016)**

- Both hydrophilic as well as lipophilic drugs can be incorporated
- Offers enhanced permeation of drug through skin
- Are composed of biodegradable and biocompatible material
- Are stable with respect to drug leakage
- Protects the drug from external environment by encapsulation
- Act as drug depot
- Can be used to sustain the drug release into the skin
- Easy to scale up and high market potential
- Can be used for vaccination and immunisation

4.2 Ethosomes

Ethosomes form the second generation novel vesicular system, tailored for enhanced drug delivery. These were first reported in 2000 by Touitou et al. They contain high amount of ethanol (From 30-45%) along with phospholipids of highly elastic nature. Ethanol acts as a penetration enhancer**(Touitou, 2000)**. Presence of ethanol imparts negative charge to the vesicles and also decreases the vesicle size. Using this approach, lipid fluidity and cell membrane permeability has been reported to increase by interacting with polar head of lipid molecules and thereby resulting in lowering of meting point of the lipid present in stratum corneum. This in turn, fluidizes lipids in the elastic vesicle as well as those present in skin **(Ainbinder and Touitou, 2005; Garg et al., 2016)**

4.2.1 Salient Features

These vesicles are capable of penetrating through pores of size smaller than their own size. They provide sustained drug delivery and can act as a carrier for both hydrophilic and hydrophobic drugs **(Jain et al., 2014)**. These vesicles can be easily applied on skin in form of gel or ointment **(Cevc and Vierl, 2007).**

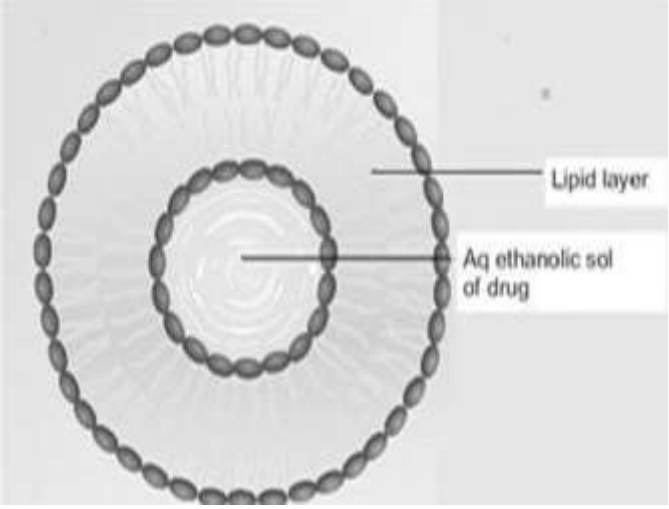


Fig.4.1. Proposed diagram of Ethosomes (Verma and Pathak, 2010)

4.2.2 Composition

Ethosomes consist mainly of phospholipids, ethanol (Upto 45 %), Glycerol and water. Different types of phospholipids like soya phosphatidylcholine, egg phosphatidylcholine, hydrogenated phosphatidylcholine etc. are used as vesicles forming agent. Ethanol plays same role as is played by surfactants in case of transferosomes by disorganising the lipid layer **(Elsayed et al., 2007; Garg et al., 2016)**

4.2.3 Mechanism of Penetration (Garg et al., 2016; Touitou, 2000)

The main advantage of using ethosomes over liposomes is the increased permeation of the drug. Though the proper mechanism behind ethosomes drug absorption is not clear, still researchers have assumed that the drug absorption probably occurs in following two phases:

1. Ethanol effect
2. Ethosome effect

4.2.3.1 Ethanol effect

Ethanol is a major ingredient and acts as a penetration enhancer. The mechanism of its penetration enhancing is well known. Ethanol interacts with lipid molecules in the polar head group region, resulting in reducing the rigidity of the stratum corneum lipids, increasing their fluidity and decreases the density of lipid multilayer of cell membrane. The intercalation of ethanol into the polar head group environment can result in an increase in the permeability of membrane. In addition to the effect of ethanol on stratum corneum structure, the ethosomes may interact with stratum corneum barrier.

4.2.3.2 Ethosomes effect

Increased cell membrane fluidity caused by the ethanol of ethosomes results in enhanced skin permeability. In the case of ethosomes encapsulating drugs, the higher positive zeta potential imparted by the drug can improve skin attachment of the vesicles. While encapsulated drug in classic liposomes remained primarily at the surface of the skin the ethosomal system showed to be highly efficient carrier for enhanced and deep drug delivery through the skin due to increased fluidity of the lipids.

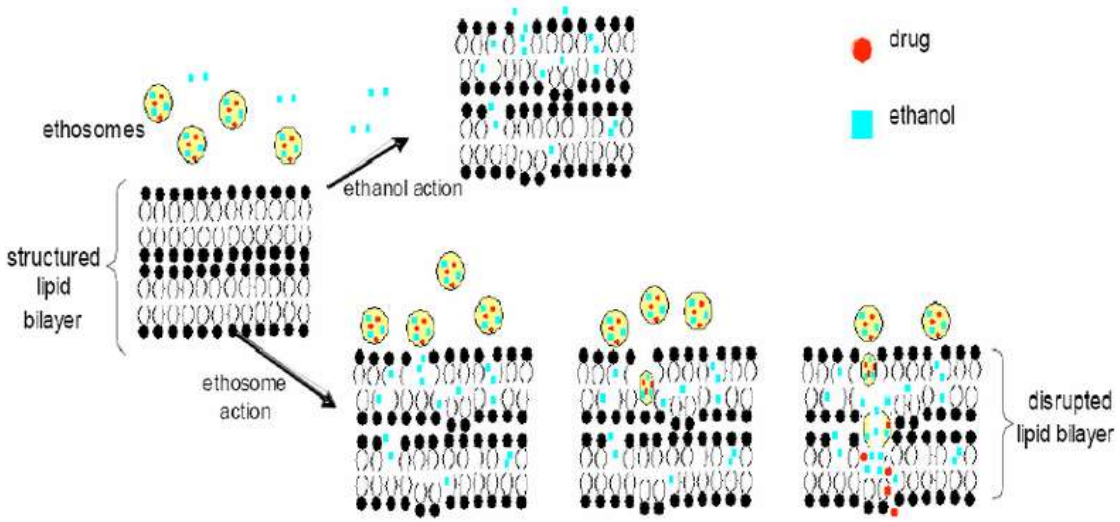


Fig.4.2 Mechanism of action of Ethosomes (Anitha et al., 2014)

4.2.4 Method of preparation

Ethosomes are prepared by following methods:

1. Hot injection method
2. Cold injection method

4.2.4.1 Hot injection method

In this method, aqueous dispersions of phospholipids are heated at 40ºC until the formation of colloidal solution. Separately, organic Solution of ethanol and propylene glycol is heated at same temperature. Organic Phase is then added to aqueous phase and drug gets solubilized in water or ethanol according to its inherent solubility properties. The vesicles so formulated are sonicated or extruded to achieve the desired size **(Garg et al., 2016; Touitou, 2000)**. The schematic flowchart of hot injection method is shown below

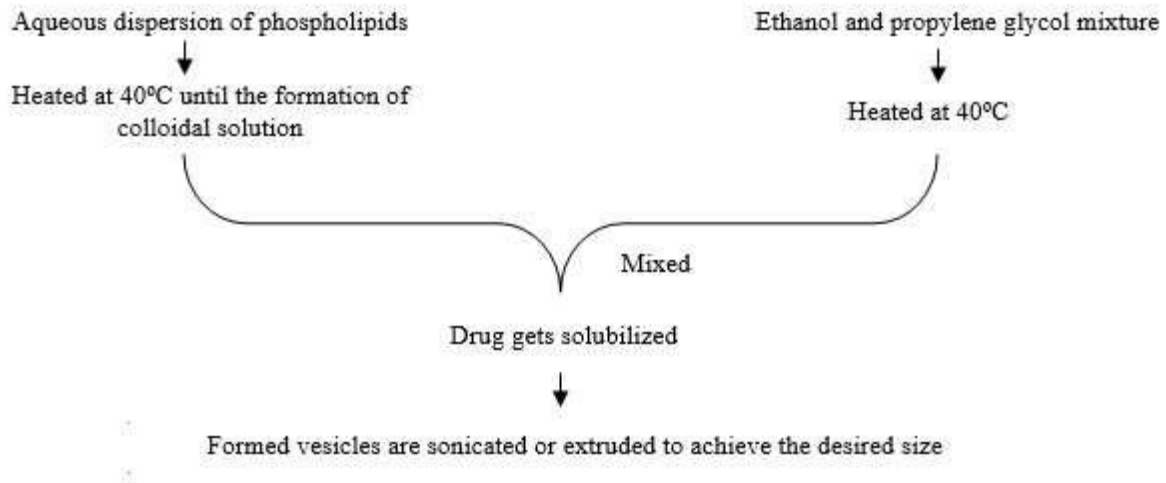


Fig. 4.3. Schematic flowchart of hot injection method (Garg et al., 2016)

4.2.4.2 Cold injection method

In this method, Ethanolic solution of lipids and drugs is prepared by vigorous stirring. To the prepared solution, polyols are added and mixture is heated at 30ºC. Preheated water is added drop wise to the mixture and stirred continuously to form uniform vesicles. Desired size of vesicles may be achieved by sonication, extrusion etc. Closed vessel should be used in the

whole process**(Chandel et al., 2012; Garg et al., 2016)**. The schematic flowchart of cold injection method is shown:

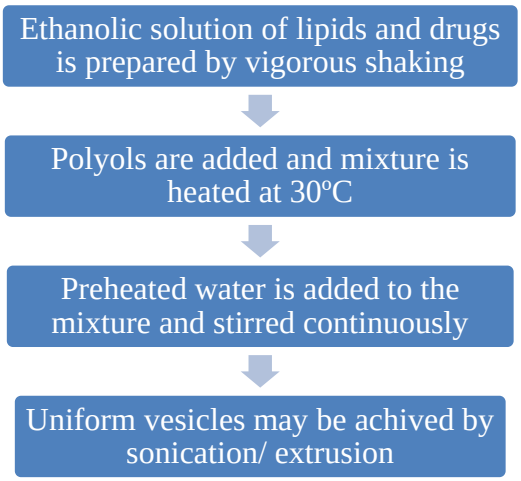


Fig. 4.4. Schematic flowchart of cold injection method (Garg et al., 2016)

AIM AND OBJECTIVE OF STUDY

CHAPTER 5

AIM AND OBJECTIVE OF STUDY

5.1 Aim of study

Formulation and evaluation of sulfadiazine loaded silver nanoparticles.

5.2 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, EQUIPMENTS AND RESEARCH METHODOLOGY

CHAPTER 6

MATERIALS AND RESEARCH METHODOLOGY

6.1. Chemicals used

Table 6.1 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

6.2. Equipment used

Table 6.2 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

6.3 Research Methodology

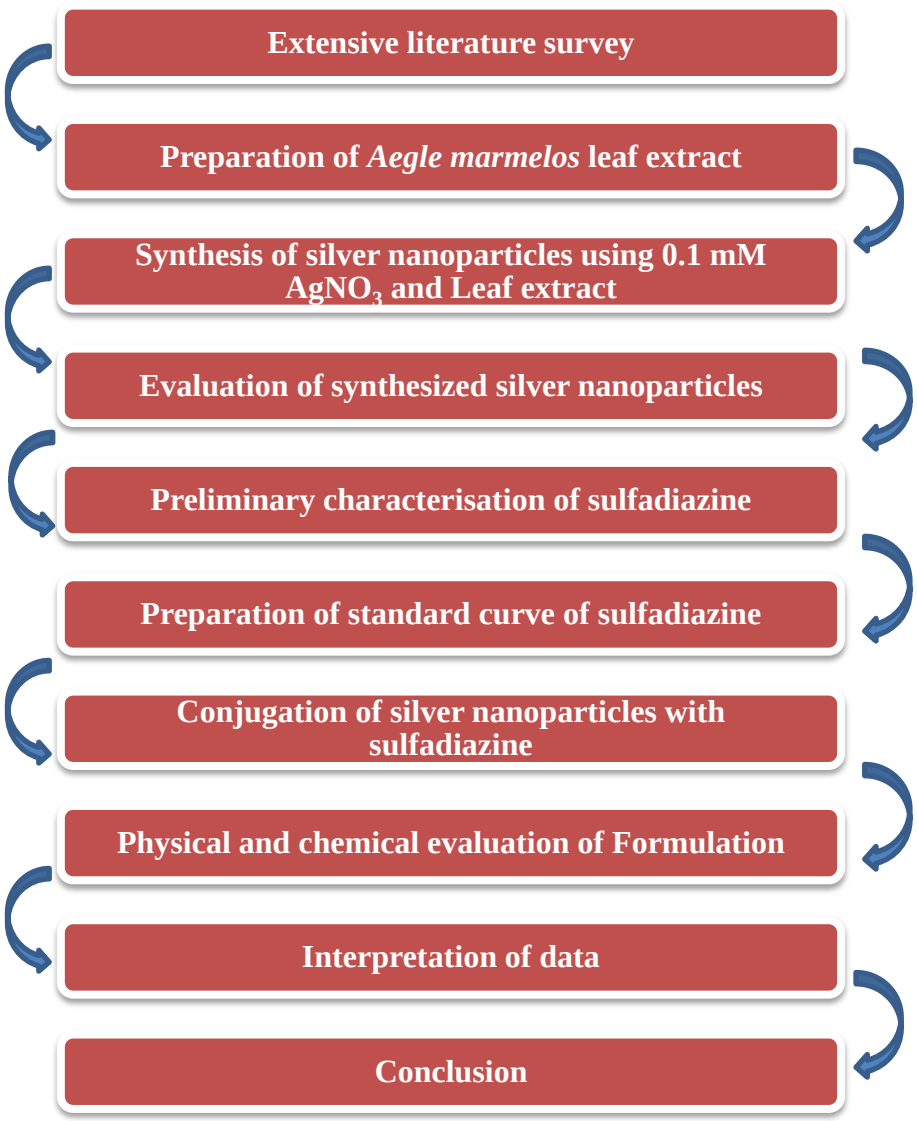


Fig.6.1. Proposed Research methodology

EXPERIMENTAL

CHAPTER 7

EXPERIMENTAL

7.1 Characterization of Sulfadiazine

7.1.1 Physical appearance test

Sulfadiazine was observed for colour, odour and physical state.

7.1.2 Melting point

Melting point of sulfadiazine was determined using capillary method. 3mm of capillary tube which was sealed at one end was filled with sulfadiazine. Capillary was introduced into the digital melting point apparatus. Melting point was noted from the temperature at which drug starts melting to the temperature at which entire sample melts.

7.1.3 FTIR spectra analysis

A FT-IR spectrum of sulfadiazine was recorded by potassium bromide (KBr) palletization method. Drug was mixed with KBr and was compressed into small thin pellet, which was subsequently analysed by FT-IR spectrophotometer. Obtained spectra were analysed for characteristic peaks corresponding to specific functional groups present in the drug molecule. These peaks were considered as a reference for further drug-excipient compatibility studies.

7.2 Analytical method development

7.2.1 Determination of absorption maxima (λ_{max}).

Sulfadiazine (10mg) was accurately weighed and transferred to a 100 ml volumetric flask. To this 0.5M Sodium Hydroxide solution poured to dissolve the drug and make up the volume Upto 100 ml. The resultant solution was having concentration of 100 µg/ml. Then they were further scanned to get the absorption maxima by using double beam UV-visible spectrophotometer.

7.2.2 Calibration plots of Sulfadiazine in 0.5M Sodium Hydroxide Solution

The calibration plot of sulfadiazine was prepared in 0.5M Sodium Hydroxide solution. From the 100µg/ml solution 02, 04, 06, 08 and 10 ml were withdrawn separately into pre calibrated volumetric flask of 10 ml volume and volume was made up to the mark by using 0.5M Sodium hydroxide solution. Absorbance was recorded at 254nm by using double beam UV spectrophotometer. All the measurements were done in triplicate and were statistically analysed. A calibration plot was constructed between concentration (µg/ml) on X- axis and

absorbance on Y- axis. The linear regression equation and linear regression coefficient were then calculated from the calibration plot.

7.2.3. Differential scanning calorimetry (DSC)

The DSC profile of pure sulfadiazine was recorded on DSC Q20 (TA Instruments, U.S.A). Thermal behaviour was studied under normal conditions with perforated and sealed quartz pans and with a nitrogen gas flow of 200ml/min. The sample was heated at 5^{o} C/min over a temperature range of 35-300^{o}C. The reference sample used for all the analysis was alumina bearing a height of 5mg. Thermograms containing peak temperatures and enthalpies were calculated with reference to reported data.

7.3. Preparation of *Aegle marmelos* leaf extract

A known weight (20 g) of freshly collected, healthy leaves of bael(*Aegle marmelos*) were washed completely in flowing tap water in the laboratory for 20 mins in order to remove the dust particles and again rinsed thoroughly in sterile distilled water. The leaf were placed in a 500ml beaker with 200ml of Double distilled water and was placed on the boiling steam bath for 25-30mins until the colour of the solution tuned into dark green. The prepared aqueous extract was brought down to room temperature, gently pressed, and filtered firstly with whatmann filter paper followed by vacuum filtration and stored in the refrigerator. This solution was further treated as a source extract and was utilised in subsequent procedures.**(Taylor et al., 2011**).

7.3.1 Detection of Phenolic compounds

To know the presence of phenolic compounds in *A.marmelos* leaf extracts; ferric ion reducing test was performed with addition of $FeCl_3$ Solution to the aqueous leaf extract before and after addition of $AgNO_3$. The change in the colour was observed. Hydrolysable tannins give blue and black colour and condensed tannins give brownish-green colour due to formation of ferrous compounds. **(Rao and Paria, 2015)**

7.4 Synthesis of Silver nanoparticles using *A.marmelos* leaf extract

Biosynthesis of AgNPs were carried out by simple reduction of 1mM Silver nitrate solution using previously prepared aqueous leaf extract of *A.marmelos* by following the standard published literature with minor modifications. In the procedure the aqueous leaf extract was added to the freshly prepared silver nitrate solution in the ratio of 1: 10 and it was incubated

in the refrigerator at 4-6ºC for 24 hrs. Then, the formation of nanoparticles was confirmed by visual colour change from light yellow to dark reddish brown. (**Krupa and Raghavan, 2014**)

7.5 Conjugation of Silver –Sulfadiazine

Synthesis of silver sulfadiazine was carried out by adding sulfadiazine in the ratio of 1:1 to the solution of previously synthesised silver nanoparticles. Addition of sulfadiazine was followed by continuous stirring using a magnetic stirrer at 600rpm for 24 hrs at a temperature of 4º-6º C to stop the aggregation of nanoparticles, after 24 hrs the Conjugate was spray dried and used for further characterization.

7.6 Spray Drying of Synthesised Silver Nanoparticles and Silver Sulfadiazine

The Synthesised nanoparticle and Silver sulfadiazine conjugate solution was converted into powder form using a laboratory spray dryer (Spray Mate, JISL, Maharashtra) operated in co-current mode. The liquid feed rate was 15 ml/min from 0.5mm diameter nozzle. Spray drying was performed at the inlet temperature of (T_{inlet}) 110ºC, corresponding to an outlet air temperature of (T_{outlet}) 60ºC. The spray drying solution contained 10% W/V of total synthesised nanoparticles and conjugate. The powder was collected from I and II cyclones of spray dryer and stored in an air tight container away from sun light.**(Raja et al., 2010)**

7.7.1 Characterisation of Silver Nanoparticles and Silver Sulfadiazine

- **UV-Visible spectroscopy analysis**

  The bio reduction of silver nanoparticles were being examined using UV-Spectrophotometer (Double beam, Shimadzu D1800) at 200-800nm for its maximum wavelength and absorbance to confirm the reduction of Silver nitrate to form Silver Nanoparticles. In case of metallic nanoparticles the conduction band and valence band lie very close to each other. Due to this the collective oscillation of electrons of silver nanoparticles in resonance with the light wave, the free electrons present in the solution gives rise to surface plasmon resonance absorption band.**(Abou El-Nour et al., 2010)**

- **Particle size and zeta potential determination**

  Particle size of silver nanoparticles in solution form was determined by Zetasizer ,Malvern Instruments Ltd. The reading was carried out at 90º angle with respect to the incident beam, Zeta-Potential of the silver nanoparticles was measured using a Beckman Coulter Delsa™Nano .Spray dried silver nanoparticles were reconstituted in

Distilled water, particle size and zeta potential was measured for the same at 25ºC.**(Sonavane et al., 2008)**

- **Differential scanning calorimetry (DSC)**

  The DSC profile of silver nanoparticles and synthesised silver sulfadiazine was recorded on DSC Q20 (TA Instruments, U.S.A). Thermal behaviour was studied under normal conditions with perforated and sealed quartz pans and with a nitrogen gas flow of 200ml/min. The sample was heated at 5^{o} C/min over a temperature range of 35-300^{o}C. The reference sample used in all the determinations was alumina with a height of 5mg. Peak temperatures and enthalpies were calculated with reference to reported data.

- **Transmission Electron Microscopy(TEM) Study**

  TEM analysis of Silver nanoparticles and silver sulfadiazine was done by using FEI Tecnai G^2 F20 model (The Netherlands) operating at 200kV. Sample was prepared by loading one drop of sample on carbon coated Copper grid and was allowed to air dry for 30 mins and analysed at various angles**(Umadevi et al., 2013).**

- **P-XRD**

  XRD was used to identify crystalline phases of sulfadiazine, Silver nanoparticles and silver sulfadiazine. A PANanalytical X'pert^3 Pro θ-2θ diffractometer using nickel filtered CuKα radiation as source and operated at 45kV and 40mA was used. Scans were typically in the 2θ range sulfadiazine (3º-50º) , silver nanoparticles (5º-100º) and silver sulfadiazine (5º-100º) , with 0.02 step sizes that were held for 2s each in the step scan mode.**(Moulton et al., 2010; Umadevi et al., 2013)**

- **FTIR**

  A FT-IR spectrum of silver nanoparticles and silver sulfadiazine was recorded by potassium bromide (KBr) palletization method. Drug was mixed with KBr and was compressed into small thin pellet, which was subsequently analysed by FT-IR spectrophotometer. Obtained spectra were analysed for characteristic peaks corresponding to specific functional groups present in the drug molecule.

- **Nuclear Magnetic Resonance (NMR) Microscopy**

  The silver sulfadiazine was characterised using NMR spectroscopy. ^{1}H-NMR and ^{13}C-NMR chemical shifts (δ) experiments were performed using a JEOL JNM ECS-400 (400MHz) spectrometer (JEOL, Japan). The solutions analysed included sulfadiazine and silver sulfadiazine. Both the solutions were prepared in Dimethyl Sulfoxide-d6 (DMSO-d6)(99.96 atom % D Isotopic purity, Sigma-aldrich, India). Tetramethylsilane (TMS) was used as internal standard for ^{1}H and ^{13}C NMR study.**(Pires et al., 2015)**

7.7.2 Antimicrobial efficacy of Silver Nanoparticles (AgNPs) and Silver Sulfadiazine (SDZ)

- **Microorganisms and inoculum preparation**

  The antimicrobial and antifungal activity of synthesised AgNPs and SDZ was evaluated against Gram positive bacteria *Bacillus subtillis* (B.subtillis, MTCC 441), Gram Negative bacteria *Escherichia coli* (E.coli, MTCC 1680) and Fungi *Aspergillus niger* (A.niger, MTCC 281). All the 3 strains were acquired from IMTECH, Chandigarh and were preserved on slants at 4ºC. Inoculums were prepared in 20 ml of Muller-Hilton broth(MHB) for bacteria and Sabouraud agar(SBA) for fungi by suspending a single bacterial and fungal colony of each test bacteria and fungi in separate broth and were incubated overnight at 37ºC and 20-25ºC for bacteria and fungi respectively on 50rpm shaking. Each inoculum prepared was expected to contain 10^{4}-10^{7} cfu/ml.**(Verma et al., 2015)**

- **Disc diffusion assay**

  The disc diffusion assay was used to assess the antimicrobial activity of AgNPs and SDZ against E.coli, B.subtillis and A.niger. The prepared bacterial and fungal inoculums were spread in Muller-Hilton agar (MHA) media plates and SBA plates respectively. 5mm sterile filter disc was soaked in (A) 10% AgNPs solution, (B) 1% SDZ solution and (C) aqueous Leaf extract of *Aegle marmelos* (AEM) and gently placed on 3 defined areas on the plates. The inoculated bacterial and fungal plates were incubated at 37ºC and 20-25ºC respectively and their Zone of inhibition was measured.

RESULTS AND DISCUSSION

CHAPTER 8

RESULTS AND DISCUSSION

8.1 Characterization of Sulfadiazine

8.1.1 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 8.1 Physical characterization of sulfadiazine

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

8.1.2 Melting point

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

Table 8.2 Melting point of Sulfadiazine

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

8.1.3 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. 8.1 and Table 8.3). Compliance between the values of characteristic peaks indicates the purity of drug

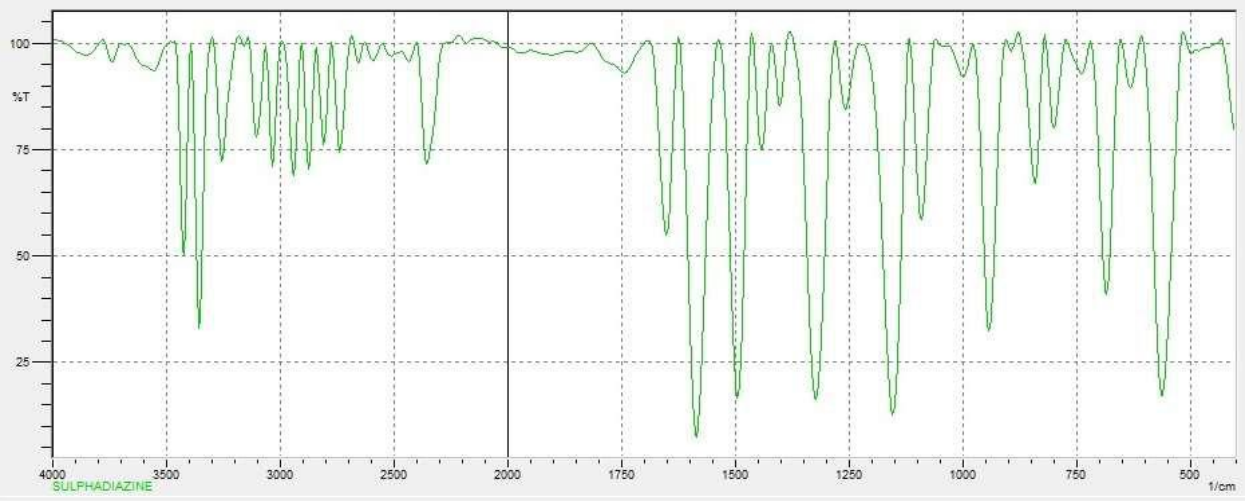


Fig. 8.1. IR spectra of Sulfadiazine

Table 8.3 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.

8.2 Analytical method development

8.2.1 Determination of absorption maxima

Irrespective to the nature of media the λmax of sulfadiazine was found to be 254nm (USP Monograph) (fig.8.2)

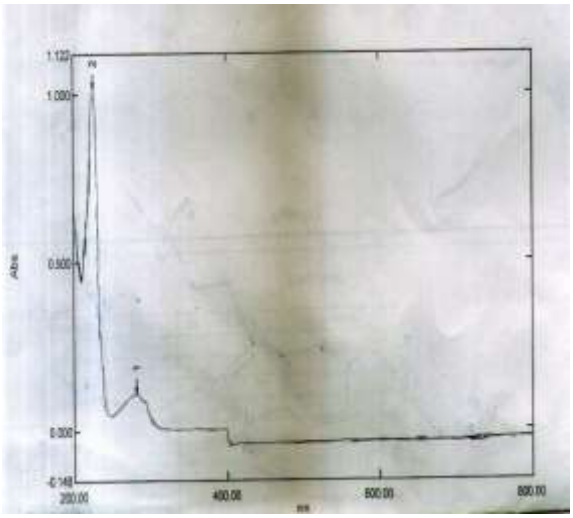


Fig.8.2 Determination of λmax of Sulfadiazine in 0.05N NaOH solution

8.2.2 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 8.4 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)

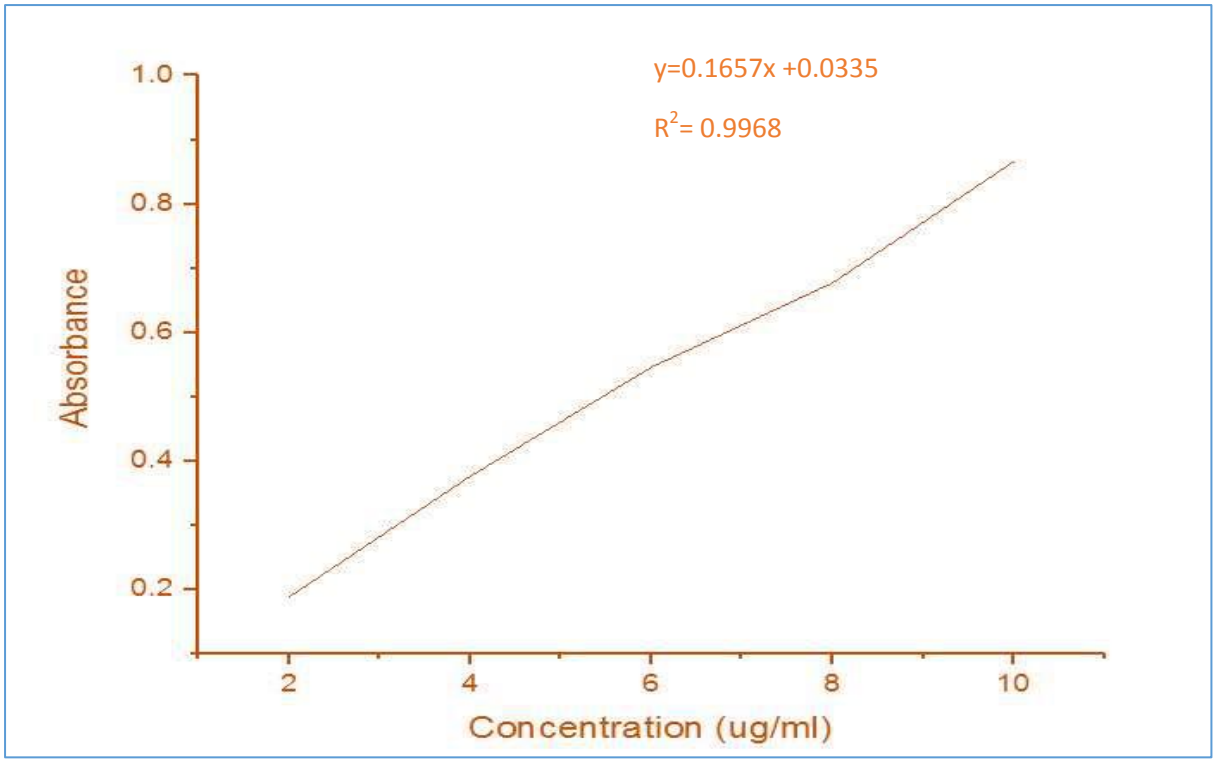


Fig.8.3. Calibration curve of Sulfadiazine in 0.05N NaOH solution

8.3 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 (Fig .8.4)

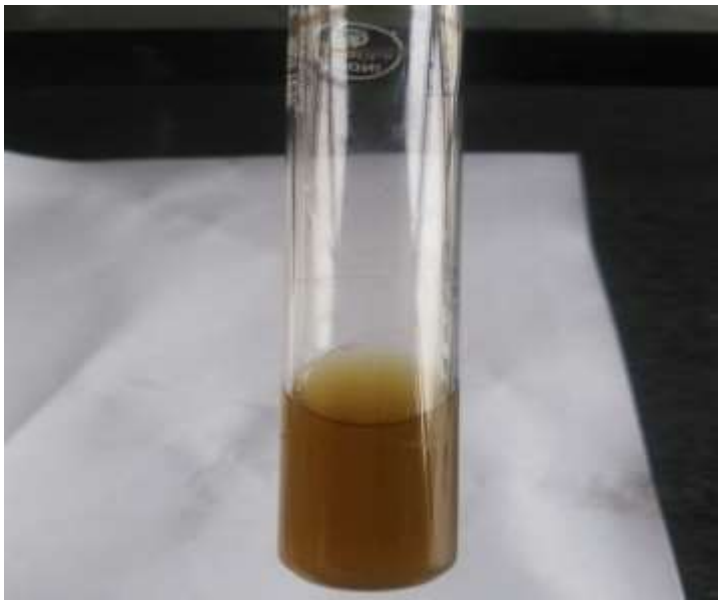


Fig.8.4. Leaf extract of *Aegle marmelos*

8.3.1 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 (Fig 8.5) which indicated presence of tannins.

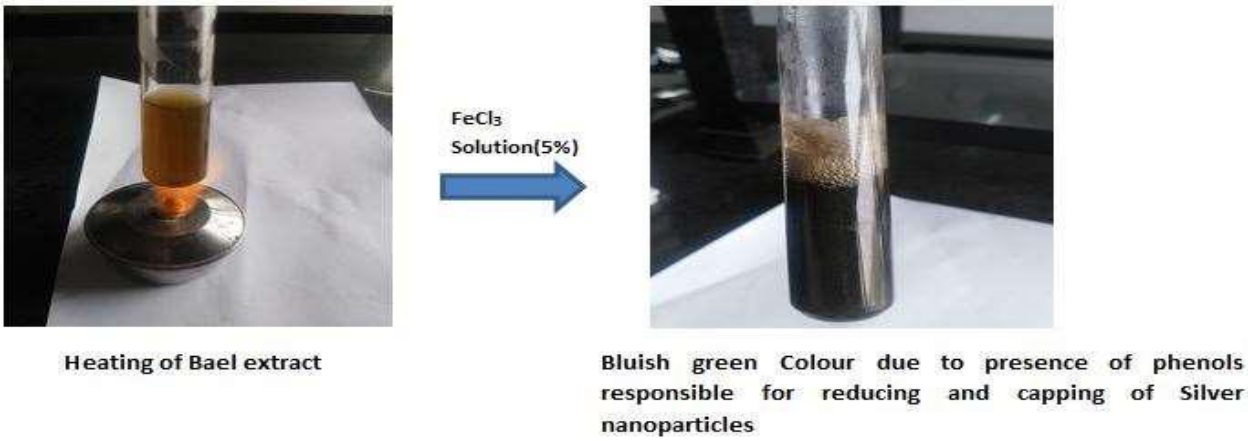


Fig.8.5. Test for presence of phenols

8.4 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 8.6)

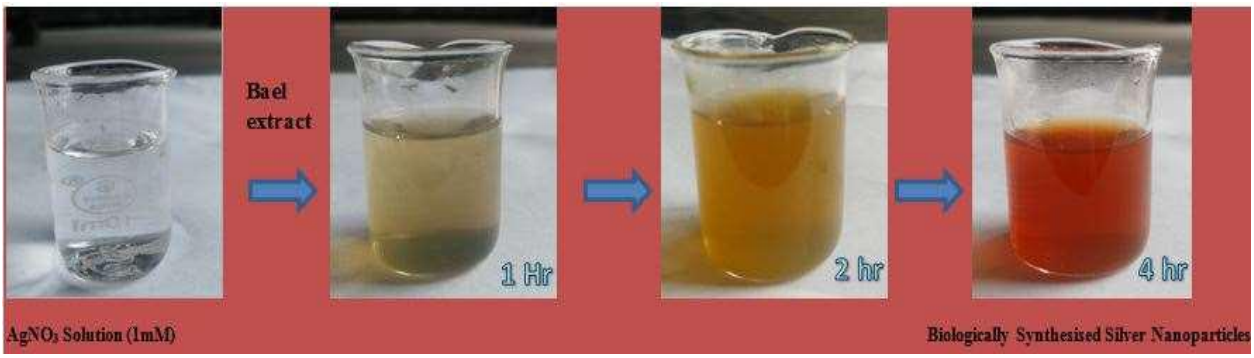
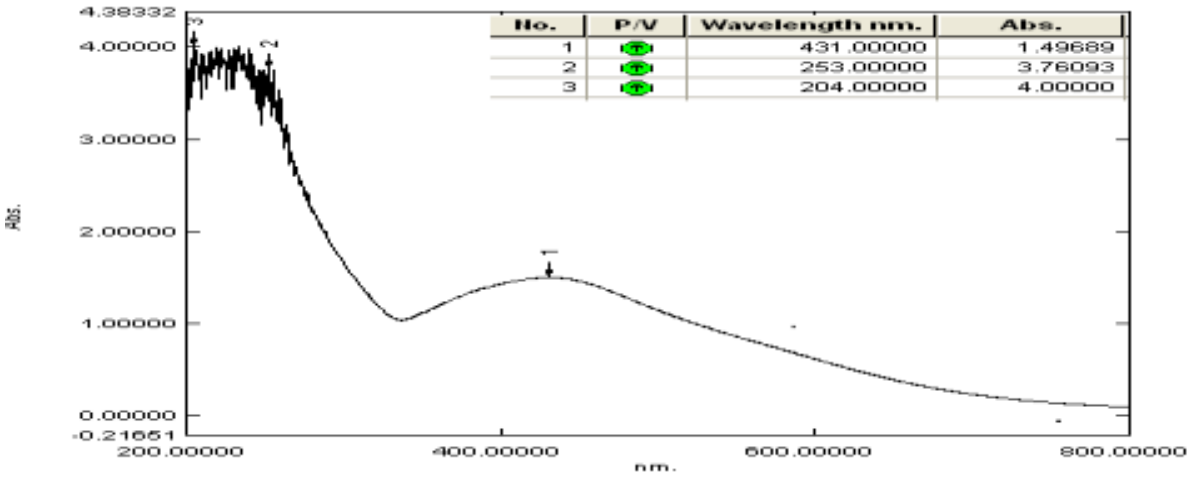


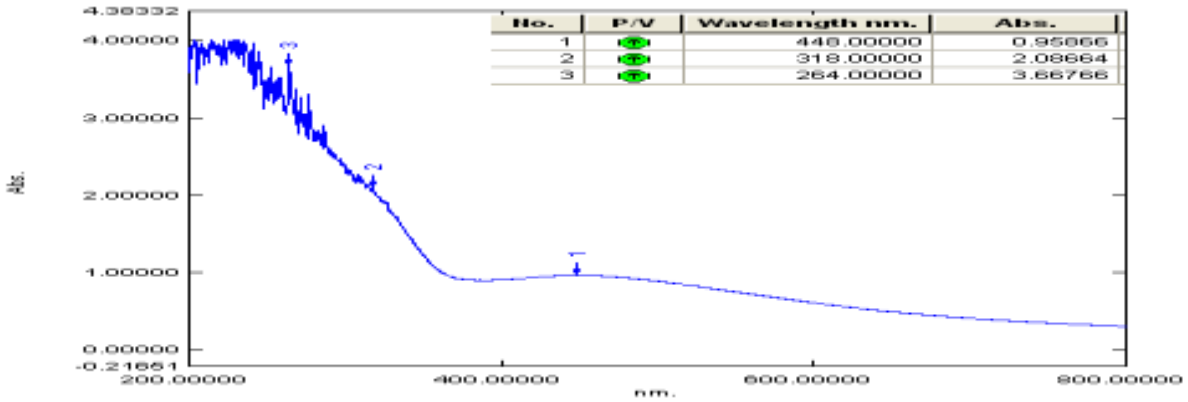
Fig.8.6. Synthesis of Silver nanoparticles at various time intervals

8.4.1 UV Spectrophotometric Analysis

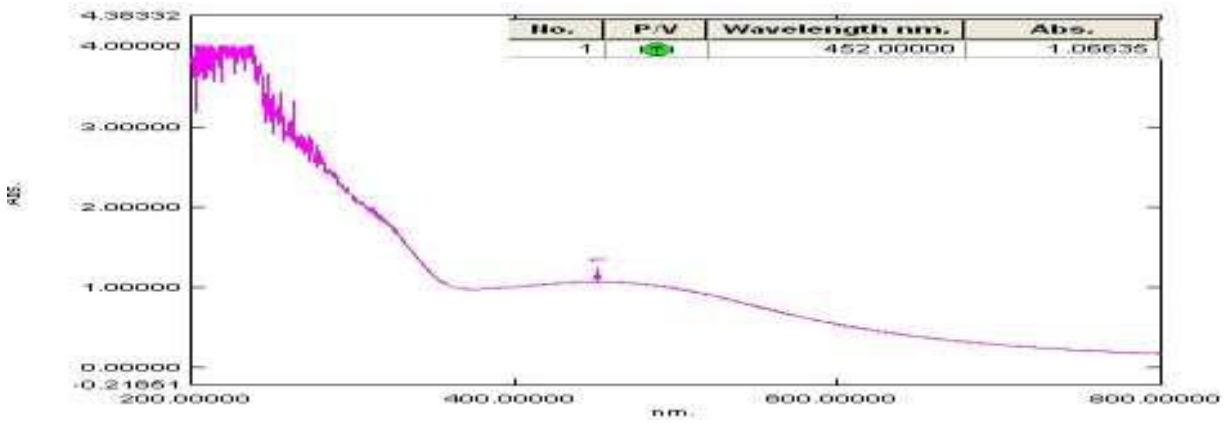
UV analysis was done in scanning mode of various concentrations of $AgNO_3$ solution and bael extract at the range of 200-800 nm. The ratio of bael extract was kept constant i.e. 1:10 and concentration of $AgNO_3$ (20ml) was varied from 1 mM -5 mM. Due to formation of silver nanoparticles we observed peaks at 370-460 nm which is known as Surface Plasmon Resonance. (Fig 8.7) 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.



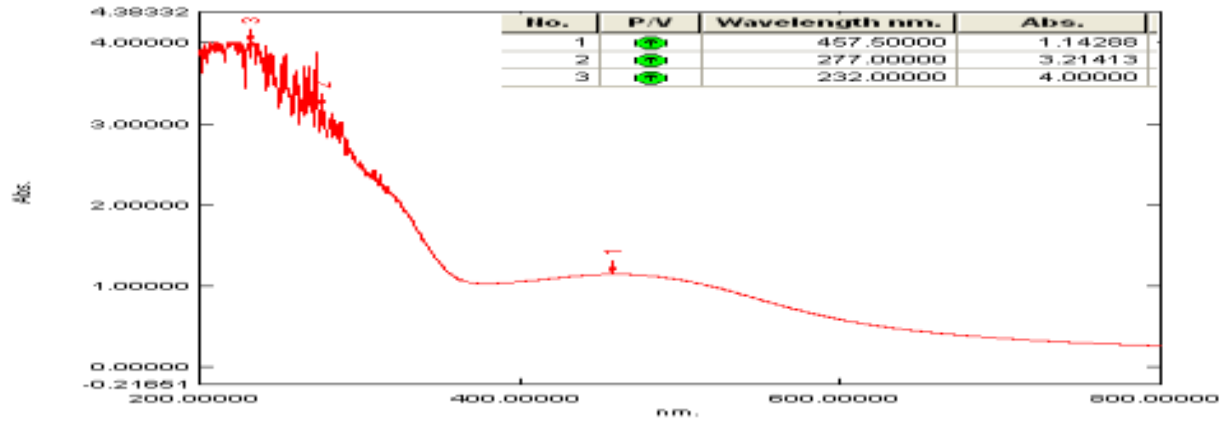
(A)



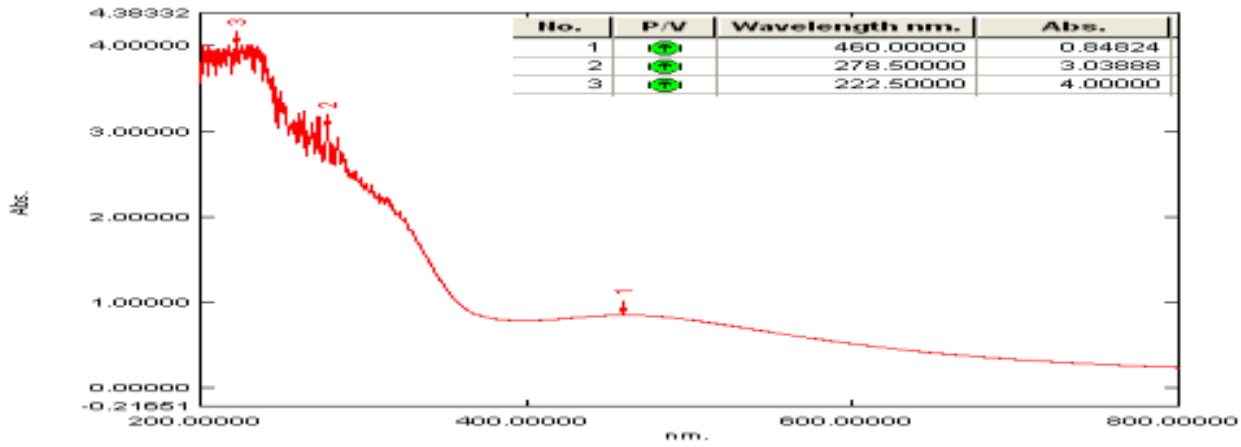
(B)



(C)



(D)



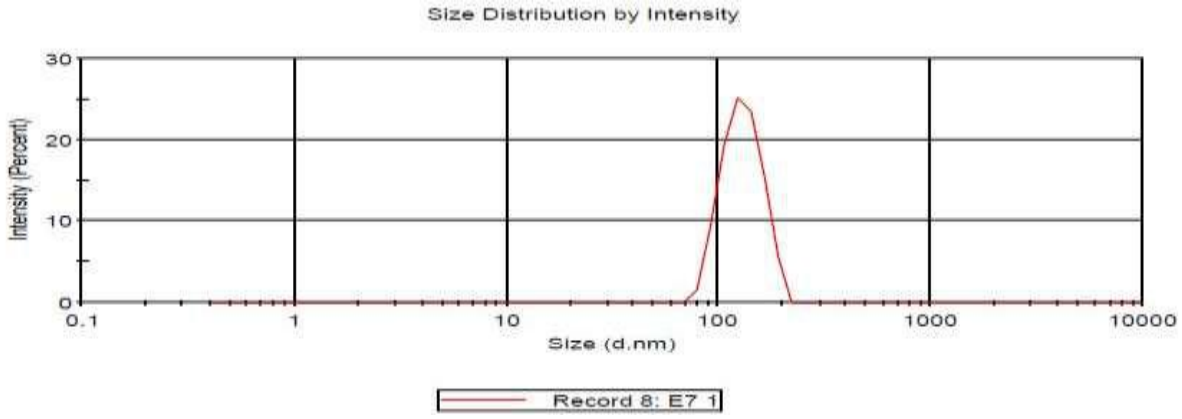
(E)

Fig.8.7. 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]

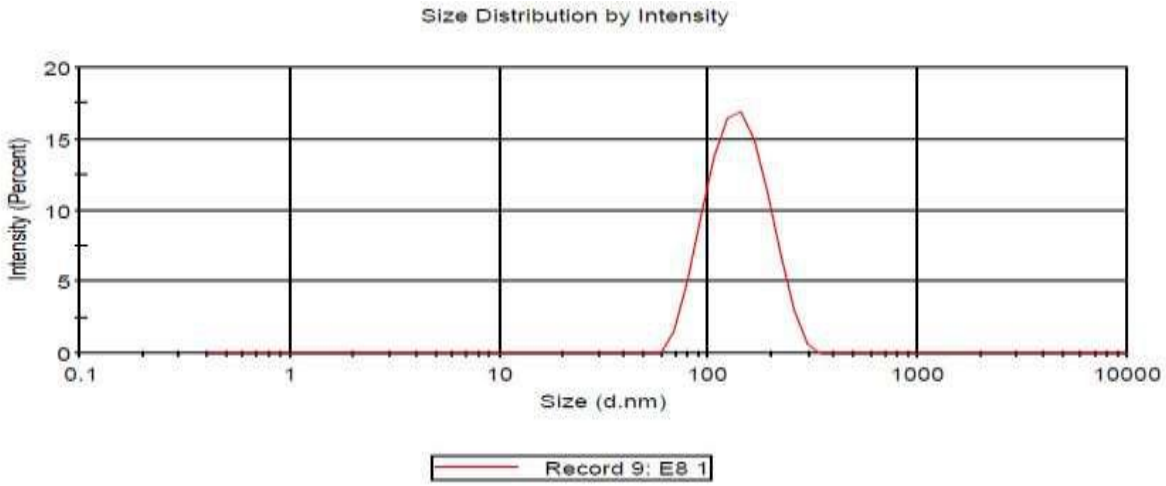
8.4.2 Particle Size and Zeta Potential Determination

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).

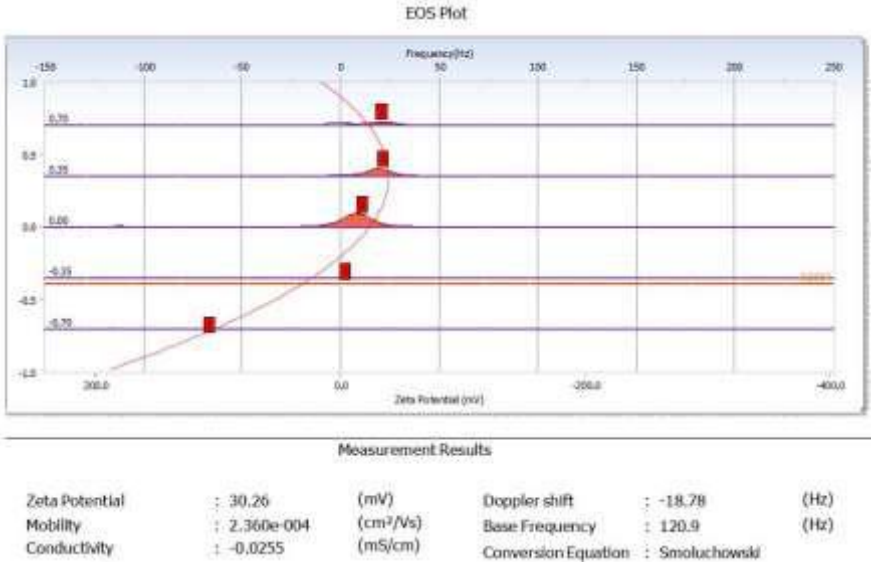
The Zeta potential of BSN was measured using Beckman Coulter Delsa™Nano and it was found to be 30.26 mV from which we can consider it to be moderately stable under provided conditions whereas Zeta potential of reconstituted SSN measured under same conditions was found to be 51.53 mV from which we considered it to be Stable.(Fig. 8.8)



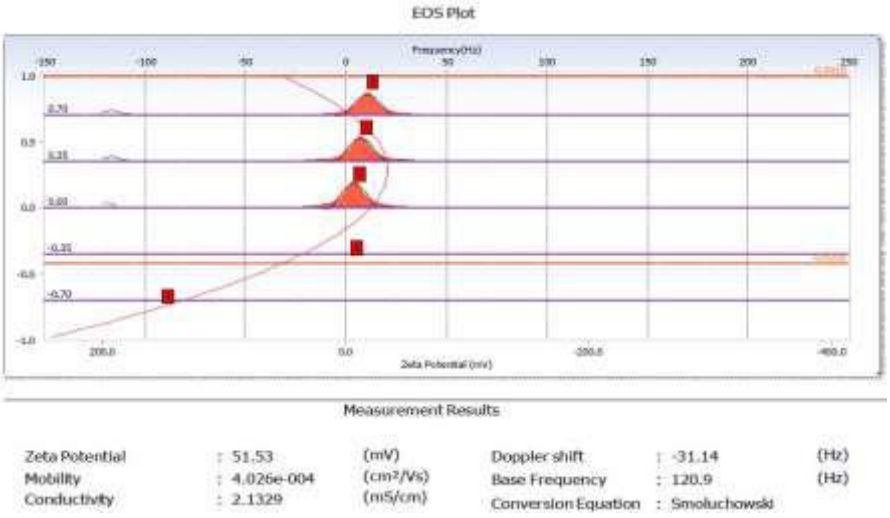
(A)



(B)



(C)

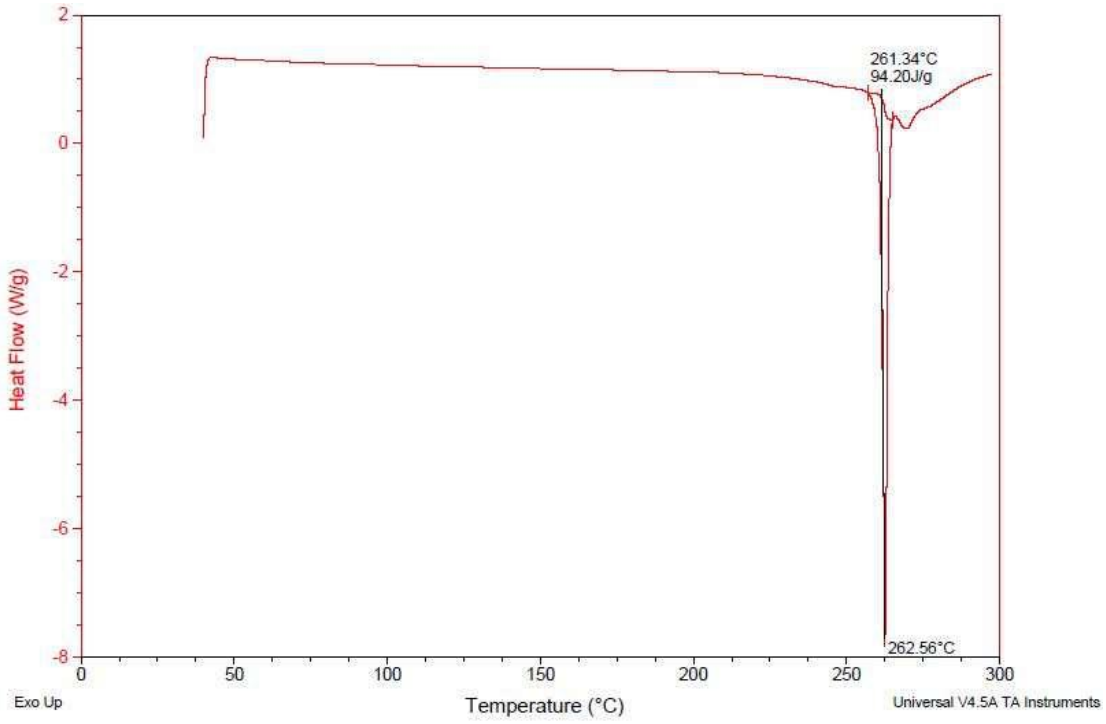


(D)

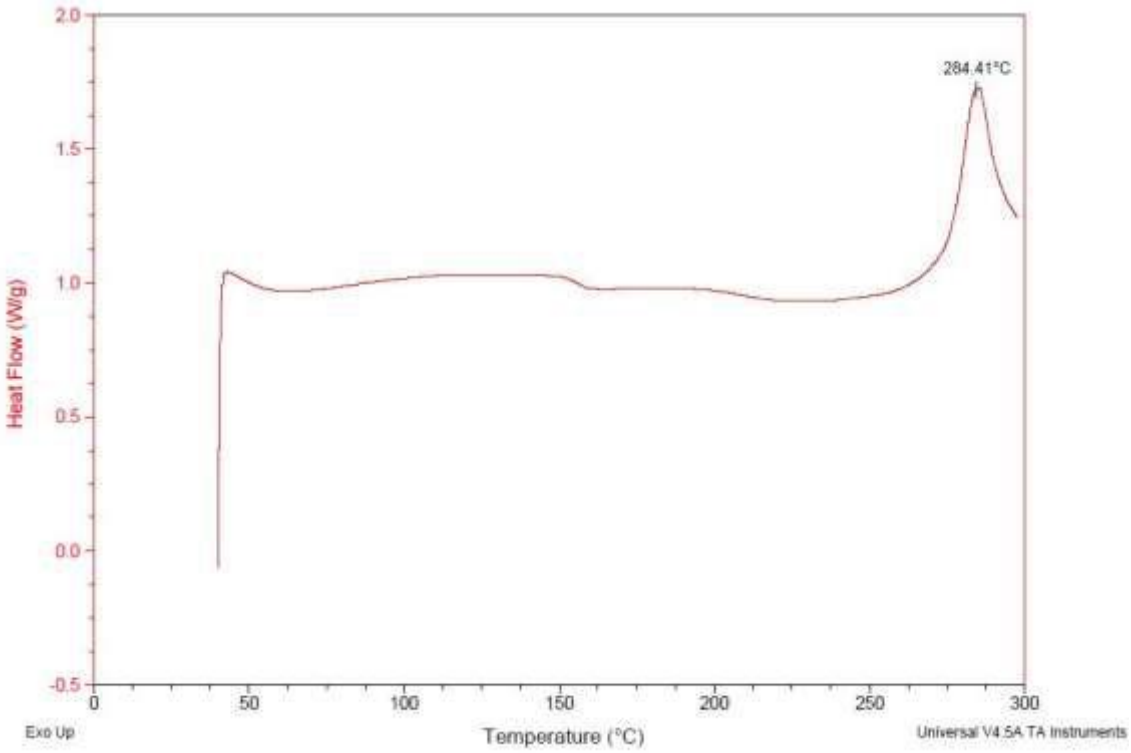
Fig.8.8. (A) Particle size distribution of biologically prepared Silver nanoparticles (B) particle size distribution of Spray dried silver nanoparticles(C) Zeta Potential of biologically synthesised silver nanoparticle (D) Zeta potential of spray dried silver nanoparticles.

8.4.3 Differential scanning calorimeter

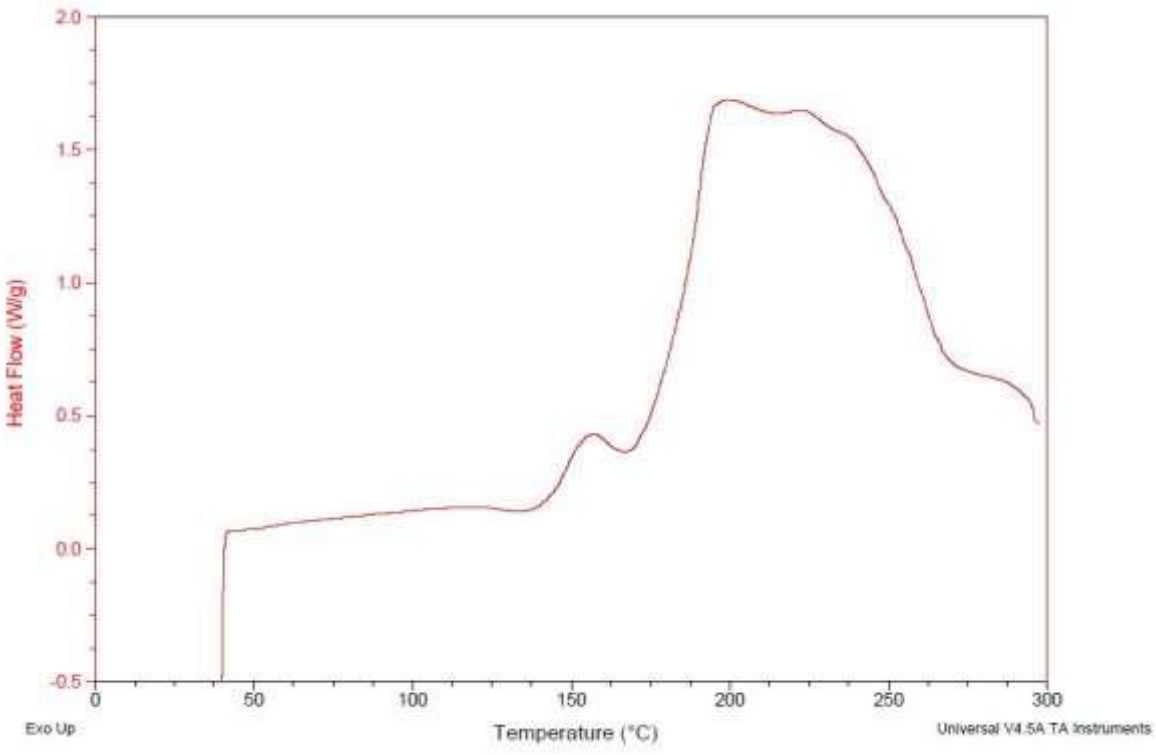
With DSC , it is possible to measure the small energy changes that occurs as matter undergo thermotropic transitions with heating from initially in solid to liquid phase. In this study, DSC thermogram for sulfadiazine was determined with DSC Q20 (TA Instruments, U.S.A) and sample size was 1.00 mg. DSC 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. (Fig.8.9)



(A)



(B)



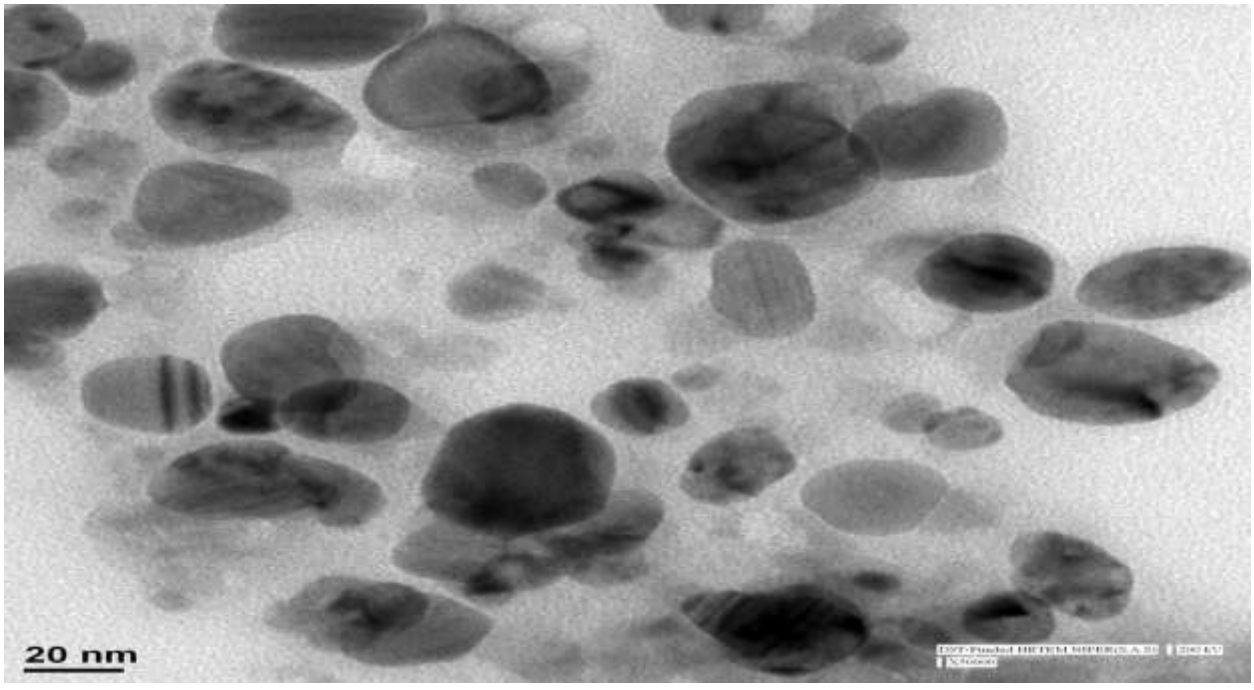
(C)

Fig.8.9. DSC Thermograms of (A) Sulfadiazine (B) Silver Nanoparticles (C) Silver Sulfadiazine

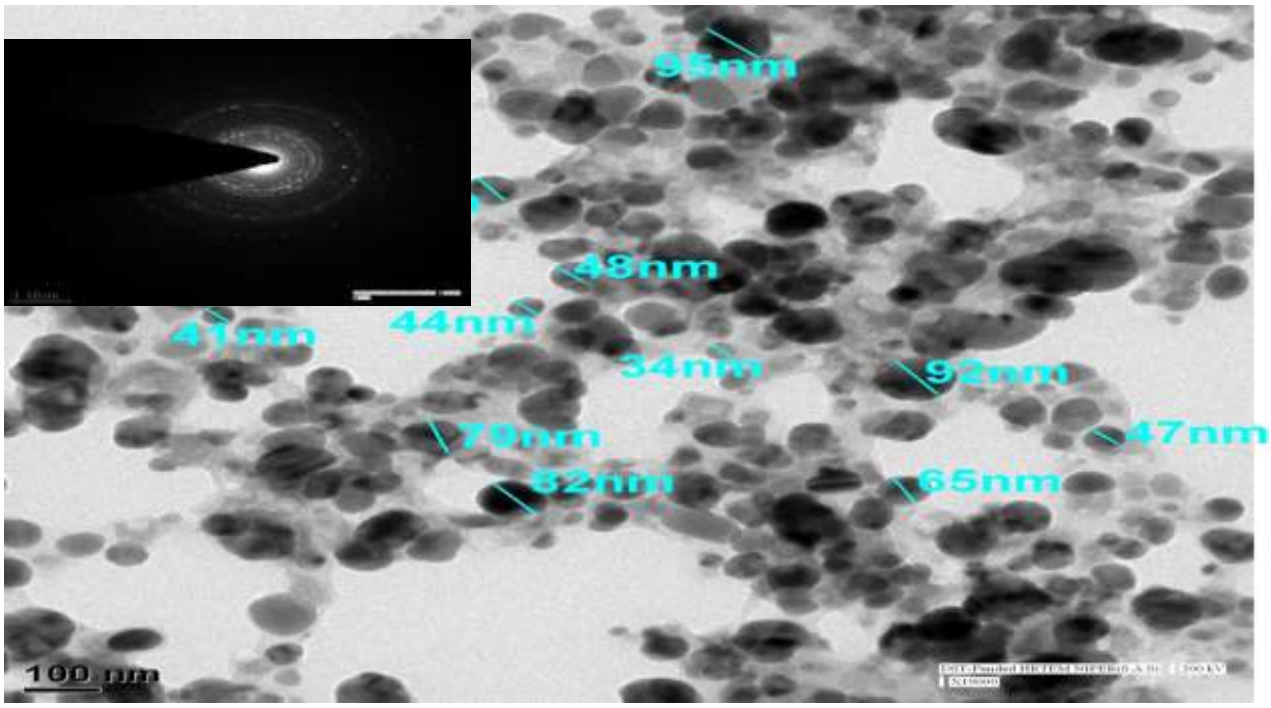
8.4.4 Transmission Electron Microscopy(TEM)

TEM images of silver nanoparticles derived from the extract of *Aegle marmelos* are shown in figure 8.10. Nanoparticles are roughly spherical in shape. Some of the nanoparticles were found to be oval and/or elliptical. This type of variation in shape and size of nanoparticles synthesized by green chemistry are common. It was being noted that the edges of the particles were lighter than that in the centre, suggesting that biomolecules present in leaf extract capped the silver nanoparticles. TEM analysis showed that most of the particles had a size in the range of 25 nm-100 nm. In Selected area electron Diffraction pattern (SAED) of the silver nanoparticles, they were found to be nanocrystalline in nature as can be seen from the SAED recorded from one of the nanoparticles in aggregate. SAED spots that corresponded to the different crystallographic planes of face-centered cubic (fcc) structure of elemental silver are seen in insight of 8.10 (B)

TEM study of Silver Sulfadiazine (SDZ) complex was done by the previously described method. The SDZ particles were found to have spherical-ovoid shape and particle size in the range of 10-60 nm, lesser in comparison to biosynthesised silver nanoparticles. The drug was found to be entrapped over the silver nanoparticles depicted by black portions in the structures. (Fig.8.11)



(A)



(B)

Fig.8.10 Transmission Electron Microscopy image of Silver nanoparticles (A) 20 nm and (B) 100 nm (Insight SAED image)

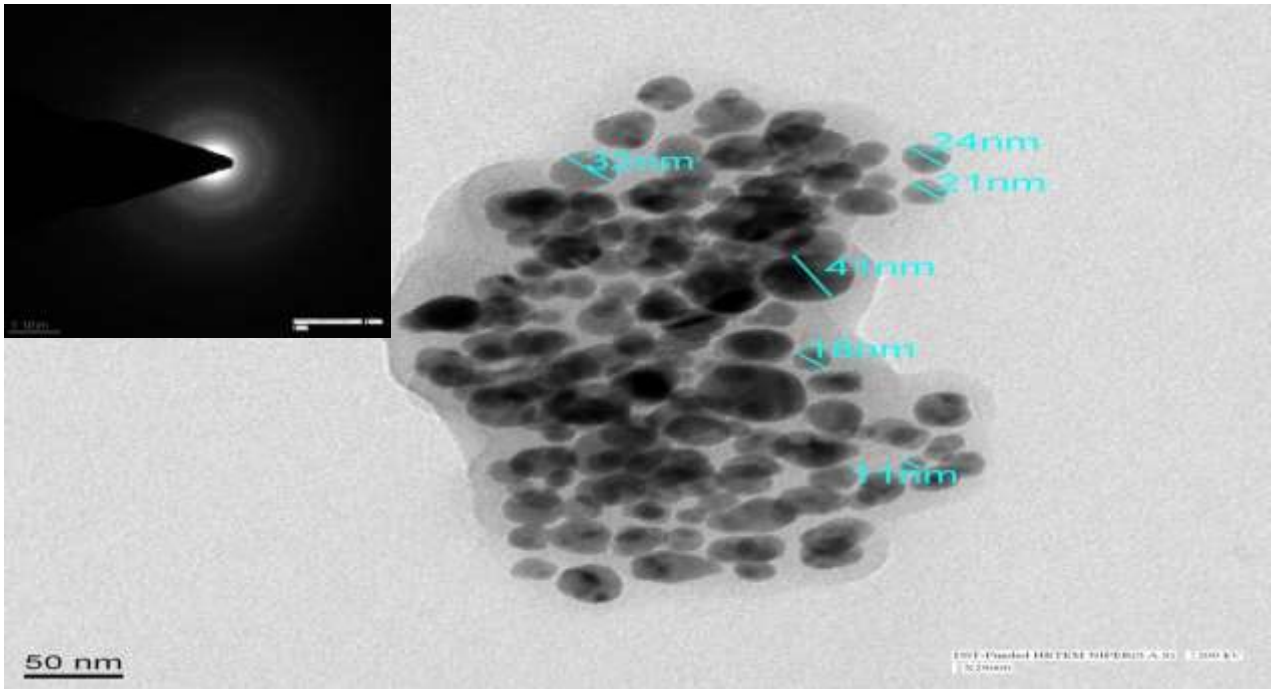
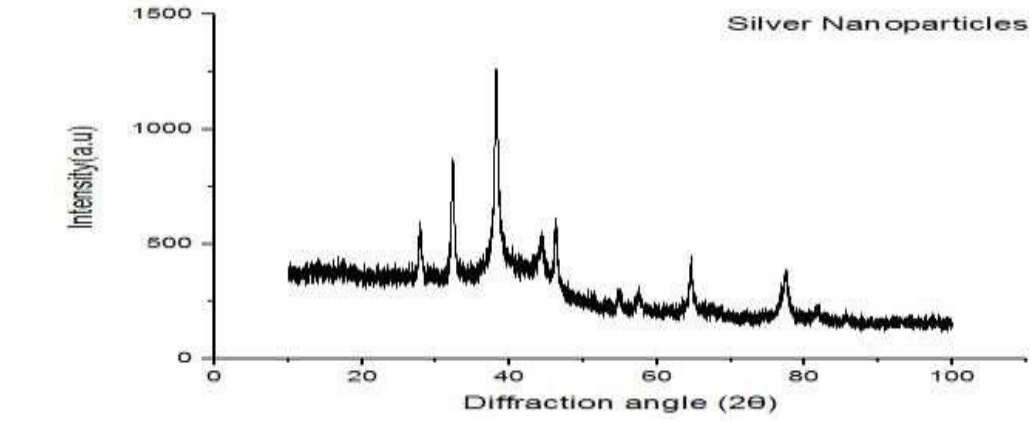


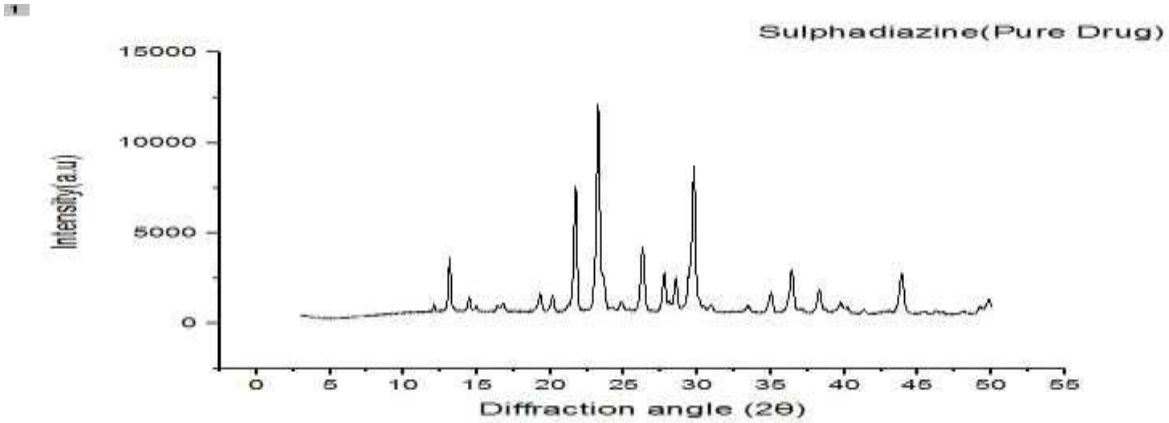
Fig.8.11. Transmission Electron Microscopy image of Silver Sulfadiazine complex (Insight SAED image)

8.4.5 PXRD analysis

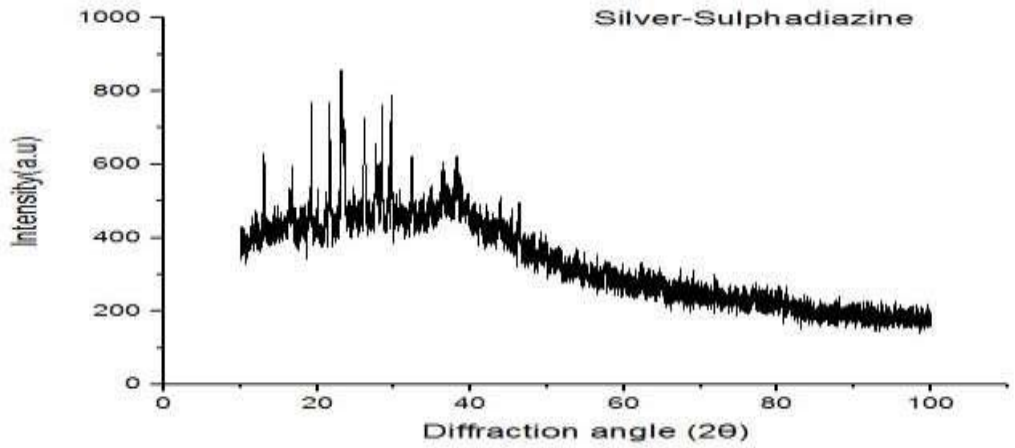
In the XRD patterns, prominent Bragg reflections at 2θ values of 38.45º, 44.48º, 64.69º and 77.62º, which corresponds to (111), (200), (220) and (311) face centered cubic silver, respectively, which were closely matched with the reported reference values of International Centre for Diffraction Data (ICDD) card number 01-087-0717 .The X-ray diffraction peaks were found to be broad around their bases indicating that the silver particles are in nano sizes. In another pattern of monoclinic sulfadiazine , the peaks at scattering angles 2θ of 11.7º, 12.8º, 14.1º, 16.5º, 19.7º, 20.9º, 21.3º, 26.0º, 27.4º and 28.7º are attributed to the crystal planes of (-201), (200), (011), (-210), (012), (003), (-402), (400), (212) and (004) (JCPDS 87-0598). The diffraction pattern obtained from silver sulfadiazine shows that, crystallinity decreases as compared to sulfadiazine alone structure as indicated by the numerous distinctive peaks with major characteristic diffraction peaks at diffraction angle of 2θ at 13.27º, 25.70º and 41.43º (Fig .8.12)



(A)



(B)

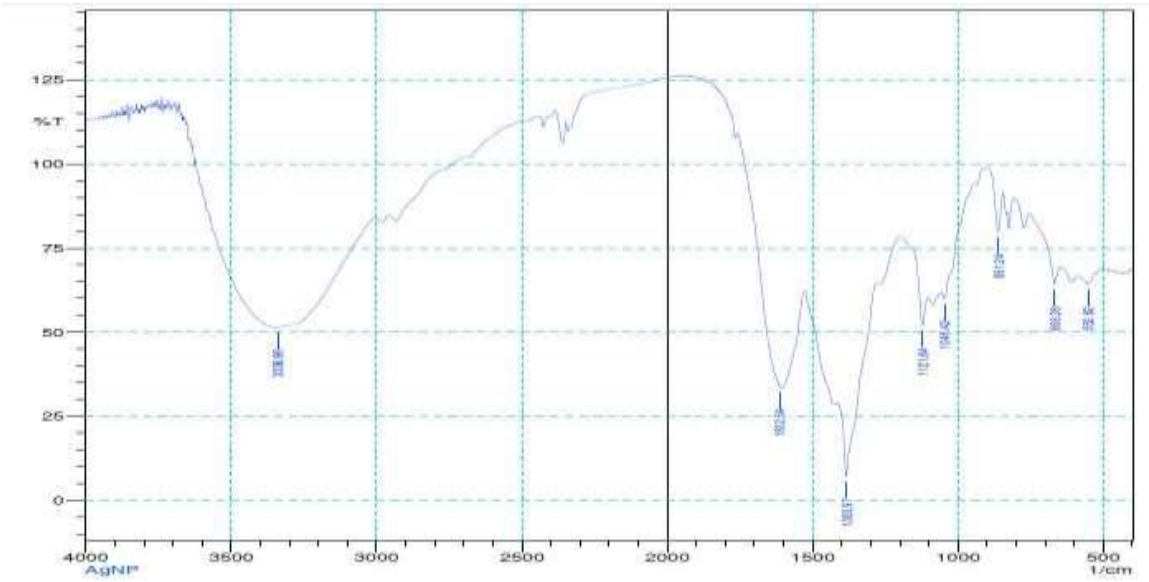


(C)

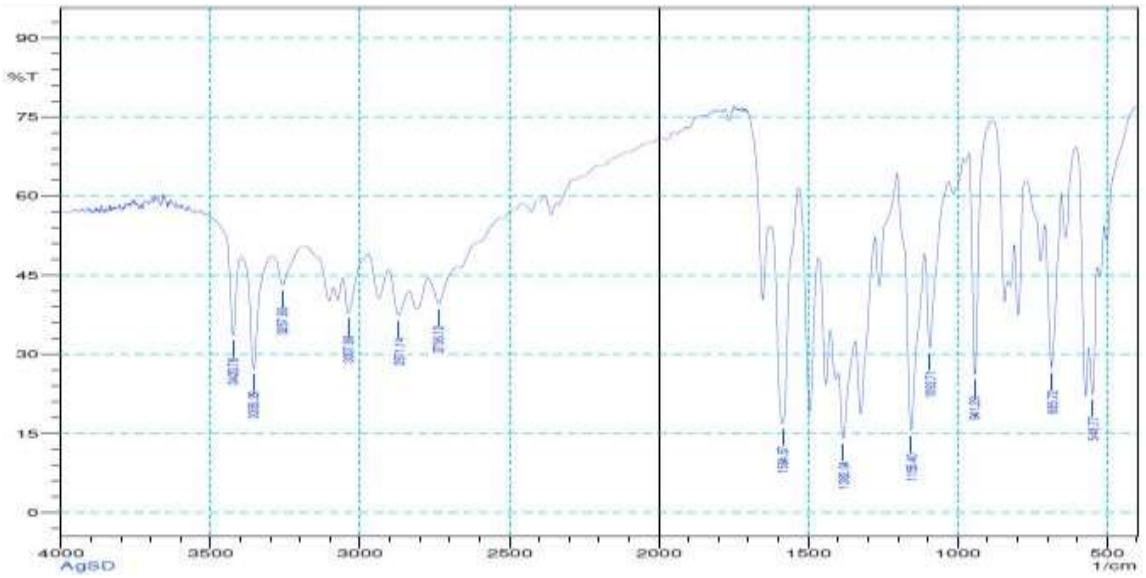
Fig. 8.12 (A) X-ray diffraction pattern of synthesised AgNPs (B) X-ray diffraction pattern of pure sulfadiazine (C) X-ray diffraction pattern of synthesised silver sulfadiazine.

8.4.6 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.8.13 FTIR spectra of (A) Silver nanoparticles (B) Silver sulfadiazine

Table 8.5 FTIR spectra analysis of Silver nanoparticles

Sr.No.	Standard value range (cm^{-1})	Observed value	Interpretation
1.	3200-3600	3336.96	O-H Stretch (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 $AgNO_3$.

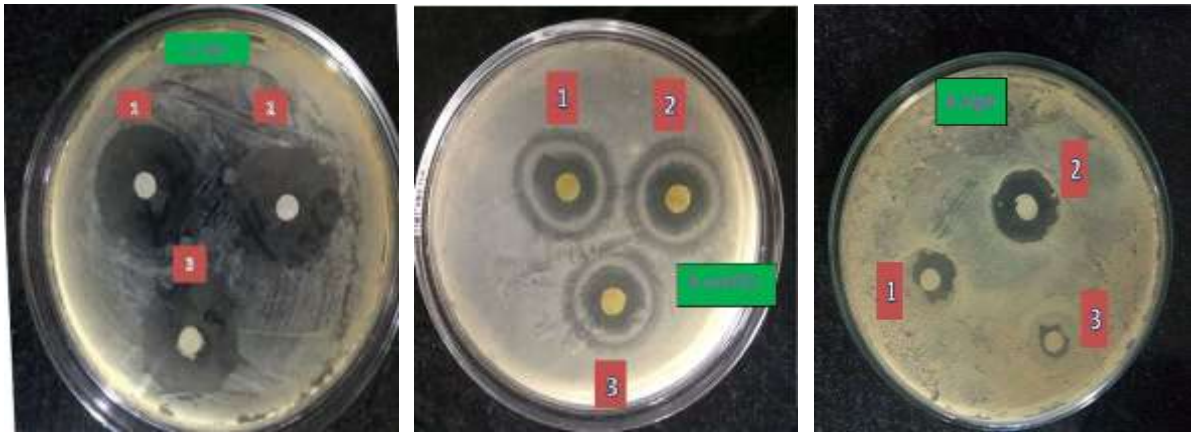
The Spectra of Silver sulfadiazine (Table 8.6) resembles quiet to that of sulfadiazine depicted above. The loss of peak for –NH bending resembles the conjugation of Silver ion to the $–N^{(-)}$.

Table 8.6 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 (Aromatic)
4.	2850-3000	2871.14	C-H Stretch
5.	2720-2750	2736.12	=C-H Stretch

8.5 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.



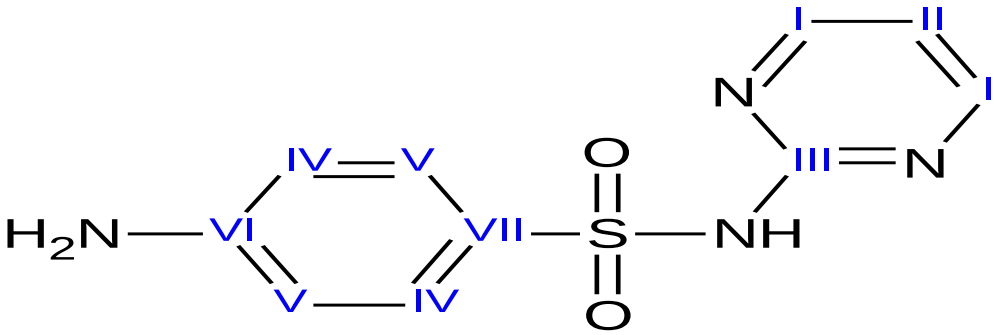
(A) **(B)** **(C)**

Fig.8.14 Disc (A) E.coli (B) B.subtillis (C) A.niger and (1) ZOI of AgNPs (2) ZOI of SDZ (3) ZOI of AEM

Table 8.7 Zone of inhibition of various test compounds

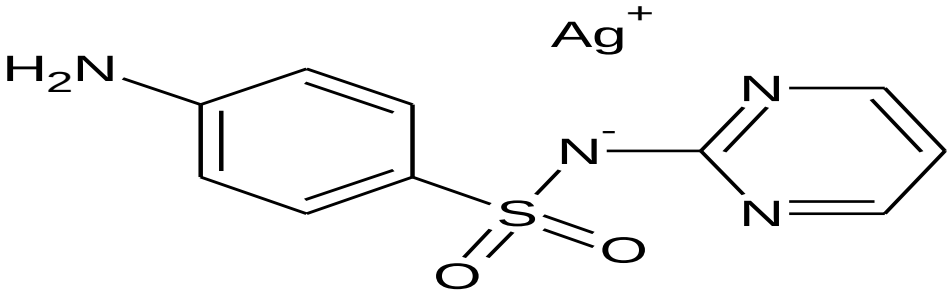
S.no	Culture	AgNPs	SDZ	AEM
1.	***Escherichia coli*** **(Gram '-'Ve bacteria)**	9mm	12mm	10mm
2.	***Bacillus subtillis*** **(Gram '+'Ve Bacteria)**	5mm	6mm	5mm
3.	***Aspergillus niger*** **(Fungi)**	3mm	6mm	2mm

8.6 Nuclear magnetic resonance (NMR) (Supplementary data)



(A)

Silver Nanoparticles (Ag^+)



(B)

Reaction between Sulfadiazine (A) and Silver nanoparticles to produce Silver Sulfadiazine complex (B) Probable structure after conjugation.

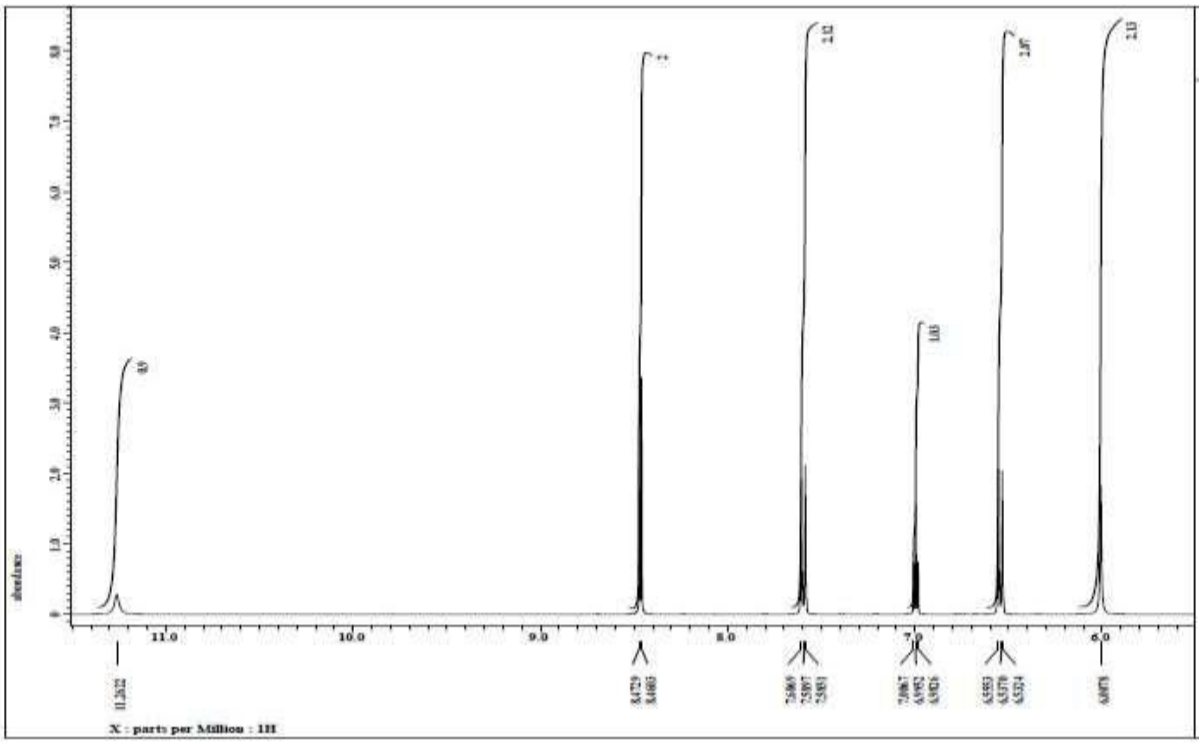


Fig.8.15 ^{1}H NMR of Sulfadiazine

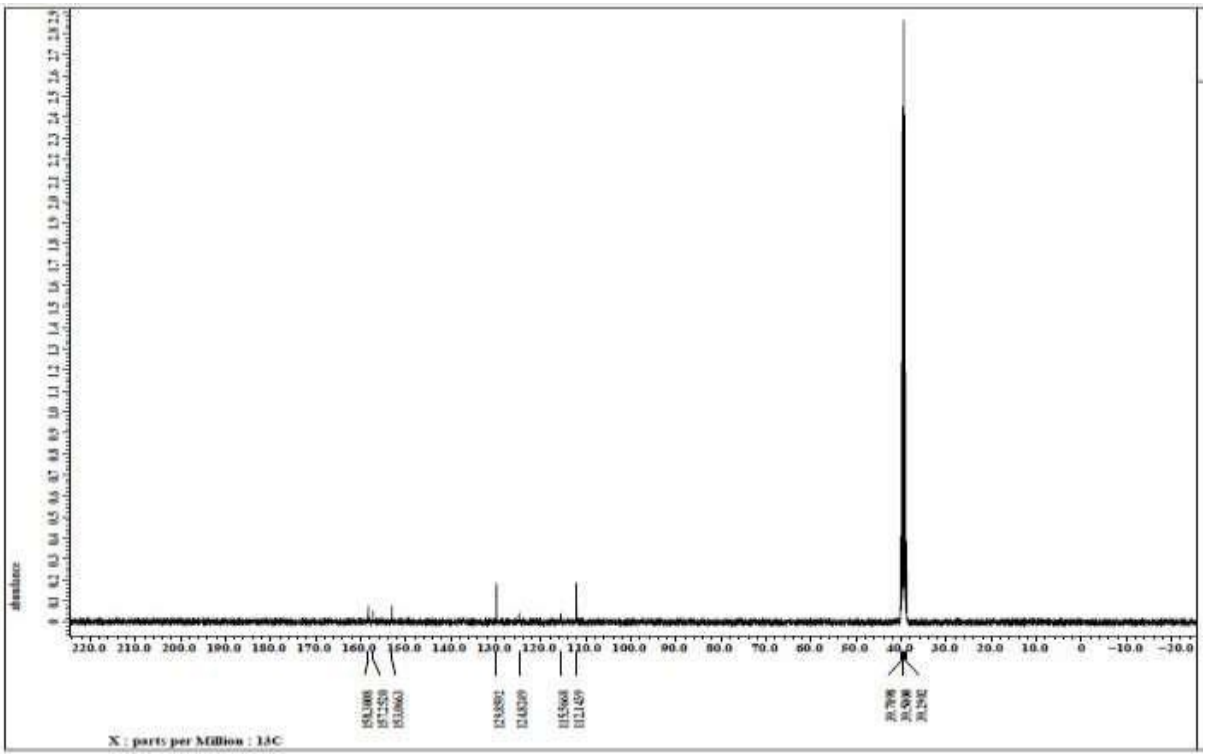
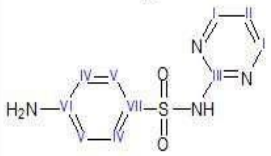
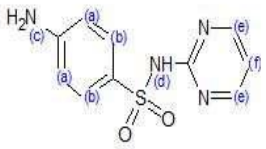
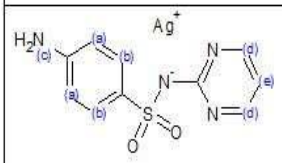


Fig.8.16. ^{13}C NMR of Sulfadiazine

Table 8.8 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

CHAPTER 9

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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