Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

DEVELOPMENT AND CHARACTERIZATION OF SILVER SULFADIAZINE EMULGEL FOR TOPICAL DRUG DELIVERY

SONAL V. GHODEKAR*, SHILPA P. CHAUDHARI, MUKESH. P. RATNAPARAKHI

College of Pharmacy, Thergaon, Pune-33 Email: shilpapchaudhari78@yahoo.com.

Received: 27 July 2012, Revised and Accepted: 15 Sep 2012

ABSTRACT

The study was carried out keeping three aims in mind, firstly to increase the solubility and bioavailability of silver sulphadiazine by formulating solid dispersions, secondly compare between polaxomer188 and 407 for increasing the solubility using different techniques of solid dispersion formulation and thirdly combine the advantages of emulsions and gels by formulating emulgel of silver sulphadiazine. It was found that between Poloxamer 407 and188, solid dispersions of poloxamer 407 were more amorphous than that of Poloxamer 188 and by using both type of Poloxamer we got nanosized particles on dispersing these dispersions in water. On formulation of emulgel with different gelling agents, SepineoP600 lecithin gel (SLO) as gelling agent were found to have more release and better gel characteristics compared to pluronic lecithin gel (PLO) and Carbopol lecithin gel (CLO) containing formulations.

Keywords: Silver sulphadiazine, Solid dispersions, Emulgel, histopathology

INTRODUCTION

In the last decade it has been found that nearly 75% of patients with burns who were autopsied have died of invasive bacterial infections originating in the burn wound[1]. Silver sulfadiazine is found to have a broad spectrum of activity against most microorganisms, particularly the gram negatives and it has also proved to have good tolerability by the patient and has low toxicity and also widely used in third & fourth degree burn[2]. In the silver sulfadiazine drug, sulfadiazine did not act as antibacterial agent but it produced synergism in combination with sub inhibitory levels of the silver sulfadiazine[3]. There are many silver sulphadiazine creams available in the market with identical composition and also water soluble gel has been prepared of silver sulphadiazine, but these creams have tendency to form pseudo eschar, which is difficult to distinguish from burn – eschar and these may also impede the penetration of SSD into the burn wound. Mainly the fatty acids or lipid soluble carrier are relatively insoluble in water, making it difficult to remove from the burn wound[4], whereas water soluble gel are easily washed of or removed. Our aim was to design the formulation that will not be difficult to remove at the same time not easily washed off. i.e. combine the advantages of emulsions and gels. So emulgel were formulated using various gelling agents. But silver sulphadiazine has less solubility in water as well as oil. Therefore solid dispersions of Silver sulphadiazine were prepared and then emulsified and gelled. The additives in formulation which may be affect on drug permeation through skin[5]. In this olive oil, almond oil,coconut oil is use as an oleaginous phase with lecithin penetration enhancer and also Poloxamer 407,188 added in the formulation, it act as wound healing agent[6,9]. This emulgel has advantage of delivering the drug directly to the site of action and for extended period of time like the other topical drug delivery systems[7].These increase the contact time and mean resident time of SSD at the applied site leading to increase in local concentration while pharmacological activity may not change as rapidly as the solution form.

MATERIALS AND METHODS

Materials

Silver sulfadiazine was purchased from Anju Drug Chem. Private Limited, Indore, India. Poloxamer 407 (Pluronic F127) and Poloxamer 188 (Pluronic F-68) was purchased from Sigma Aldrich. Lecithin (PHOSPHOLIPON® 85 G) as gift sample of Lipoid Company, Sepineo P600 gift sample of Yasham Bio-Science Pvt Ltd. Mumbai. Carbopol 934, Tween 80, Span 60, calcium hydroxide, Methyl paraben, Propyl paraben was purchased from loba chemie Mumbai. Ethanol was purchased from loba chemie. All extra pure oils are purchased from Local market. All other chemicals were pharmaceutical grade and used without further modification.

Methods

Preparation of solid dispersions

Lyophilization method

The processes of lyophilization have three stages: freezing, primary drying (ice sublimation) and secondary drying (water desorption). In this method solid dispersion were prepared by using different ratios (1:1, 1:2 &1:3) of silver sulfadiazine and carrying agent (Poloxamer 407, Poloxamer 188). The weighed amount of drug and polymers were mixed properly and dissolved in 1:1 proportion of ethanol and water, then solution was kept in freeze dryer for 24 hr, to get completely freeze product. Then lyophilize this product by using lyophilizer (Christ ALPHA 1-2 LD Plus) for 48 hr to get complete dried product of solid dispersion. Lyophilized preparations were stored in desiccators at room temperature (RT). (Table 1)

Melt method

In this method solid dispersion were prepared by using different ratios (1:1,1:2 &1:3) of silver sulfadiazine and carrying agents (Poloxamer407,Poloxamer 188).The weighed amount of drug sample and carrying agent mixed properly and then melted by using hot plat at 50^0C. Then melted dispersion was allowed to cool and kept in desiccators at RT.

Solvent melts method

Solid dispersion were prepared by dissolving the silver sulfadiazine & carrying agent such as (Poloxamer 407,Poloxamer 188) in ethanol various proportion such as (1:1, 1:2 &1:3),which is then evaporated by heat melting the polymer in solvent until a clear, solvent free film is left. Then this dispersion is further dried to constant weight and kept in desiccators at RT.

Solvent evaporation method

The first step in this method was to dissolve the drug and polymer in the solvent (ethanol). Then the solvent is allowed to evaporate at RT. The drug and polymers were taken in different proportions (1:1, 1:2&1:3). The dried product stored in desiccators for further characterization.

Evaluation of solid dispersion

Solid dispersion obtained from the above method was evaluated for their phase solubility, Drug content, FTIR, XRD, DSC, and SEM.

Table 1: Compositions of batches containing silver sulfadiazine and Poloxamer 407 &Poloxamer 188

Carrying agents	Drug: Carrier ratio	Formulation code			
		Lyophilization method	Melt method	Solvent evaporation method	Solvent melt method
Poloxamer 407	1:1	L1	M1	SE1	SM1
	1:2	L2	M2	SE2	SM2
	1:3	L3	M3	SE3	SM3
Poloxamer 188	1:1	LA	MA	SEA	SMA
	1:2	LB	MB	SEB	SMB
	1:3	LC	MC	SEC	SMC

Drug content

The amount of solid dispersion equivalent to10 mg drug was weighed accurately. Then it was dissolved in 100 ml of water, suitably diluted and UV absorbance was measured at 254 nm.

Phase solubility study

The solubility of silver sulfadiazine in distilled water was determined. An excess amount of silver sulfadiazine was placed in glass bottles containing 5 ml of water. The bottles were thoroughly shaken for 24 hr and kept aside for 24 hr at room temperature. After 24 hr solution was filtered, suitably diluted filtrate was for silver sulfadiazine content.

Fourier transforms Infrared spectroscopy (FTIR)

The possible interactions between the drug and carrier in the solid state were characterized by FTIR spectrophotometer using ATR technique. The spectra were scanned over a frequency range 4000-400 cm^{-1}.

Differential Scanning Calorimetry (DSC)

The interaction between the pure drug and the carriers during preparation of solid dispersion were assessed by carrying out thermal analysis of drug and polymer alone as well solid dispersion using DSC. Samples(10 mg) were heated in an open aluminium pan at a rate of 20°C/min conducted over a temperature range of 40 to 300°C under a nitrogen flow of 50 mL/min. (DSC 6220 SII Nanotechnology).

X-ray powder diffractometry (XRD)

To determine the sample powder characteristics, X-ray powder diffraction studies of drug and polymer alone as well as solid dispersion was performed. X-ray powder diffraction patterns were recorded on Brukar AXS, DH Advance, and Germany. The scanning rate employed was 6° min–1 over 10 to 50°diffraction angle (2θ) range. To determine the powder characteristics, X-ray powder diffraction studies of drug and polymer alone as well as solid dispersion was performed.

SEM (Scanning electron microscope) Studies

The surface morphology of the drug sample examined in this studies the small amount of sample was manually placed onto a carbon tab. after that it is coated and then sample were examined by SEM and take images under various magnification onto computer. The SEM was used to characterize the morphological changes in solid dispersion. The samples were coated with gold palladium alloy using Jeol/EO fine coat sputter. The samples were then observed at different magnification using Jeol JSM 6360A.

Silver Sulfadiazine Emulgel preparation

The optimized solid dispersion with Poloxamer 407 (1:1) proportion by melt method was used further for the preparation of emulgel. The oil phase of the emulsion was prepared by dissolving (Span 60, calcium oleate,) surfactant's in oil. Tween 80 was dissolved in water to give aqueous phase. Preservative (methyl paraben and Propyl paraben) and solid dispersion then added to aqueous phase. Both the oily and aqueous phases were separately heated to 70° to 80°C. Then heated aqueous phase was added to oily phase continuous constant stirring and cooled to room temperature. Then lavender oil was added. Gelling phase was prepared by the dispersing gelling agents in purified water with constant stirring and the organic phase (oil) with lecithin and both are mixed well to form gel, the pH was adjusted to 5.5 to 6.5 using Triethanolamine. Finally emulgel was prepared by mixing gelling phase and emulsion phase in 1:1 proportion using mortar and pestle to obtain the emulgel.

Table 2: Factors and Levels for the 3^2 Factorial Designs

Sr.no	Factors	Variables	Levels
1	Gelling agents	Pluronic lecithin Gel(PLO)(A1)	-1
		Sepineo P600 lecithin Gel (SLO)(A2)	0
		Carbopol 934 lecithin Gel (CLO)(A3)	+1
2	Oils	Almond oil(A)	-1
		Olive oil (O)	0
		Coconut oil(C)	+1

Where A1 =PLO (Pluronic lecithin), A2= Sepineo P600 lecithin (SLO),

A3= Carbopol 934 lecithin (CLO)

A=Almond oil, O =Olive oil, C = Coconut oil

Table 3: Composition of silver sulfadiazine emulgel formulation

Sr. No.	Formulation code	Oils	Gelling agents
1	AA1	-1	-1
2	AA2	-1	0
3	AA3	+1	+1
4	OA1	0	-1
5	OA2	0	0
6	OA3	0	+1
7	CA1	+1	-1
8	CA2	+1	0
9	CA3	+1	+1

Optimization of Emulgel

Experimental design

Nine silver sulfadiazine emulgel formulations were prepared according to a 3^2 factorial design employing the qualitative factors and levels shown in Table 2. Composition of silver sulfadiazine emulgel formulation was shown in Table 3.

Emulgel Evaluation

Physical appearance

The prepared emulsified gel formulations containing silver Sulfadiazine were inspected visually for their colour, consistency and phase separation.

Consistency

The cone attached to holding rod was dropped from the fix distance of 10 cm such that it should fall on the centre of measuring cylinder filled with emulgel. The distance travelled by cone was noted down after 10 sec.

Measurement of pH

One gram of gel was dissolved in100 ml distilled water and stored for two hrs and pH measured with digital pH meter.

Spreadability

One gram of emulgel was placed between the two glass slides and load of 500 g was applied. The time required to slip off the slides was measured and Spreadability was calculated using formula

$S = M. L / T$

Where M = wt. tied to upper slide

L = length of glass slides

T = time taken to separate the slides.

Rheological Study

The viscosity of different emulsified gel formulation was determined at 37^0C using a Brook field viscometer (Brookfield DV-E viscometer) with helipath spindle no F at three different rpm (10, 50,100).

Globule size and Polydispersity index

Globule size and its distribution were determined by Motic microscope. The 1.0 gm of sample was dissolved in purified water and agitated to get homogeneous dispersion. Sample was added on slide and observes under microscope. Mean globule diameter and Polydispersity index was determined.

Drug Content

Silver sulfadiazine content in emulsified gel was measured by dissolving emulgel in suitable solvent by Sonication. Absorbance was measured after suitable dilution at 254 nm in UV/VIS spectrophotometer (UV-1800 CE, Shimadzu Corporation, Japan).

Swelling index

The swelling index can be calculated by placing 1 gm of emulgel formulation on porous aluminium foil. It was placed in petriplate containing 0.1N NaOH. The sample was weighed after specific time interval and swelling index was calculated using formula.

$\% SW = [(W_t - W_0) / W_0] \times 100$

W_t = weight of swollen emulgel after time t.

W_0 = original weight of emulgel at zero time.

Extrudability

The Extrudability was measured by application of force to the aluminium collapsible tube (weight in grams) containing emulgel. The area of ribbon of emulgel was measured and Extrudability was measured by formula

Extrudability = Applied weight to extrude emulgel from tube (in gm) / Area (in cm^2)

Gel Strength

A sample of 5gm of emulgel was placed in a 100 mL graduated cylinder. Gel strength at physiological temperature, was determined by the time (in seconds) taken by the apparatus to sink down 5 cm through the prepared emulgel[17].

***In Vitro* diffusion study through cellophane membrane**

Franz diffusion cell (with effective diffusion area 3 cm^2 and 30 ml cell volume) was used for the drug release studies. Emulsified gel (500 mg) was applied onto the surface of cellophane membrane evenly. The cellophane membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared PBS (pH 5.8) solution to solubilize the drug. The receptor chamber was stirred by magnetic stirrer. The samples (1.0 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV visible spectrophotometer at 254 nm after appropriate dilutions. Cumulative corrections were made to obtain the total amount of drug release at each time interval.The cumulative amount of drug released across the cellophane membrane was determined as a function of time.

***In Vitro* permeation study by using burn eschar skin**

In this permeation study was used for the find out % drug diffusion from the burn eschar skin. Study done as follows.

Preparation of eschar

Burn Eschar samples were obtained from 2 female (mean age of 30 ± 7 years) patients. The burning in all selected patients was due to flame and only the eschar samples collected from leg regions. Samples were stored at -20°C until use. At this temperature no any damage to the eschar tissue. Before each experiment, eschar samples were thawed, initially in a refrigerator for 24 h and then for another 1 h at room temperature. Samples were then washed with buffered solution and then with water and measured for thickness at room temperature. The samples were then cut into smaller pieces suitable for permeation studies. Eschar samples used for this study showed a thickness of 0.09 ± 0.015 cm (Mean ± SD, n = 3).

General permeation procedure

Franz-type diffusion cells with an effective diffusion area of approximately 3 cm^2 were used for permeation studies. Eschar samples were placed between donor and receptor chambers of the cells, while the epidermal side faced the donor compartment. To control level of Eschar skin hydration initial pre-treatment was given with phosphate buffer for about 24 h, in the diffusion cell only, then the receptor chamber was washed with distilled water and then filled with 30 ml of fresh receptor phase.500 mg of the formulation optimized emulgel was then placed in the donor chamber. The receptor chamber was magnetically stirred. 1 ml samples were collected from the receptor solution at designated time intervals and replaced with the same amount of fresh receptor solution and their drug contents determined. The % drug release permeated was plotted against time. Experiments were performed at Room Temp (32°C), which is the skin surface temperature.

Hydration level

To evaluate the effect of hydration on permeability of eschar towards silver sulfadiazine emulgel, three hydration levels of fully hydrated, semi-hydrated and dry eschar tissues were used. These hydration levels were obtained by controlling the contents of receptor and donor chambers of the diffusion cells in the 24 h-pretreatment phase described above while the eschar was mounted on the diffusion cells. Fully- hydrated samples were prepared by placing phosphate buffer 5.8 in both donor (5ml) and receptor (30ml) chambers throughout the pretreatment phase. For semi-hydrated samples, 30 ml water was placed in each receptor

chamber, while the donor chambers were kept empty. In case of the dry eschar samples there was no water within donor or receptor chambers, during the pretreatment phase. During the pretreatment phase, cells were refrigerated for 24 hours, followed by keeping at room temperature for 1 hour. Then the different levels of hydrated eschar were used for diffusion studies.

Antimicrobial activity

Weighed 29.5gm of sabouraud dextrose agar was transferred in a 500 ml of conical flask and 500 ml of purified water and some amount of heat is applied to dissolve it completely. Sterilized for 15 min at 121°C at 15 lb pressure in autoclave for about 20 min. Then cooled it at room temperature and the bacterial strain(staphylococcus aurous) was dispersed in the medium and then the medium was poured it in to the three petridish and allowed it cool it for sometime at room temperature until it forms solidifies at room temperature and then the three cups are bored in each petridish with the help of sterile steel bore of 6 mm and calculated concentration of the standard drug (silver sulfadiazine),formulation (CA2) and without drug Gels were placed in the bores with help of 18 gauges needles and incubated the petri plates for 18 h at 37°C in incubators. Then the zone of inhibition was observed and calculated the radius of the zone of inhibition.

RESULT AND DISCUSSION

Silver sulfadiazine was found to be soluble in organic solvents such as 0.005 % ammonium solution. A simple reproducible method of estimation was carried out in ammonium solution at 254 nm against the blank the standard graph obtained was linear; with regression coefficient 0.996.Silver sulfadiazine is very slightly soluble in water and having poor bioavailability and coming under the category of class 4 of biopharmaceutical classification (BCS) system. So in order to improve its solubility we prepared solid dispersions using various techniques[8].The solubility of silver sulfadiazine at 30^{0}C was found to be 0.0034±0.24mg/ml, therefore silver sulfadiazine is considered as a practically less soluble drug. The drug content in all the tested combinations was found to be in the range of 89 to 98% for solid dispersion respectively. The results of phase solubility were confusing as the solubility values varied a lot due to the generation of nanosized particles due precipitation reactions. In the phase solubility study solubility is increase in Melt method with Poloxamer 407[10]. Therefore use of FTIR, XRD and DSC was done to optimize the formulation for further studies. According to XRD, FTIR, DSC it found that due to formation of solid dispersion with Poloxamer the form of drug change it's converted to amorphous form and finally solubility increased.

Table 4: Drug content, saturation solubility of silver sulfadiazine solid dispersion

S. No.	Method	Polymer	Formulation Code	Saturation solubility mg/ml
1.			Pure drug	0.0034 ±0.24
2.	Lyophilization method	Poloxamer 407	L1	0.011±0.54
			L2	0.011 ±0.52
			L3	0.0037 ±0.54
		Poloxamer 188	AL	0.0060 ±0.66
			BL	0.0037 ±0.33
			CL	0.0040 ±0.20
3.	Solvent melt method	Poloxamer 407	SM1	0.0035 ±0.19
			SM2	0.0037 ±0.65
			SM3	0.0043± 0.45
		Poloxamer 188	ASM	0.0054 ±0.56
			BSM	0.011 ±0.20
			CSM	0.013 ±0.19
4.	Solvent evaporation method	Poloxamer 407	SE1	0.00603±0.18
			SE2	0.0037 ±0.12
			SE3	0.0040 ±0.13
		Poloxamer 188	ASE	0.0093± 0.80
			BSE	0.011 ±0.31
			CSE	0.16 .22
5.	Melt method	Poloxamer 407	M1	0.0188 ±0.11
			M2	0.0036 ±0.14
			M3	0.0037 ±0.25
		Poloxamer 188	AM	0.013 ±0.33
			BM	0.015 ±0.12
			CM	0.007 ±0.45

X-ray powder diffractometry (XRD)

X-ray diffractogram of silver sulfadiazine, poloxamer407, Poloxamer 188 and solid dispersion shown in Fig 1. The diffraction pattern of the pure silver sulfadiazine showed its microcrystalline nature, as indicated by the numerous distinctive peaks with major characteristic diffraction peaks appearing at a diffraction angle of 2θ at 13.27°, 25.70° and 41.43°. The spectrum of Poloxamer 407 was characterized by the complete absence of any diffraction peak. The diffraction pattern of Poloxamer 407 solid dispersion (M1:2) shows the peaks of silver sulfadiazine with reduction in peak intensities indicating that the conversion of crystalline form to partial amorphous state. These assumptions were found to be in full agreement with the results presented by the DSC and FTIR studies.

FTIR Studies for solid dispersion

The FTIR spectrum of silver sulfadiazine, Poloxamer 107, Poloxamer 188 and its solid dispersions as shown in Fig 2a- 2h. The spectrum marked as 'E' represent the drug, 'D' represent the carrier (Poloxamer 107 or Poloxamer 188) and 'A', 'B', 'C' represents FTIR of solid dispersion with drug: carrier ratio 1:1, 1:2, 1:3 respectively. FTIR spectra of silver sulfadiazine shows prominent peak in the region of 3390 cm^{-1} due to the N-H Asymmetric, a peak at 1595cm^{-1} due to phenyl skeletal, a peak at 1560 due to pyrimidine skeletal, a peak at 1230 cm^{-1}due to asymmetric (SO_2).Solid dispersion of drug with Poloxamer 107 or Poloxamer 188 shows summation of the spectra of the drug and polymer. It shows overlapping of N-H groups and broadening of the peak was observed. However other peaks remain unchanged. This indicates that overall symmetry of the molecule might not be significantly changed.so no inter action was observed in any of the formulation.

Differential Scanning Calorimetry

Thermal analysis of drug as well as polymer & solid dispersions was carried out using DSC as shown in Fig.3 The DSC curve of silver sulfadiazine profiles a sharp exothermic peak at 289.3°C nearly (reported 290^{0}C) to its melting temperature and indicating its

microcrystalline nature. The thermogram of neat Poloxamer 407 and Poloxamer 188 exhibited a broad endothermic ranging from 51.3°C, 57.4°C was observed. In the solid dispersion of Poloxamer 407 & Poloxamer 188 demonstrated a broadening of the silver sulfadiazine exothermic peak together with a shift to a higher temperature. It could be explained by the formation of crystalline microaggregates of the drug and their considerable dispersions within the amorphous polymeric matrice. It indicated that silver sulfadiazine no longer present in the crystalline form, may have got converted into the amorphous form.

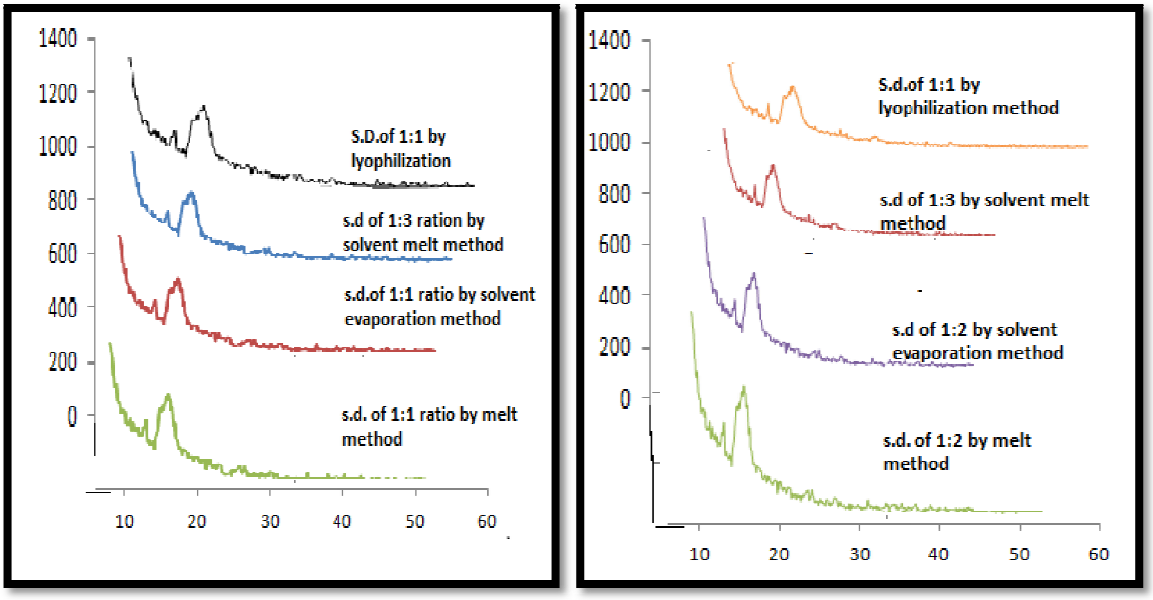


Fig. 1: XRD patterns of silver sulfadiazine and its solid dispersion of Poloxamer 407 and188.

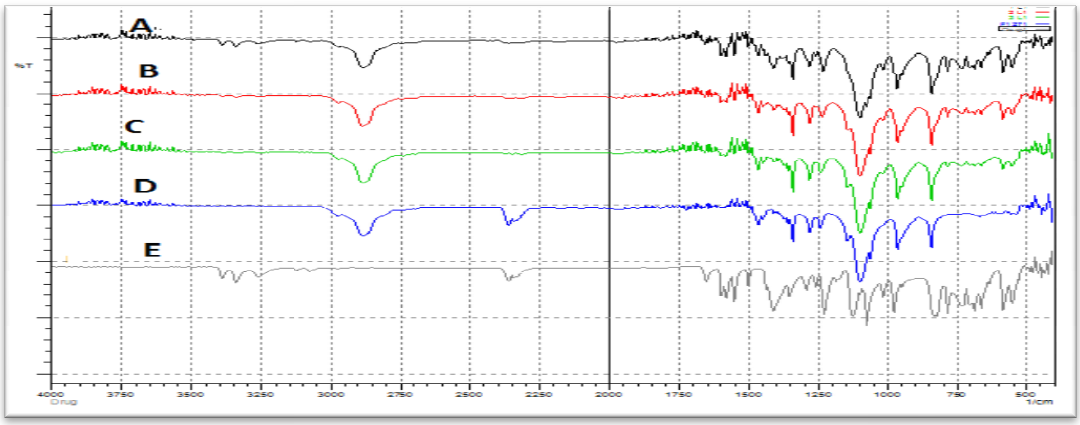


Fig. 2.a: IR of solid dispersion with Poloxamer 407& Drug by lyophilization method

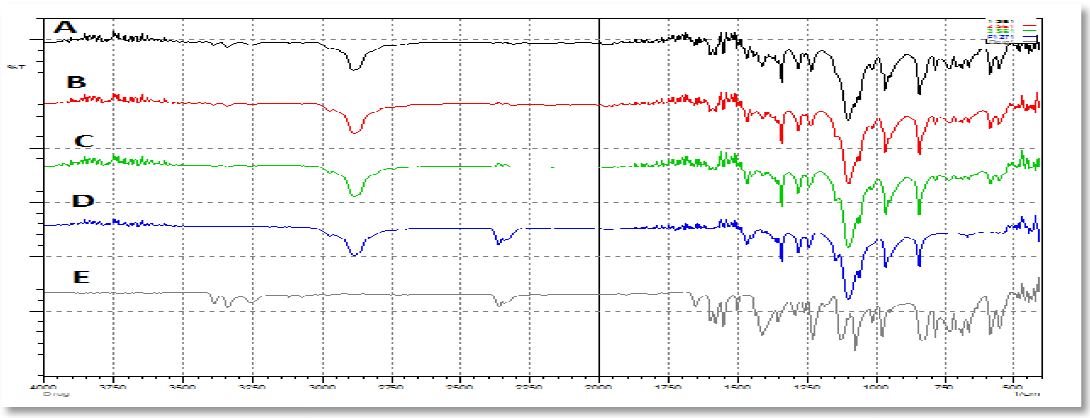


Fig. 2.b: IR of solid dispersion with Poloxamer 407&Drug by Solvent evaporation method

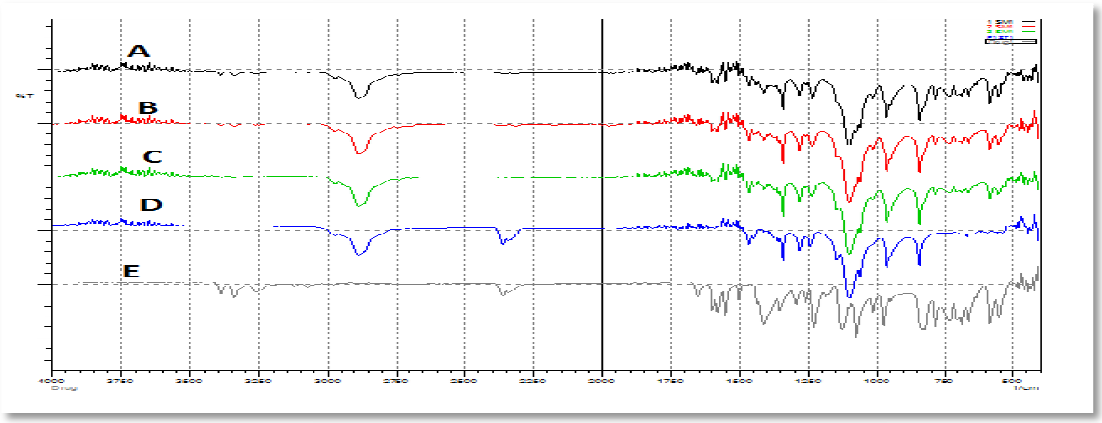


Fig. 2.c: IR of solid dispersion with Poloxamer 407 &Drug by Solvent melt method

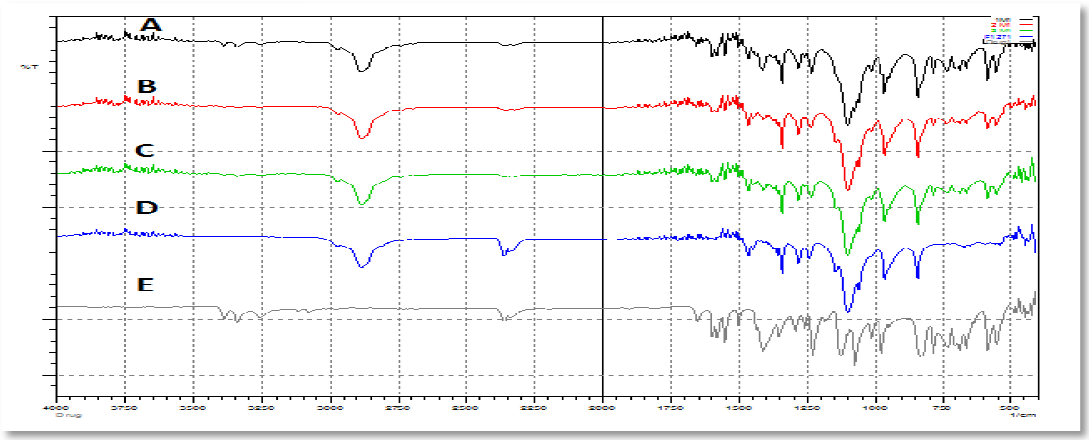


Fig. 2.d: IR of solid dispersion with Poloxamer 407 &Drug by Melt method

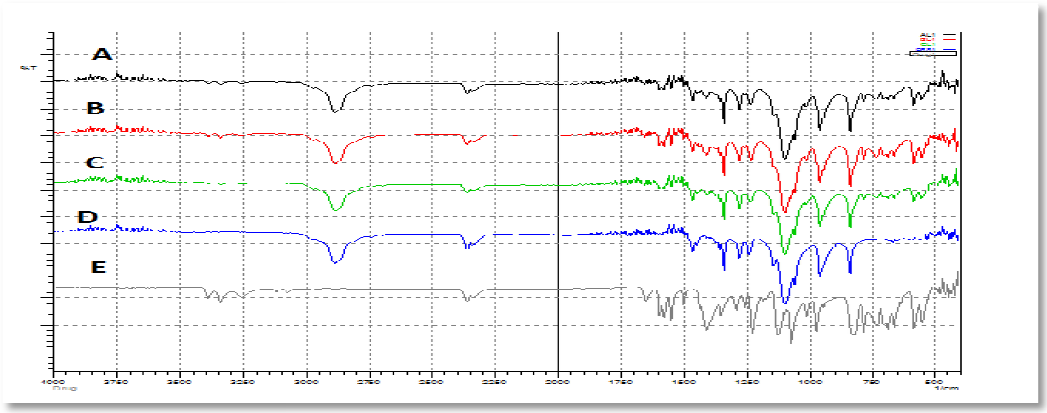


Fig. 2.e: IR of solid dispersion with Poloxamer 188 & Drug by lyophilization Method.

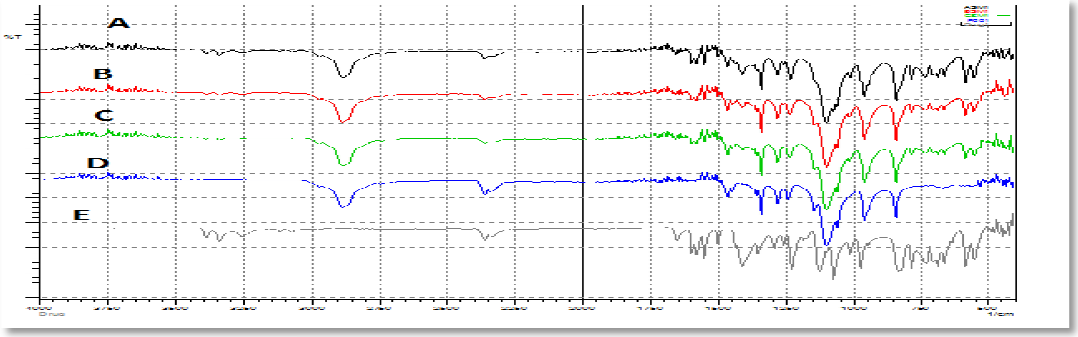


Fig. 2.f: IR of solid dispersion with Poloxamer 188 &Drug by Solvent melt method

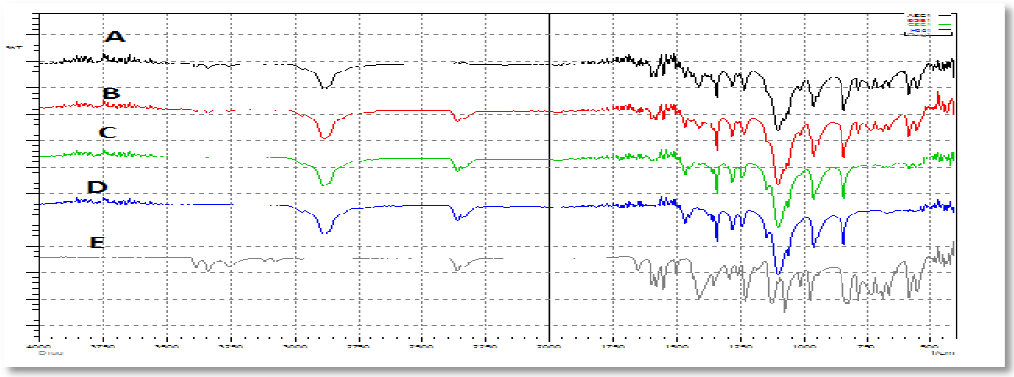


Fig. 2.g: IR of solid dispersion with Poloxamer 188&Drug by Solvent evaporation method.

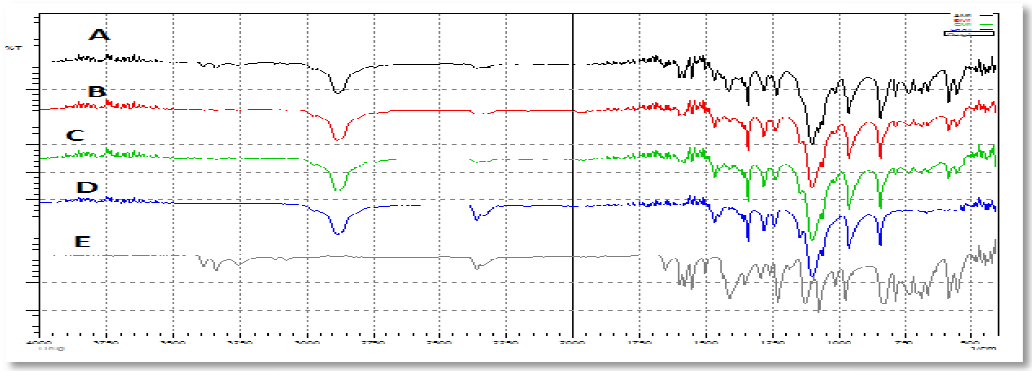


Fig. 2.h: IR of solid dispersion with Poloxamer 188 &Drug by melt method.

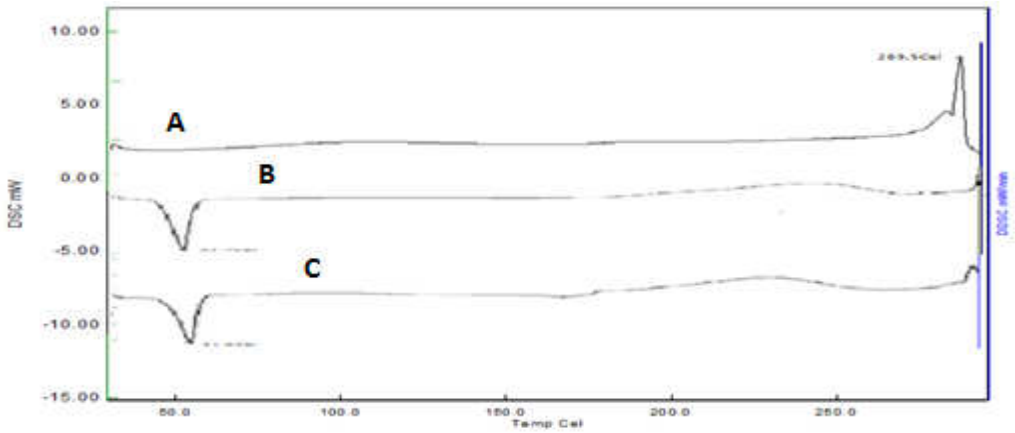
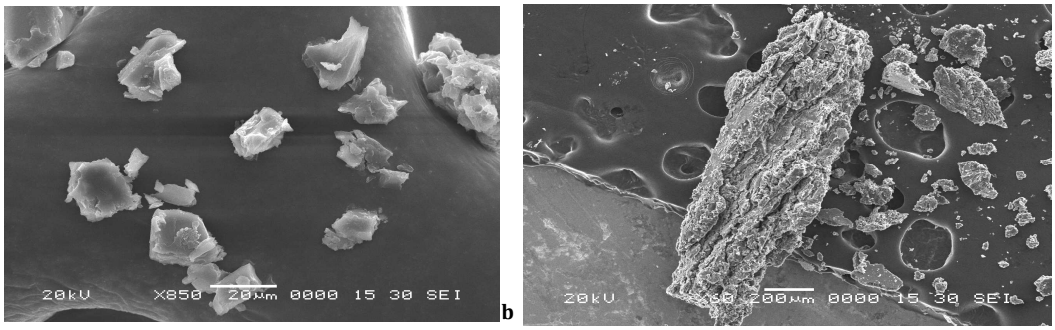


Fig. 3: DSC Thermograms of (A) silver sulfadiazine, (B) solid dispersion of Poloxamer 407 (C) solid dispersion of Poloxamer 188



a b

Fig. 4: SEM studies for solid dispersion of a) Poloxamer 407 b) solid dispersion

Therefore the Solid dispersion with poloxamer 407 (M1:1) was selected as optimized dispersion for further emulgel formation.

SEM Studies

SEM studies indicated that pure drug particles were microcrystalline in nature, the solid dispersion of silver sulfadiazine with Poloxamer 407& Poloxamer 188 showed a homogeneous dispersion indicating the drug molecule dispersed uniformly in carrier matrices prepared by melting method assuming amorphous solid dispersion state. (Fig. 4)

Emulgel evaluation

The gelling agent concentration can optimize by finding gelling concentration at body temperature. The prepared emulsified gel formulations containing silver sulfadiazine were inspected visually for their colour, Consistency and phase separation[11, 12]. The physical appearance of emulgel were as given in Table 5, all formulation were physically stable.

The pH values of all prepared formulation ranged 5 to 6, which are acceptable to avoid the risk of irritation upon application to the skin because skin pH is 5.5.

More is the value of spreadability is good for application on the skin. Spreadability of all formulation ranges form15±0.95 g.cm/sec to 60±0.45. So here AA2, CA2 have good spreading ability[13].The Extrudability is higher it means higher force required to extrude the material from the tube. So here less force is require for AA2 formulation. (Table 6) The gel strength was found in the range of 109.66 to 137.6 g/cm^2. All the emulgel formulation had small average globule size between 676.23 ± 5 nm. and Polydispersity index was found that 0.10 ± 5, so it indicated homogeneous dispersion of droplets in the formulation. Small droplet size was preferred in terms of skin penetration[14]. All the formulation showed uniformity in drug content and were within permissible range. Viscosity of the formulation varied according to type of gelling agent used in the formulation showing highest viscosity for PLO gel than CLO. The in vitro release profile of silver sulfadiazine from its various formulation are represented in Table.7.The better release of the drug from all formulation was observed and the emulgel Formulation ranked its descending order

: CA2>AA2>OA2>OA3>CA1>AA1>CA3>AA3>OA1.

Where the amount of drug release after 6 hr was calculated and the highest drug release found into CA2. This might due to presence of Sepineo P 600 lecithin gelling agent which leads to increase the hydrophilicity of the emulgel because contained acrylamide/ sodium acrloyl dimethyl taurate copolymer having self emulsifying ability[15]. It forms stable emulgel. This in turn facilitates penetration of the release medium into the emulgel and diffusion of the drug from the emulgel[16].The lower drug release in CLO, PLO gelling agent might be due to high viscosity observed in Table 6, this may be due to the network structure of Carbopol 934 and lecithin and in case PLO there is block copolymer of ethylene oxide and propylene oxide.

Table 5: Physical appearance of various formulations

S. No.	Formulation code	Colour	Consistency(mm)*	Phase separation
1	AA1	Cream colour	5	None
2	AA2	White	10	None
3	AA3	White	5	None
4	OA1	Cream colour	5	None
5	OA2	White	11	None
6	OA3	White	5	None
7	CA1	Cream colour	6	None
8	CA2	White	10	None
9	CA3	White	6	None

(*Mean ± S.D. n=3)

Table 6: Evaluation parameter of Silver Sulfadiazine Emulgel

Formulation code	pH*	Spreadability gcm/sec*	Extrudability* (g/cm^2)	Viscosity(cps)* 10 rpm	Gel strength* (g/cm^2)
AA1	5.91	30	15.1	930	109.66
AA2	5.45	60	10	460	115.33
AA3	5.05	17.41	20	330	117.50
OA1	5.08	24	22	1370	125.33
OA2	5.6	50	12	650	128.33
OA3	5.8	15	15	1125	131.64
CA1	5.66	26.08	30	925	134.66
CA2	5.59	54.54	25	705	135.66
CA3	5.49	15.78	40	605	137.6

(*Mean ± S.D. n=3)

Table 7: Evaluation parameter of Silver Sulfadiazine Emulgel

Formulation code	Globule size (nm)*	Polydispersity Index*	% Drug content*	% Drug diffuse through cellophane membrane at 6hr*
AA1	676.08	0.13	88.00	68.62
AA2	675.21	0.10	95	77.16
AA3	674.12	0.13	85.62	51.04
OA1	678.2	0.12	81.42	50.35
OA2	676.4	0.14	96	76.43
OA3	676.54	0.15	84.37	75.55
CA1	673.21	0.14	84.29	68.94
CA2	675.32	0.16	98.00	80.51
CA3	676.28	0.15	85.71	66.13

*(Mean ±S.D.n=3)

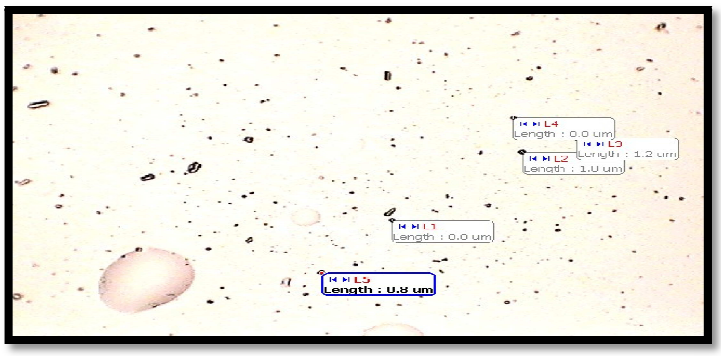


Fig. 5: Globule size of emulgel

Optimization

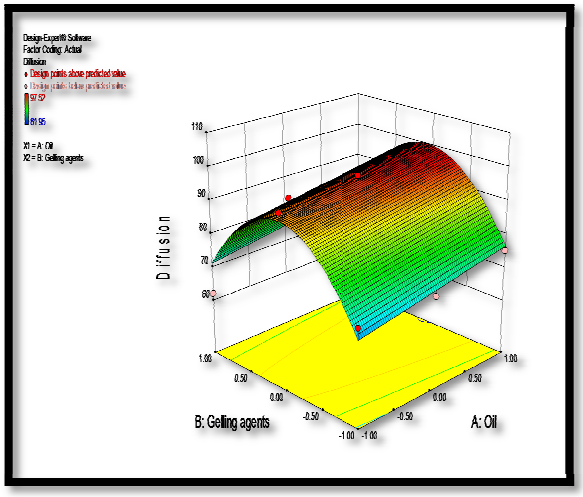
The data obtained were treated using Stat Ease Design Expert Software and analysed stastically using analysis of variance (ANOVA). The model F- value of 9.48 implies the model is significant. The values in this model Probe value found was 0.0039. The data clearly indicates that the %drug diffusion and viscosity values are strongly dependent on the selected independent variables. The fitted regression equations relating the responses %drug diffusion and viscosity are shown in the equations, respectively. The equation conveyed the basis to study of the effects of variables. The regression coefficient values are the estimates of the model fitting. The r^2 was high indicating the adequate fitting of the quadratic model. The polynomial equations can also be used to draw conclusions considering the magnitude of co-efficient and the mathematical sign it carries i.e. positive or negative. The positive coefficient of variable X_1 i.e. Oils in case of response diffusion indicates that as the types of oils vary, diffusion increased accordingly for coconut oil. However, the positive coefficient for viscosity t indicating that the changing the oil leads to increased viscosity value and changing gelling agent decreased the viscosity. The polynomial equation as follows:

Final equations in Terms of Actual Factors (Release)

Drug release = 96.08 + 4.08 (oils) + 1.45 (gelling agent) - 22.10(gelling agent) ^{2}

Final equations in Terms of Actual Factors (viscosity)

Viscosity = 109.88+12.16(oils) - 39.41(gelling agents)



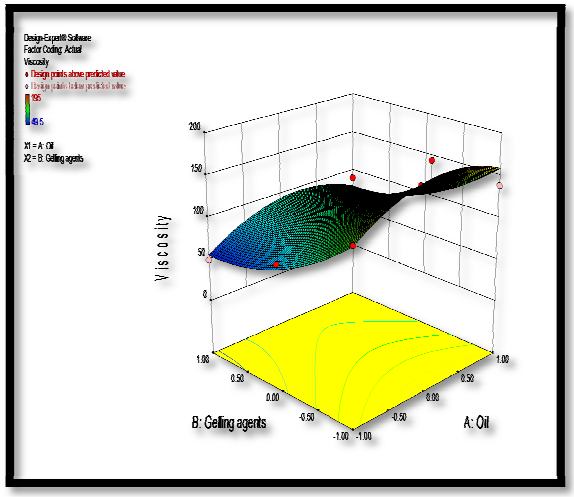


Fig. 6: 3D plot for diffusion response Fig. 7: 3D plot for viscosity study

In the 3D plot for diffusion response indicate that in the combination of SLO gelling agent shows maximum diffusion of drug.

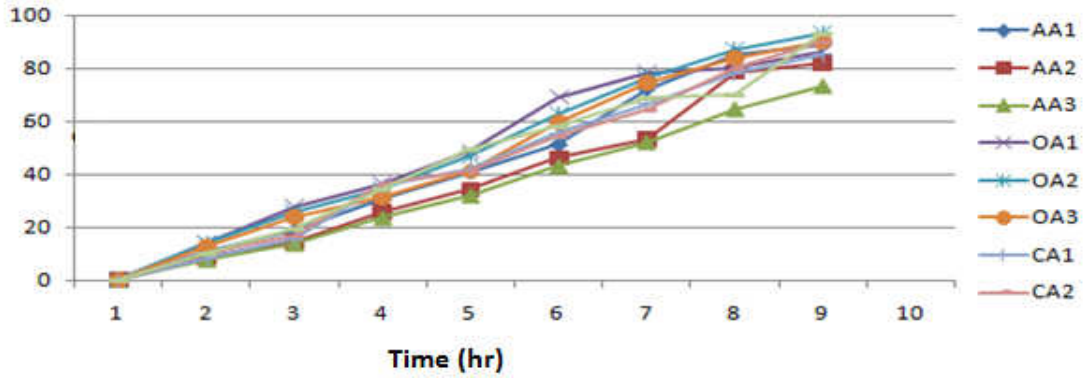


Fig. 8: swelling index of silver Sulfadiazine emulgel formulation

Swelling index

The formulation OA2, CA3, OA3 having maximum swelling index properties in comparison of other it means emulgel formulating having greater swelling tendency i.e. tendency to absorb extrudates from wound. (Fig. 8)

***In Vitro* permeation study by using burn eschar skin**

Based on diffusion, viscosity and drug content optimized formulation CA2 was selected for permeation through fully hydrated, semi hydrated and dry eschar samples. Results in Fig. 9 shows that when dry skin is used for permeation, permeation is high for 6-8 hrs where as permeation is slow in fully hydrated and semihydrated for first few hours i.e. 1-3 hrs then there is sudden increase in permeability. This increase in permeability is more for hydrated Escher skin then semihydrated Escher skin. Permeation through dry eschar ceases and reaches a plateau after 6-8 hrs while permeation continues to increase in other two systems. This might show that the dry eschar tissue has microscopic cracks which could be closed during hydration. The cracks, which may possibly in dry tissues, provide permeable pathways (possibly channels filled with surrounding medium) and, after closure, e.g. due to hydration, permeation ceases[18]. The results of the other two systems show that higher hydration levels will eventually overcome this problem by providing more permeable pathways in the hydrated systems. Semi-hydrated eschar showed a biphasic permeation profile. This system was hydrated only from the receptor side during the pretreatment phase. Therefore, the system is not fully-hydrated, and therefore its permeability, can change during the experiment, which could be the reason behind observing the biphasic system. Comparison of the fully-hydrated and semi-hydrated systems shows that after these stages, greater hydration levels result in higher permeabilities. Water interacts with proteins which are present in both the eschar tissue and the stratum corneum. Water also interacts with polar groups of stratum corneum's lipid bilayers and disorders packing at the polar plane and improves diffusion of drug through the barrier[19, 20]. A mechanism that might still be important in the burn eschar. These results show that permeation of drugs through burn eschar could be severely increased by full hydration of the tissue, a process that is easily achievable in burn patients by covering the eschar with occlusive dressings. These results show that hydration promotes eschar permeation to a large extent. The emulgel as a vehicle can change the efficacy, by providing hydration and occlusive dressing.

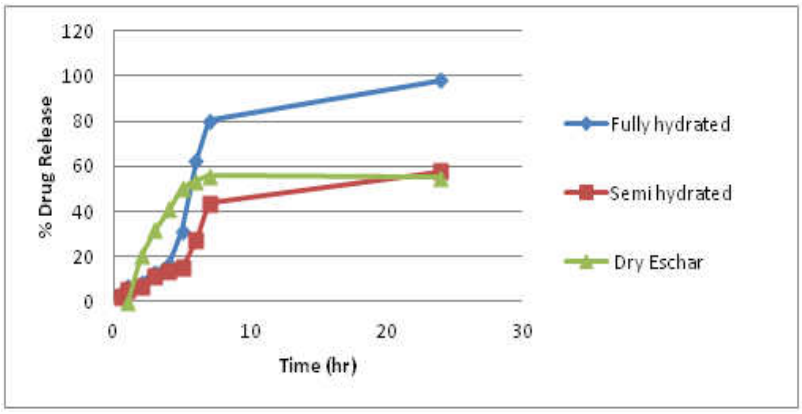
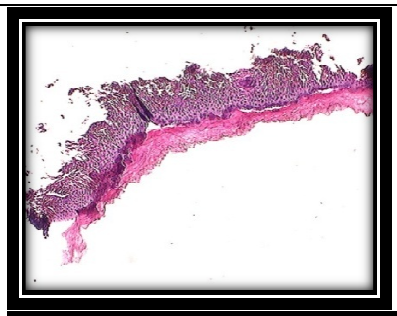


Fig. 9: Cumulative amount of silver sulfadiazine permeated through, fully hydrated, semi-hydrated and dry burn eschar tissue.

Histopathology examination

Histopathology observation of burn eschar skin after diffusion as follows in Table 8:

Table 8: Histopathophathological evaluation of burn eschar skin

S. No.	Burn eschar skin Type	Observation	Images
1	Untreated	In this section epidermis with minimal dehydration with adequate keratin layer. Skin Coagulation observed	
2	Treated Full hydrated	In this no evidence of dehydration.No microscopic cracks observed. Epidermal layer show, Stratum corneum intact. No coagulation is observed. There is no evidence of necrosis of the upper Dermis with treatment.	

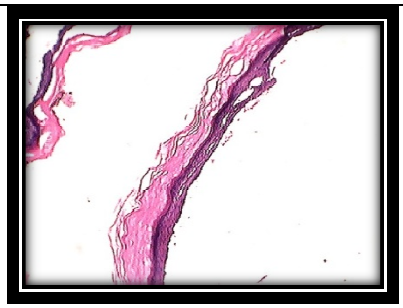
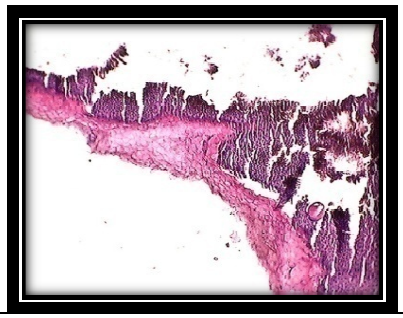
3	Treated semi hydrated	In this section moderate dehydration with atrophic changes. Keratin layer is reduced. No microscopic cracks. Intact epidermal layer. Intact stratum corneum. There is no evidence of necrosis of the upper Dermis with treatment.	
4	Treated dry hydrated	In this section revealed severe atrophic epidermis with minimal keratin layer microscopic cracks noted. Stratum corneum breaks.	

Table 9: Zone of inhibition of formulation

Test micro-organism	**Zone of inhibition (mm)**		
	CA2	**Control (Without drug)**	**Silver sulfadiazine Cream (1%)**
Staphylococcus aurous	53.20	52.26	51.22

Antimicrobial activity of emulgel

As per the Table 9 maximum zone of inhibition was found for emulgel formulation when compared with marketed formulation and control i.e. emulgel without drug. In control formulation zone of inhibition is shown might be due to coconut oil itself act as antimicrobial agent. It contain Lauric acid present in the oil can kill bacteria. It also have antibacterial and anti fungal activity[21].

CONCLUSION

Silver sulfadiazine was slightly soluble in water so solubility is enhanced by solid dispersion formulations. Melt method with 1:1 drug poloxamer 407 ratio show maximum increased solubility. Sepineo P600 lecithin Gel (SLO) and coconut oil containing emulgel shows more in vitro diffusion and good Spreadability. Histopathology study of burn eschar skin shows intact stratum corneum with no tissue necrosis and damage and also gives maximum diffusion of drug from fully hydrated burn skin.

ACKNOWLEDGEMENT

We would to thank Mr M.J. Patil, Principal MMM's College of Pharmacy, and Pune for his constant support and encouragement and also thankful to BJ medical college, Sassoon hospital, Pune for providing burn eschar skin.

REFERENCE

1. Kominos SD, Yee RB. In Vitro Penetration of Topical Antiseptics through Eschar of Burn Patients; Ann. Surg 1975; 183 (4):358-364.
2. Nascimento EG D, Sampaio TBM.Evaluation of chitosan gel with 1% silver sulfadiazine as an alternative for burn wound treatment in rats, Acta Cirúrgica Brasileira 2009; 24 (6):460-465.
3. Fox C L, Modak S. Mechanism of Silver Sulfadiazine Action on Burn Wound Infections, Antimicrobial agent and chemotherapy 1974; 5 (6): 582-588.
4. Gear AJL, Hellewell TB.A new silver Sulfadiazine water soluble gel, Burn 1997; 23(5):387-381.
5. Laithy H M, Shaboury KMF.The Development of Cutina Lipogels and Gel Microemulsion for Topical Administration of Fluconazole AAPS Pharm Sci tech 2002; 3 (4): 77–85.
6. Escobar JJ.et al.Applications of Thermoreversible Pluronic F-127 Gels in Pharmaceutical Formulations, J Pharm pharmaceut sci 2006; 9 (3): 339-358.
7. Jain A, Gautam SP, Gupta Y et.al. Development and Characterization of Ketoconazole Emulgel for Topical Drug Delivery. Der Pharmacia sinica 2010; 1(3):221-231.
8. Kalyanwat R, Patel S.Solid Dispersion, a Method for Enhancing Drug Dissolution. International Journal of Drug Formulation & Research 2010; 1(3):1-14.
9. Ranalwaya N, Pokharna G, Deb L, Jain N. Wound Healing Activity of Latex of Calotropis Gigantea. International Journal of Pharmacy and Pharmaceutical Sciences 2009; 1(1):176:181.
10. Kumar P, Chander M et al. Physiochemical Characterization and Release Rate Studies of Solid Dispersions of Ketoconazole with Pluronic F127 and PVP K-30 Services. Iranian Journal of Pharmaceutical Research 2011; 10 (4): 685-694.
11. Shivhare UD et.al. Formulation Development and Evalution of Diclofenac Sodium Gel Using Water Soluble Polyacrylamide Polymer.Digest Journal of Nanomaterials and Biostructure 2009; 4(2):285-290.
12. Kumar L, Verma R.Formulation, In Vitro Evaluation and Stability Studies of Optimized Bioadhesive Topical Gel, Journal of Pharmacy Research 2010; 3(7):1506-1509.
13. Khullar R, Saini S, Seth N, Rana AC. Emulgels, a Surrogate Approach for Topically Used Hydrophobic Drugs. IJPBS 2011 ;(1):117-128.
14. Vijayan V, Rao SD. et al. Preparation and Characterization of Anti Diabetic Drug Loaded Solid Lipid Nanoparticles Jitps 2010; 1 (8): 320-328.
15. Bonacucina G.et al. Characterization and Stability of Emulsion Gels Based on Acrylamide/Sodium Acryloyldimethyl Taurate Copolymer. AAPS Pharm SciTech 2009; 10(2):368-375.
16. Kumar L, Verma R.In Vitro Evaluation of Topical Gel Prepared Using Natural Polymer. International Journal of Drug Delivery 2010 ;(2): 58-63.

17. Badgujar SD, Sontakke MA et al. Formulation and Evaluation of Sumatriptan Succinate Nasal In-Situ Gel Using Fulvic Acid as Novel Permeation Enhancer, IJPRD 2010; 2(8): 007.
18. Sharif B, Zadeh M.et al. Effect of Hydration on Barrier Performance of Third-Degree Burn Eschar. Iranian Journal of Pharmaceutical Research 2006; 5(3): 155-161.
19. EL-Kattan AF, Asbill CS and Michniak BB. The Effect of Terpene Enhancer Lipophilicity on the Percutaneous Permeation of Hydrocortisone Formulated In HPMC Gel Systems. International Journal of Pharmaceutics 2000; 198(2):179–189.
20. Hamid R. Moghimi A.et.al. Enhancement Effect of Terpenes on Silver Sulphadiazine Permeation through Third-Degree Burn Eschar, Burns 2009 ;(35): 1165 –1170.
21. Deveda P, Jain A et al. Gellified Emulsion For Sustain Delivery Of Itraconazole For Topical Fungal Diseases. International Journal of Pharmacy and Pharmaceutical Sciences 2010; (2)1:104-111.