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## FORMULATION AND INVITRO EVALUATION OF ETHOSOMAL GELS CONTAINING SULFASALAZINE AS THE MODEL DRUG

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### ABSTRACT

The method described by Touitou was employed with little modification for the preparation of various ethosomal formulations containing different concentration of ethanol (20 % to 50 %) with sonication. The techniques used were simple and reproducible. The prepared ethosomes were spherical and discrete in shape. However ethosomes prepared by sonication method were more uniform and small in size which is essential for skin penetration. While comparing the entrapment efficiency, ethosomes containing 30% w/w IPA and prepared by sonication showed highest value respect to all other formulation; so it is concluded ethosomal prepared by sonication and containing 30 % w/w IPA as the best formulation considering all other aspects. Increase in the polymer concentration led to increase in, % Drug entrapment efficiency, Particle size. The *invitro* drug release decreased with increase in the polymer and copolymer concentration. Among all formulations F6 shows Maximum drug release in 1440 min when compared with other formulations. Analysis of drug release mechanism showed that the drug release from the formulations followed the Non fickian diffusion mechanism and follows First order kinetics. Based on the results of evaluation tests formulation coded F6 was concluded as best formulation.

**Key Words:** Ethosomes, Sonication, Transdermal, Entrapment, Stability

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### INTRODUCTION

Optimization of drug delivery through human skin is important in modern therapy. Recently, the transdermal route vied with oral treatment as the most

That delivers precise amount of drug through the skin for systemic action. Improved methods of drug delivery for biopharmaceuticals are important for two reasons; these drugs represent rapidly growing portion of new therapeutics, and are most often given by injection. Discovery of new medicinal agents and related innovation in drug delivery system have not been only enabled the successful implementation of novel pharmaceutical, but also permitted the development of new medical treatment with existing drugs.

Throughout the past two decades, the transdermal patches have become a proven technology holding the promise that new compound could be delivered in a safe and convenient way through the skin. Since the first transdermal patch was approved in 1981 to prevent nausea and vomiting associated with motion sickness, the FDA has approved through the past 22 years more than 35 transdermal patch products spanning 13 molecules. Transdermal route offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, and most importantly, it provides patient convince. But one of the major problems in transdermal drug delivery is the low penetration rate through the outer most layer of skin. Liposomes were discovered in the early 1960's by Bangham and colleagues (Bangham et al., 1965) and subsequently became the most extensively explored drug delivery system. In early 1960's a great knowledge of vesicle derivatives have been tested for their abilities. Most experiments, however, have centered on liposomes, since derivations only add to their basic properties. Vesicles are closed, spherical membrane that separate a solvent from the surrounding solvent. Possible use of liposomes in topical drug delivery vehicles for both water and lipid soluble drug has been investigated. While it has been suggested that the external envelop of a liposomes would allow it to pass through lipophilic skin, most researches show that liposomal vesicles become trapped within the top layer of the stratum corneum cells<sup>2</sup>. Generally liposomes are not expected to penetrate into viable skin, although occasional transport processes were reported<sup>1</sup>. This behavior is useful both for local treatment of skin disorders and for cosmetic formulations. Specific drug accumulation at the site of action and decreased systemic drug absorption can impart increased efficiency as well as decreased side effect to a compound applied topically. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicle structures for use in better drug delivery within

their cavities that would allow tagging the vesicle for cell specificity. Vesicles would also allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and would be able to release just the right amount of drug and keep that concentration constant for longer periods of time. One of the major advances in vesicle research was the finding a vesicle derivative, known as an ethosomes. Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipid (Phosphotidyl choline; PC), ethanol at relatively high concentration and water. It was found that ethosomes penetrate the skin and allow enhanced delivery of various compound to the deep strata of the skin or to the systemic circulation (1-6).

The increasing need to deliver medication to patients efficiently with fewer side effects and improved compliance has accelerated the pace of invention of new drug delivery system. Revolutionary drug delivery technology is extended to transdermal route apart from oral. The ability to increase the transdermal permeation can be valuable aid when oral administration of drug is associated with problems. Hence there is a need to modify route of administration for better absorption of the drug. The transdermal route of administration may be better suited. Transdermal penetration of Sulfasalazine cannot be increased by niosomes or liposomes because of its size and rigid character of lipid layer. Recent advancement to increase permeation by reducing the size of carrier and making the lipid layer malleable gave novel drug carrier "Ethosomes" which has shown its effectiveness to increase skin penetration of drugs to several folds then that of simple cream, liposomal carrier and hydroalcoholic solutions. Hence there is a need for preparation of Sulfasalazine ethosomes for enhanced penetration through the skin, thereby reducing dose, minimizing frequency of administration and adverse affects, hence better patient compliance. These advantages of Sulfasalazine ethosomal gel includes avoidance of first pass metabolism, predictable and extended duration of action, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in the blood levels, and most

important, it provides patient convenience. The purpose of the present investigation is aimed to prepare and evaluate Sulfasalazine ethosomes containing different concentration of ethanol and phospholipids by sonication for size reduction of vesicles.

## MATERIALS AND METHODS

### Preparation of sulfasalazine ethosomes (by cold method) (7-10)

Preparation of Sulfasalazine ethosomes was followed by method suggested by Touitou et al., with little modification. The ethosomal system of Sulfasalazine comprised of 2-4 % phospholipids, 20-40 % isopropyl

Alcohol, 10 % of propylene glycol, 0.005g of cholesterol and aqueous phase to 100 % w/w.

Sulfasalazine 0.5 g was dissolved in IPA in a covered vessel at room temperature by vigorous stirring (Table-1). Propylene glycol was added during stirring. This mixture was heated to 30<sup>0</sup> in a separate vessel and was added to the mixture drop wise in the center of the vessel, which was stirred for 5min at 700rpm in a covered vessel the vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation is stored under refrigeration.

**Table-1 Composition of different ethosomal formulations**

Ethosomal formulation	Lecithin (Soya lecithin%)	IPA(%)	Propylene glycol (%)	Drug (g)	Cholesterol(g)	Water
IF <sub>1</sub>	2ml	20ml	5ml	0.5g	0.005g	100ml
IF <sub>2</sub>	3ml	20ml	5ml	0.5g	0.005g	100ml
IF <sub>3</sub>	4ml	20ml	5ml	0.5g	0.005g	100ml
IF <sub>4</sub>	2ml	30ml	5ml	0.5g	0.005g	100ml
IF <sub>5</sub>	3ml	30ml	5ml	0.5g	0.005g	100ml
IF <sub>6</sub>	4ml	30ml	5ml	0.5g	0.005g	100ml
IF <sub>7</sub>	2ml	40ml	5ml	0.5g	0.005g	100ml
IF <sub>8</sub>	3ml	40ml	5ml	0.5g	0.005g	100ml
IF <sub>9</sub>	4ml	40ml	5ml	0.5g	0.005g	100ml

### Preparation of Sulfasalazine ethosomal gel

The best achieved ethosomal vesicles suspension, was incorporated into carbopol gel (1%, 1.5%, 2% w/w).the specified amount of carbopol 934 powder was slowly added to ultrapure water and kept at 100<sup>0</sup>c for 20min. tri ethanolamine was added to it drop wise. Appropriate amount of formula IF-2 containing Sulfasalazine (1.5% w/w) was then incorporated into gel-base. Water q.s was added with other formulation ingredients with continuous stirring until homogenous formulation were achieved (G-1, G-2 and G-3).Gel containing free Sulfasalazine was prepared by similar method using 1.5% carbopol (Table-2).

**Table-2 Composition of different ethosomal gel formulation**

Gel formulation	Sulfasalazine ethosomal suspension(ml)	Carbopol 934(%)	Triethanolamine (ml)	Phosphate buffer (pH 6.8)
G-1	20ml	1	0.5	q.s
G-2	20ml	1.5	0.5	q.s
G-3	20ml	2	0.5	q.s

## Characterization of ethosomes (7-10)

### Entrapment efficiency

The entrapment efficiency of Sulfasalazine by ethosomal vesicle was determined by ultracentrifugation 10 ml of ( ethosomal suspension )<sup>18</sup>. each sample was vortexed for 2 cycles of 5 min with 2 minutes rest between the cycles.

1.5ml of each vortexed sample and fresh untreated ethosomal formulations were taken into different centrifugal tubes. These samples were centrifuged at 20,000 rpm for 3 hours. The supernatant layer was separated, diluted with water suitably and drug concentration was determined at 237 nm in both vortexed and unvortexed samples.

### In-vitro release studies

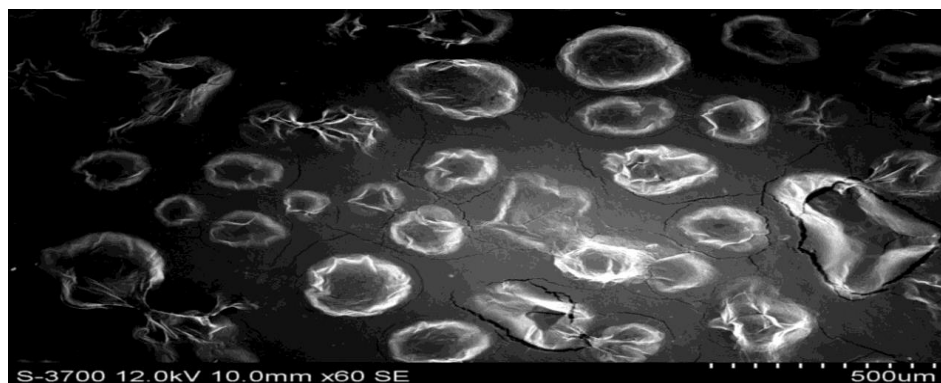
#### Drug release study from dialysis membrane

The skin permeation of Sulfasalazine from ethosomal formulation was studied using open ended diffusion cell specially designed in our laboratory according to

the literates. The effective permeation area of the diffusion cell and receptor cell volume was 2.4 cm and 200 ml respectively. The temperature was maintained at  $37 \pm 0.5^\circ\text{C}$ . The receptor compartment contained 200 ml of pH 6.8 buffer and was constantly stirred by magnetic stirrer at 100 rpm. Prepared dialysis was mounted between the donor and the receptor compartments. Ethosomal formulation was applied to the dialysis membrane and the content of diffusion cell was kept under constant stirring then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analysed by spectrometric method at 237 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh pH 6.8 buffer. Triplicate experiments were conducted for drug release studies.

## RESULTS AND DISCUSSION

Ethosomal formulations composed of phospholipid, drug and ethanol were prepared using the method detailed in last chapter materials and methods and also according to literature with little modification. Ethosomal suspension obtained with sonication were slight yellowish in colour and hazy in appearance. Different characteristics of ethosomes and the effect of sonication were further evaluated and results are reported under the characterization. Microscopic analysis was performed under different magnification to visualize the vesicular structure, lamellarity and to determine the size of ethosomal preparations (Fig-1).



**Fig-1 Scanning electron microscope image**

The maximum entrapment efficiency of ethosomal vesicles as determined by ultracentrifugation was 87.4% for ethosomal formulation containing 40% ethanol (F9). As the IPA concentration increased from 20% to 40% w/w, there was increase in the entrapment efficiency and with further increase in the ethanol concentration (>40% w/w) the vesicle membrane becomes more permeable that lead to decrease in the entrapment efficiency. Results of entrapment efficiency also suggest that 4% phospholipid is optimal concentration for entrapment efficiency and hence increased or decreased in concentration of phospholipid reduces the entrapment efficiency of vesicles.

These result further supported by observation made by Jain NK. Entrapment efficiency of ethosomal formulations are significantly different and are reported in Table-3. Increase in entrapment efficiency may be due to the possible reduction in vesicle size. The detrimental effect on the vesicle during ultra-centrifugation which are larger in size. Sonication gives the more uniform lamellae, smaller vesicle and uniform size and hence it may be the reason for higher vesicular stability and lesser vesicular disruption during ultra centrifugation.

**Table-3 Drug entrapment efficiency of Sulfasalazine Ethosomal Gel**

Formulation code	Entrapment efficiency (%)
F1	71.0
F2	75.4
F3	77.1
F4	75.4
F5	80.2
F6	82.3
F7	80.5
F8	83.1
F9	87.4

From the table-4, it was confirmed that the F1, F2, F3, F4, F6, F7 of ethosomal gel release theory up to 24 hrs. And also from fig-2, 3 it was also confirmed that the formulation (F6) showed maximum drug release up to 24hrs.

**Table-4 In-vitro cumulative % drug release profile for Sulfasalazine Ethosomes**

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.08	39.6	3.6	4.57	0.75	5.42	6.26	3.6	2.31	1.46
0.16	53.3	10.08	11.8	6.26	13.5	15.95	10.08	9.42	8.4
0.25	60.44	25.2	26.04	19.68	27.15	30	25.2	24.4	22.97
0.5	67.1	32.04	33.02	28.48	35.68	41.68	32.04	31.24	30.01
1	77.3	75.5	67.1	33.02	41.68	53.3	38.7	37.02	35.68
2	89.3	87.11	77.3	42.6	55.5	57.7	52.4	49.7	47.1
4	100.2	92.6	89.3	49.7	62.22	64.4	58.6	55.5	53.5
6		100.8	100.9	57.77	68.5	70.6	65.7	64.4	62.22
12				67.1	78.2	82.6	75.5	73.3	70.6
24				72.44	88.4	93.7	87.11	78.2	75.5

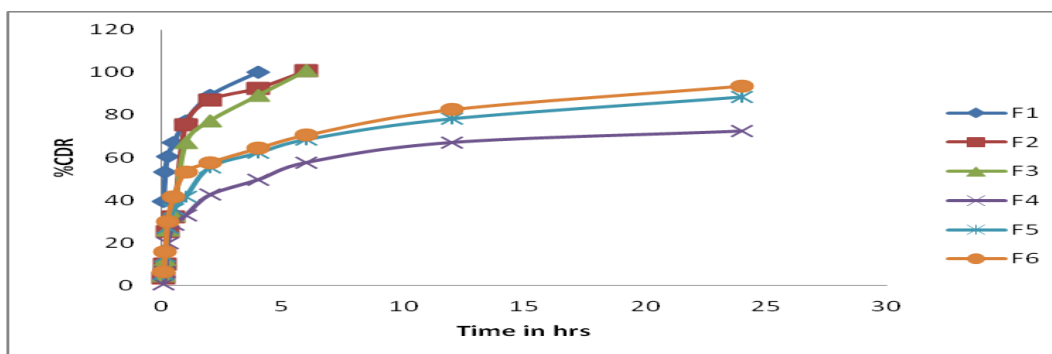


Fig-2 Graph showing dissolution profile for formulations F1-F6

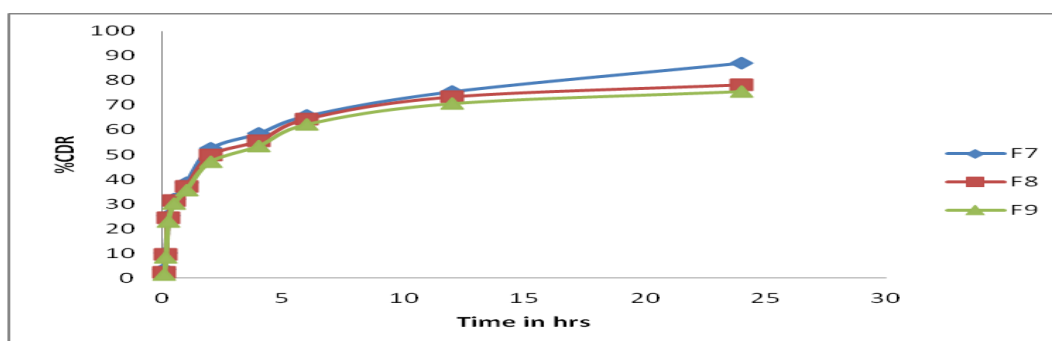


Fig-3 Graph showing dissolution profile for formulations F7-F9

The stability studies were carried out according to the procedure described in the section of chapter. The results are shown in the table-5.

Number of Days	% Entrapment Efficiency at temperatures			% Drug Content at temperatures		
	4±2°C	25±2°C	37±2°C	4±2°C	25±2°C	37±2°C
15	87.6	87.60	87.56	99.2	99.17	99.80
30	87.5	86.81	86.74	98.14	98.94	98.76
45	87.24	86.65	86.55	97.21	98.46	98.55
90	86.92	86.38	85.83	97.43	98.37	98.04

Table-5 % Entrapment efficiency and % Drug content after stability studies

## CONCLUSION

The results of this investigation indicate that Ion gelation method can be successfully employed to fabricate Sulfasalazine acids ethosomal gels. FT-IR spectra of the physical mixture revealed that the drug is compatible with the polymers and copolymer used. Ethosomal gels containing carbopol and ethanol and phospholipids had a least size range of 613µm. Increase in the polymer concentration led to increase in, % Drug

entrapment efficiency, Particle size. The *invitro* drug release decreased with increase in the polymer and copolymer concentration. Among all formulations F6 shows Maximum drug release in 1440 min when compared with other formulations. Analysis of drug release mechanism showed that the drug release from the formulations followed the Non fickian diffusion mechanism and follows First order kinetics.

Based on the results of evaluation tests formulation coded F6 was concluded as best formulation.

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