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Design and development of Ethosomal Gel of Diacerein by using cold method

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A B S T R A C T

Trans dermal route offers several potential advantages over conventional routes. These advantages 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. Recently advancement in liposomes was done and result obtained was "Ethosomal system". Ethosomal system showed topical delivery with higher trans dermal flux and higher skin deposition and became an attractive option as it has several desirable advantages. Diacerein is under investigation for the treatment of Insulin Resistance, Diabetes Mellitus (Type 2), and Diabetes-Related Complications. Diacerein Ethosomes were prepared using the method reported by Touitou et al.,(2000) with little modification. Studies were performed on Ethosomes containing 20%, 30%, 40% and 50% w/w ethanol with sonication used for their size reduction. To confirm the presence of vesicular structure, formulations were visualized under microscope at different magnified fields, which showed presence of lipid bilayer as well as spherical structure of vesicles. Using the same microscopic method and special software "particle size analysis", size of vesicle was determined for sonicated Ethosomes. Vesicular size was found to be in the range of 0 – 5.483 µm. Vesicular size was reduced up to 3 folds by sonication. In-vitro release was carried out using dialysis membrane. The values of drug release were EF_1 (20% alcohol) 76.89%, EF_2 (20% alcohol) 82.31%, EF_3 (20% alcohol) 73.62, EF_4 (30% alcohol) 86.42%, EF_5 (40% alcohol) 72.09%, EF_6 (50% alcohol) 63.21%. The order of drug release was found to be first order for all the formulations. Percentage drug accumulation into skin was also found to be maximum by the Ethosomes containing 30 % w/w ethanol and 3% lecithin which showed effective subdermal deposition and indicated better subdermal action for hypertension

Keywords: Trans dermal, lipid bilayer, alcohol, lecithin, Ethosomal system

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CONTENTS

1. Introduction

Optimization of drug delivery through human skin is important in modern therapy. Recently, the transdermal route vied with oral treatment as the most successful innovative research area in drug delivery[1].Transdermal drug delivery system was first introduced more than 20 years ago. Transdermal drug delivery system is a type of convenient drug delivery system where drug goes to the systemic circulation through the protective barrier i.e. Skin. Only the lipophilic drugs having molecular weight < 500 Daltons can pass through it. Ethosomes have been found to be much more efficient in delivering drug to the skin[2].

Diacerein is a prodrug which is metabolized to rhein. It is currently approved in France for the treatment of osteoarthritis although the use of diacerein is restricted due to the side effects including severe diarrhea. Diacerein is under investigation for the treatment of Insulin Resistance, Diabetes Mellitus (Type 2), and Diabetes-Related Complications. Chemically named as 4,5-bis(acetyloxy)-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid.

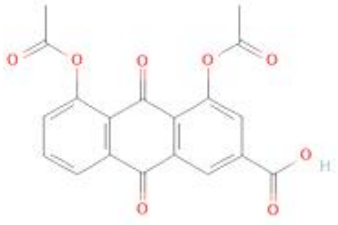

Figure 1

Lecithin 8002-43-5 1,2-diacyl-sn-glycero-3-phosphocholine (trivial chemical name, Phosphotidyl choline). The composition of lecithin (and hence also its physical properties) varies enormously depending upon the source of the lecithin and the degree of purification. Egg lecithin, for example, contains 69% Phosphotidyl choline and 24% Phosphotidyl ethanolamine, while soybean lecithin contains 21% Phosphotidyl choline, 22% Phosphotidyl ethanolamine, and 19% Phosphotidyl inositol, along with other components.

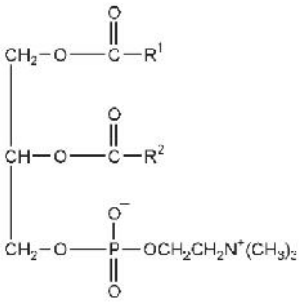

Figure 2

Propylene glycol: 1, 2-Dihydroxypropane; E1520; 2-hydroxypropanol; methyl ethyl- ene glycol; methyl glycol; propane-1,2-diol. Propylene glycol has become widely used as a solvent extractant, and preservative in a variety of parenteral and nonparenteral pharmaceutical formulations. It is a better general solvent than glycerin and dissolves a wide variety of materials, such as corticosteroids, phenols, sulfa drugs, barbiturates, vitamins (A and D), most alkaloids, and many local anesthetics.

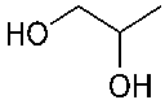

Figure 3

2. Methodology

Scanning of model drug (Diacerein)

10 mg of pure model drug (Diacerein) was dissolved in water and was diluted to give concentration of 10µg/ml and was scanned between 190 nm to 380 nm fort the determination of λ_{max}. The wavelength of 252 nm was selected as for λ_{max}. The same was used for further analysis of drug solution and absorbance of final standard solution was also measured at 252 nm.

Preparation of pH 6.8 phosphate buffer[46]

Dissolve 28.8g of disodium hydrogen phosphate and 11.45g of potassium Di hydrogen phosphate in distilled water and then make up the volume to 1000ml with distilled water.

Preparation of calibration curve

10mg of pure Diacerein drug was taken in a 10ml standard flask and dissolved in distilled water. The volume of stock solution was made up to 10 ml with pH 6.8 phosphate buffer. From the above stock solution, 1 ml was transferred into a 10ml volumetric flask and volume was adjusted to 10 ml that corresponded to 100µg/ml Diacerein in solution. From that solution different aliquots of 0.2, 0.4, 0.6, 0.8, 1.0 and1.2 ml were transferred to 10ml volumetric flask and volume was adjusted to 10ml with ph 6.8 phosphate buffer, which gave a concentration of 2, 4, 6, 8,10 and 12 µg/ml respectively of final standard.

Compatibility Studies

IR spectroscopy can be used to investigate and predict any physicochemical interactions between different components in a formulation and therefore it can be applied to the selection of suitable chemically compatible excipients. The aim of present study was to test whether there is any interaction between the carriers and the drug.

Preparation of Diacerein Ethosomes

Preparation of Diacerein Ethosomes was followed by method suggested by Touitou et al., with little modification[10.] The Ethosomal system of Diacerein comprised of

- 2-5% phospholipids (soy lecithin),
- 20-50% ethanol,
- 10% of propylene glycol,
- 0.005g of cholesterol
- Aqueous phase to 100% w/w

100mg of Diacerein was dissolved in 6ml of water in a vessel and cholesterol was added to it with vigorous

stirring. Propylene glycol was also added during stirring. The contents were heated to 30^0c. In another closed vessel, soy lecithin was dissolved in ethanol with continuous stirring and heated to 30^0 c. When both the solutions reached to same temperature slowly ethanol solution was added drop wise in the centre of vessel containing drug mixture. Then the stirring was continued for another 10min in a covered vessel. Water was added to adjust the volume up to 20 ml. The vesicle size of Ethosomal formulation can be decreased to desire extent using sonication[31] or extrusion[32] method. Finally the formulation was stored under refrigeration[33]. Ethosomes were formed spontaneously by this process.

Preparation of Diacerein Ethosomal gel

The best achieved Ethosomal vesicle suspension formula EF_4 was incorporated into Carbapol gel (1%, 1.5%, 2% w/w). The specified amount of Carbapol-934 powder was slowly added to pure water and kept at 100^0c for 20min. Triethanolamine was added to it drop wise. Appropriate amount of formula EF_4 containing Diacerein was then incorporated into gel-base. Sufficient water was finally added with other formulation ingredients with continuous stirring until homogenous formulation was achieved (G_1, G_2 and G_3). Gel containing free Diacerein drug (100mg) was also prepared by similar method using 1.5% Carbapol.

Table 1: Composition of different Ethosomal formulations

Ethosomal formulation (EF)	Lecithin (%)	Propylene Glycol (%)	Ethanol (%)	Cholesterol (mg)	Drug (mg)	Water
EF_1	2	10	20	0.05	100	Q.s
EF_2	3	10	20	0.05	100	Q.s
EF_3	4	10	20	0.05	100	Q.s
EF_4	**3**	**10**	**30**	**0.05**	**100**	**Q.s**
EF_5	3	10	40	0.05	100	Q.s
EF_6	3	10	50	0.05	100	Q.s
*EF_7	-	10	30	0.05	100	Q.s

*EF7- Free suspension without vehicle forming agent.

Table 2 Composition of different Ethosomal gel formulation

Gel formulation	Diacerein Ethosomal suspension(ml)	Carbapol (%)	Tri ethanol amine (ml)	Water
EF_4G_1	20	1	0.5	Q.s
EF_4G_2	**20**	**1.5**	**0.5**	**Q.s**
EF_4G_3	20	2	0.5	Q.s
*G_4	0.025g	1.5	0.5	Q.s

*G-4 free drug gel

3. Results and Discussion

Calibration curve in water (make up with ph 6.8 phosphate buffer): Standard solutions of different concentrations were prepared and their absorbance was measured at 234 nm (Table 3). Calibration curve was plotted against drug concentrations versus absorbance as given in the (Figure.4).

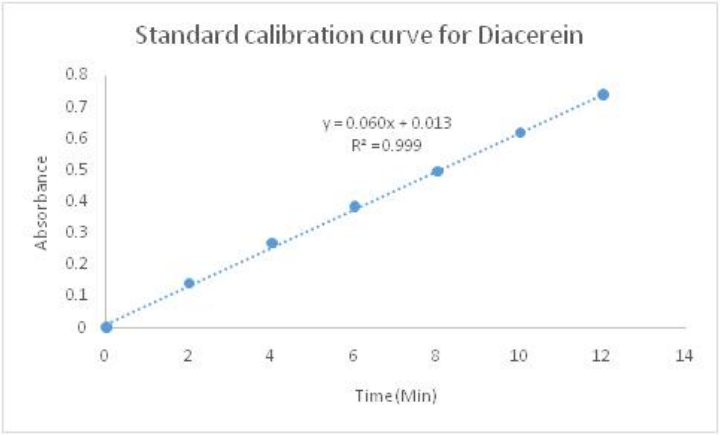

Figure 4: Standard graph of Diacerein

Table 3 Determination of λ_{max} of Diacereinin methanol-- λ_{max} = 252 nm

Concentration (µg /ml)	Absorbance
0	0
2	0.14
4	0.269
6	0.383
8	0.496
10	0.618
12	0.740

Table 4 Assessment of peaks for functional groups

S. No.	Functional groups	Range of groups (drug) (cm^{-1})	Assessment peak of pure drug (cm^{-1})	Assessment peak of Ethosomal gel formulation (cm^{-1})
1	Alkyl C-H Stretch	2950 - 2850 (/cm)	2697-2980cm^{-1}	2507-2851 cm^{-1}
2	Alkenyl C-H Stretch	3010 - 3100 (/cm)	3028-3123cm^{-1}	2981 -3010 cm^{-1}
3	Alkenyl C=C Stretch	~3300(/cm)	-------	------
4	Amine N-H Stretch	3500 – 3300 (/cm)	------	------

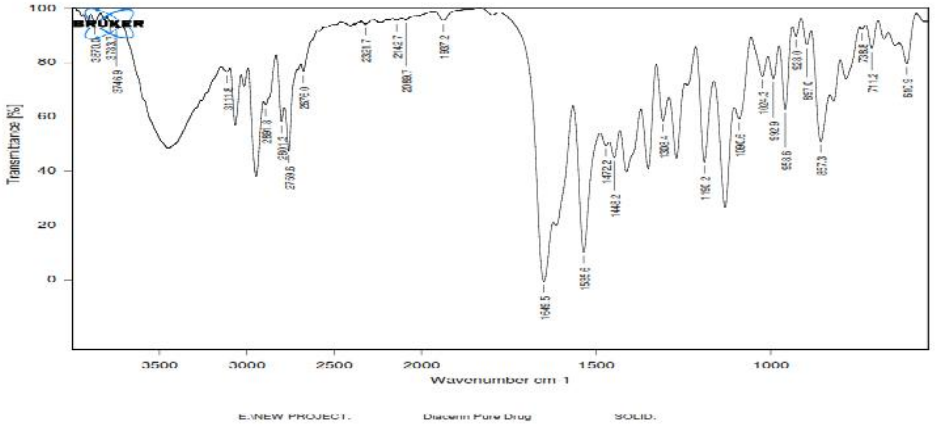

Figure 5: FTIR of pure Diacerein drug

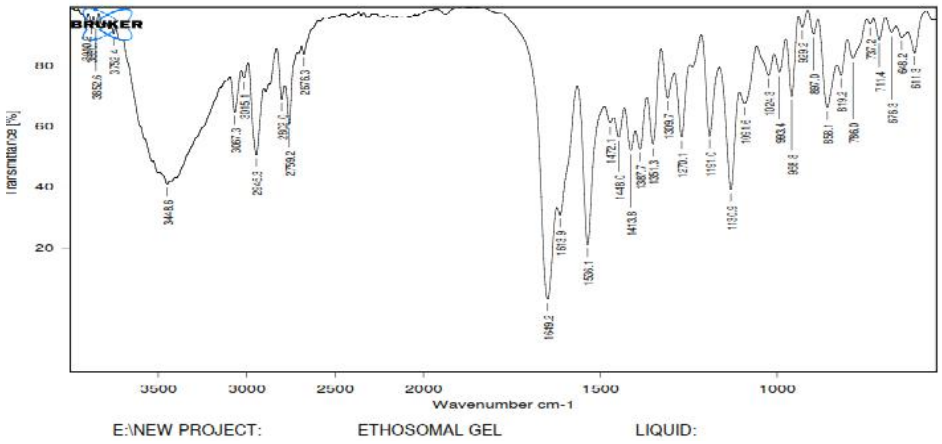

Figure 6: FTIR of Diacerein Ethosomal gel

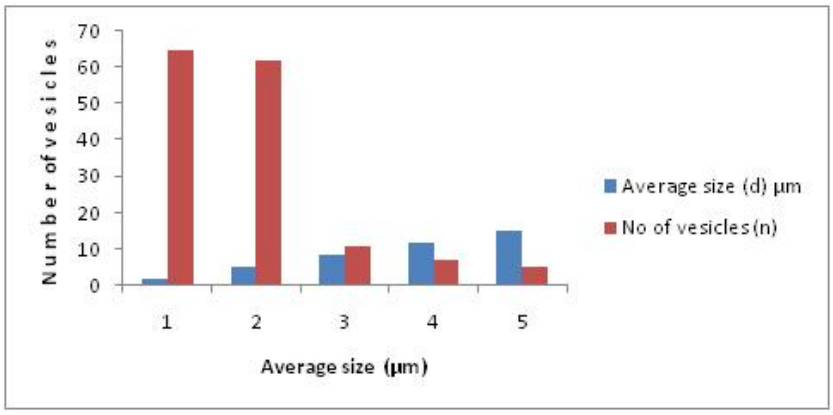

Figure 7 Size distribution range of EF_1

Table 5 Size distribution of Ethosomal formulation#2 EF_2 (3% Lecithin, 20% ethanol)

SIZE RANGE					
Eye piece micrometer	**In µm**	**Average size (d) µm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**n x d**
0-1	0.00-3.33	1.665	60	40.000	99.9
1-2	3.33-6.66	4.995	45	30.000	224.775
2-3	6.66-9.99	8.325	30	20.000	249.75
3-4	9.99-13.32	11.655	10	6.667	116.55
4-5	13.32-16.65	14.985	5	3.333	74.925
			Σn = 150		**Σnd = 765.9**

Average diameter (d avg) = $\frac{\Sigma nd}{\Sigma n} = 5.106$ µm

Table 6 Drug entrapment efficiency of Diacerein Ethosomal gel

Formulation code	Entrapment efficiency (%)			MEAN
EF1	72.19	71.75	71.82	71.92
EF2	66.91	67.12	68.53	67.52
EF3	60.05	60.00	60.01	60.02
EF4	**79.91**	**79.62**	**79.33**	**79.62**
EF5	58.01	55.96	54.96	56.31
EF6	39.39	42.32	42.76	41.49

Table 7 In-vitro cumulative % drug release profile for Diacerein Ethosomes

	Cumulative % drug release								
Time	EF_1	EF_2	EF_3	EF_4	EF_5	EF_6	EF_7	EF_4G_2	F_4G_4
0	0	0	0	0	0	0	0	0	0
30min	14.56	17.5	12.09	18.09	11.39	10.01	11.21	17.82	8.46
2hr	25.62	31.52	23.26	32.51	20.21	18.09	23.62	22.31	15.31
4hr	30.1	38.3	28.21	39.42	25.22	22.31	29.72	28.81	19.71
6hr	33.08	42.52	32.71	46.42	30.09	27.69	33.32	32.71	23.04
8hr	38.62	47.81	37.21	49.31	36.21	30.03	37.29	37.61	25.31
10hr	40.07	54.32	39.05	56.51	37.02	33.61	40.25	41.62	28.32
12hr	43.62	58.50	43.02	60.41	42.3	37.32	47.91	46.32	33.13
14hr	49.30	61.32	48.05	63.21	46.31	40.65	52.41	50.02	37.04
16hr	52.41	64.92	51.51	66.72	49.85	43.32	52.56	57.42	39.21
18hr	59.32	69.07	58.37	71.46	57.31	48.32	52.56	60.21	40.32
20hr	66.02	75.41	66.42	76.32	65.21	52.09	52.56	66.32	43.01
22hr	70.52	78.2	70.31	82.31	68.71	56.31	52.56	72.02	45.32
24hr	76.89	82.31	73.62	86.42	72.09	63.21	52.56	75.62	47.62

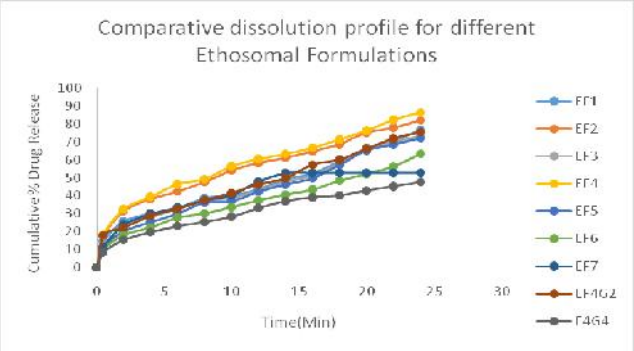

Figure 8 In-vitro drug release studies of different Ethosomal formulations

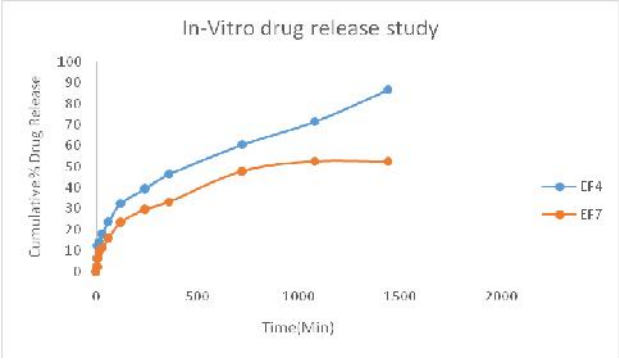

Figure 9 In-vitro release studies of Optimized Ethosomal suspension formulation (EF4) and free drug solution (EF7)

4. Conclusion

From the present study it can be concluded that Ethosomal gel is promising route of drug administration. Even though the TDDS faces the problem of drug permeation because of the rigid stratum corneum, it can be overcome by the use of penetration enhancers such as ethanol. The size of the ethosomes can be reduced by sonication thereby improving the skin permeation properties of ethosomes. By encapsulating Diacerein into ethosomes the frequency of dosing can be reduced as ethosomes cause the delivery of drug for almost 24hrs. Since the overall drug administered is reduced, the adverse drug reactions of Diacerein such as dizziness, allergy, hypotension, etc can also be reduced. Finally it can be said that many drugs such as NSAIDS, anti hypertensive drugs, anti fungal drugs, anti HIV agents, etc have the scope to be incorporated into ethosomes and thereby enhancing their activity.

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