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FORMULATION DEVELOPMENT USING QBD APPROACH AND EVALUATION FOR PREPARATION OF ROFECOXIB ETHOSOMES FOR TOPICAL GEL ADMINISTRATION

***Pingale Prashant. L., Boraste Sahebrao S., Walke Prashant S.**

Associate Professor, Department of Pharmaceutics, GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Prin. T. A. Kulkarni Vidyanagar, Nashik-422005, India.

***Corresponding Author: Pingale Prashant. L.**
Associate Professor, Department of Pharmaceutics, GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Prin. T. A. Kulkarni Vidyanagar, Nashik-422005, India.

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ABSTRACT

Transdermal delivery embodies an attractive alternative to oral delivery of drugs and is composed to provide an alternative to hypodermic injection too. Transdermal delivery has a variety of benefits compared with the oral route. In specific, it is used when there is a significant first-pass effect of the liver that can prematurely metabolize drugs. Transdermal delivery also has leads over hypodermic injections, which are painful, generate dangerous medical waste and stance the risk of disease transmission by needle re-use, especially in developing countries. In a cited research work, we attempted to prepare Rofecoxib Ethosomes for Topical Gel Administration using QbD approach and Evaluation of prepared ethosomes.

KEYWORDS: QbD, Rofecoxib Ethosomes, ethosomes.

INTRODUCTION

Transdermal delivery can provide a number of advantages over conventional methods of drug administration, including enhanced efficacy, increased safety, greater convenience and improved patient compliance. By delivering a steady flow of drugs into the bloodstream over an extended period of time, transdermal systems can avoid the peak and valley effect of oral injectable therapy and can enable more controlled, effective treatment. By avoiding first pass metabolism through the gastrointestinal tract and the liver, the therapeutically equivalent for the transdermal delivery of certain compounds can be significantly less than the corresponding oral dosage, potentially reducing dosage related side effects.[1]

Ethosomes: The "Somes" are the cell like formulations of novel drug delivery system. There are different types of somes, viz. Liposomes, Phytosomes, Niosomes, Colloidosomes, Ethosomes, Cubosomes etc.[2] Ethosome are novel carrier system used for delivery of drugs having low penetration through the transdermal route. Ethosomes are the trivial modification of well-known drug carrier liposome. Ethosomes are lipid vesicles that contain phospholipids, alcohol (ethanol and isopropyl alcohol) in fairly high concentration and water.[3] Ethosomes are flexible vesicles made up of phospholipids and ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers to microns (μ).[4] Ethosomes have got a property of permeation through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes.[3, 5.8]

Rheumatoid Arthritis: Rheumatoid Arthritis is an autoimmune disease which means that certain cells of the immune system do not work correctly and start confronting healthy tissues — the joints in RA. The cause of RA is not known. Yet, new research is giving us a better idea of what makes the immune system attack the body and create inflammation. In RA, the emphasis of the inflammation is in the synovium, the tissue that lines the joint. Immune cells release inflammation-causing chemicals. These chemicals can destruct cartilage (the tissue that cushions between joints) and bone.

Additional things possible play a role in RA as well. For instance, genes that affect the immune system may make some people further prone to getting RA.

RA is the most common form of autoimmune arthritis, affecting more than 1.3 million Americans. Of these, about 75% are women. In fact, 1–3% of women may get rheumatoid arthritis in their lifetime. The disease most often begins between the fourth and sixth decades of life. However, RA can start at any age.

Therapy for RA has improved significantly in the past 30 years. Current treatments give most patients good or

excellent relief of symptoms and let them keep functioning at, or near, normal stages. With the right medications, many patients can achieve "remission" — that is, have no signs of active disease.

There is no cure for RA. The goal of treatment is to lessen your symptoms and poor function. Doctors do this by starting proper medical therapy as soon as possible, before your joints have lasting damage. No single treatment works for all patients. Many people with RA must change their treatment at least once during their lifetime.

The selective cyclooxygenase 2 (COX-2) inhibitors have emerged as an important option in the treatment of rheumatoid arthritis (RA). Rofecoxib and celecoxib, the selective COX-2 inhibitors currently available, have shown efficacy in reducing symptoms of RA comparable with that of traditional non-steroidal anti-inflammatory drugs (NSAIDs).[9]

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY

In comparison to other transdermal & dermal delivery sy stems

- Enhanced permeation of drug through skin for trans dermal drug delivery.
- Delivery of large molecules (peptides, protein molec ules) is possible.
- It contains non-toxic raw material in formulation.
- High patient compliance: The ethosomal drug is ad ministrated insemisolid form (gel or cream) hence producing high patient compliance.
- The Ethosomal system is passive, non-invasive and i s available for immediate commercialization. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
- Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.

MECHANISM OF DRUG PENETRATION

The main advantage of ethosomes over liposomes is the increased permeation of the drug. The mechanism of the drug absorption from ethosomes is not clear. The drug absorption probably occurs in following t wo phases:

1. Ethanol effect
2. Ethosomes effect

1. Ethanol effect

Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhaningeffect is well known. Ethanol penetrates into intercellular lipids and in creases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. Ethosomes effect

Increased cell membrane lipid fluidity caused by the etha nol ofethosomes results increased skin permeability. So t he Ethosomes permeates very easily inside the deep skin layers, where it got permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.

Characterization of ethosomes

Parameter	Importance	Method
Size and shape	Determine skin penetration	SEM, TEM, DLS
Zeta potential	Size of vesicles	Zeta meter
Entrapment efficiency	Suitability of method	Ultracentrifugation
Drug content	Important in deciding the amount of vesicle preparation to be used	UV, HPLC
Stability studies	To determine the shelf life of vesicle formulation	SEM, TEM, HPLC
In-vitro dissolution	Determine the drug release rate from the vesicle	Franz diffusion cell
Skin permeation	Determines rate of drug transport through the skin	CLSM

Therapeutic applications of ethosomes

Ethosomes can be used for many purposes in drug delivery. Ethosomes are mainly used as replacement of liposomes. Mainly the transdermal route of drug delivery is preferred. Ethosomes can be used for the transdermal delivery of hydrophilic and impermeable drugs through the skin. Various drugs have been used with ethosomal carrier.

Sr. No.	Class	Example	Uses
1	Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmitylphosphatidyl choline Distearylphosphatidyl choline	Vesicles forming component
2	Polydlycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
3	Alcohol	Ethanol, isopropyl alcohol	For providing the softness for vesicle membrane
4	Cholesterol	Cholesterol	For providing the stability to vesicle membrane
5	Vehicle	Carbopol 934	As a gel former

EXPERIMANTAL

- **CHEMICALS:** Ethanol, Soy Lecithin, Rofecoxib, DMSO
- **GLASSWARE:** Beaker, glass rod, measuring cylinder
- **EQUIPMENTS:** Franz diffusion cell, UV spectrophotometer, Sonicator, Magnetic hot plate, Magnetic bead.

EXPERIMENTAL DESIGN

Based on literature survey ethosome vesicles was found to form with phospholipid with concentration in the range of 1-5% and ethanol in the range of 20-50%. Concentration of lipid and ethanol plays most important role in formation of ethosome vesicles and also in the drug entrapment. Thus these two factors were considered for design of experiment. Vesicle size also has an influence on the formulation therefore sonication time for the vesicle size reduction was also considered as an independent factor.

Table: 1 Composition of ethosomal formulation.

Sr. No.	Ingredients	Concentration
1	Drug	0.25% w/v
2	Ethanol	20-50 % v/v
3	Phosphoipid	1-5 % w/v
4	Water	Upto 100 % v/v

The most popular response surface method design is the central composite design. Thus, for optimization of ethosome vesicle central composite design was used in which 2 factor at three level analysed using entrapment efficiency and vesicle size as a dependant variable.

Table: 2 Factors and their level.

Independent factor	Level		
	Low(-1)	Medium(0)	High(1)
Concentration of ethanol	20	35	50
Concentration of lipid	1	3	5
Dependable factor	(Y_1)% Entrapment		
	(Y_2)Vesicle Size (micron)		
	(Y_3)% release in 10 hr		

Different factor combinations were obtained and experimentally run to measure the following responses:

The composition and quantities of different formulations are given in **Table 2**.

Table: 2 Formulation according to design.

Formulation	Ethanol Conc. (%v/v)	Phospholipid Conc. (%w/v)	Drug (%w/v)	DMSO (% v/v)	Water (% v/v)
F1	50	1	0.25	6.67	up to 100
F2	20	1	0.25	6.67	up to 100
F3	35	1	0.25	6.67	up to 100
F4	50	5	0.25	6.67	up to 100
F5	35	3	0.25	6.67	up to 100
F6	35	5	0.25	6.67	up to 100
F7	20	3	0.25	6.67	up to 100
F8	50	3	0.25	6.67	up to 100
F9	20	5	0.25	6.67	up to 100

METHOD OF PREPARATION

1. Weigh all the ingredients accurately.
2. Phospholipid & ethanol are stirred using magnetic stirrer at 40°C until phospholipid gets completely dissolved in ethanol.
3. In a volumetric flask, dissolve drug in DMSO.
4. Add this drug solution to the lipid-ethanol solution with continuous stirring.
5. Add water and stir for some more time until a homogeneous product is achieved.
6. Sonicate for 10 mins.
7. Store in well closed container in a cool place.

Addition of gel forming agent

Carbopol 934 forms very good consistency transparent gel at low concentration. Carbopol 934 is not toxic and does not cause any irritation on skin. So, carbopol 934 is selected as a gelling agent. 1% carbopol gel base is prepared by directly adding Carbopol 934 to the formulation with vigorous stirring.

EVALUATION PARAMETERS

Entrapment efficiency, vesicle size and % release in 10 hr were done of optimized formulation as mentioned above.

1. Optical microscopy

Morphology of vesicles was evaluated using optical microscopy. Slides of optimized formulation were prepared and were seen in Motic microscope in 40x lens. The photographs were taken for the optimized formulation.

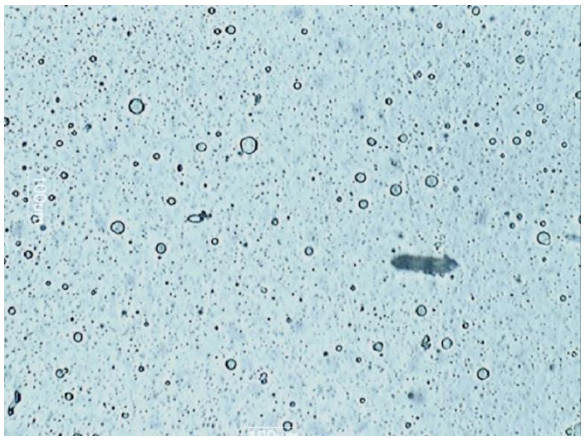

Fig. 1: Rofecoxib Ethosomes observed under Motic Microscope.

2. Entrapment efficiency: Entrapment of drug in ethosome vesicle was determined using cooling-centrifugation method.

- 4 ml of ethosomal formulation was taken each in two Eppendorf tube and was centrifugated with following specifications:
  Speed=12000 rpm, Time= 2 hrs, Temp. = 2-4°C
- After taking out the supernatant solution, the remained part was again centrifugated for another 10 min. so as to separate the remained part of supernatant solution.
- Supernatant and sediment were recovered and their volume were measured. Both were diluted with hydro-ethanolic solution and entrapped DRUG was analysed using UV-Spectroscopy at 267 nm.

Percentage entrapment was calculated using equation = T–C/T * 100

T =total amount of drug found in supernatant and sediment.

C =amount of drug found in sediment.

3. Diffusion studies

It was done by using modified Franz diffusion cell for 2 hrs. Most stable formulation was taken forward to perform diffusion studies, sample were analysed at λ_{max} 267nm and concentration was observed from the calibration curve. The ethosomal vesicles showed maximum release of 96.80% in 105 min.

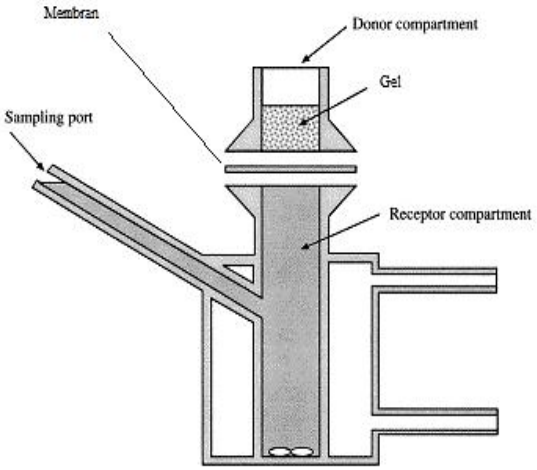

Fig. 2: Assembly used for diffusion study.

RESULTS AND DISCUSSION

Formulation	Ethanol Conc. (%v/v)	Phospholipid Conc. (%w/v)	Entrapment efficiency (%)	Average vesicular size (microns)
F1	50	1	50.29	17.8um
F2	20	1	39.89	30.1um
F3	35	1	34.06	16.0um
F4	50	5	107.89	20.7um
F5	35	3	65.71	23.5um
F6	35	5	82.86	23.0um
F7	20	3	38.40	34.3um
F8	50	3	85.60	18.2um
F9	20	5	50.63	24.0um

1. Effect of lipid concentration

Entrapment efficiency is the percentage fraction of the total drug incorporated into the ethosome formulation. The maximum and minimum value of % EE obtained were 107.89% for F4, 85.60% for F8 and 82.86% for F6. It was also found that increase in lipid concentration from 1% to 5% the % EE value increase 50.29% to 107.89% significantly. It is obvious that increase in lipid concentration leads to increase in entrapment efficiency.

2. Effect on Vesicle size

The mean vesicle sizes of all formulation are presented in table. The smallest mean vesicle size of 16.0 µm was observed from DRUG loaded ethosome formulation F3where as maximum vesicle size was obtained as 34.3 µm for F7 formulation. The formulation with same ethanol concentration and sonication time shows increase in vesicle size with respect to increase in lipid concentration. Though with increase in ethanol

concentration the vesicular size decreased, a phenomenon observed by number of scientific groups. This indicates that at higher ethanol concentration the membrane thickness is reduced considerably, probably due to the formation of a phase with interpenetrating hydrocarbon chains.

CONCLUSION

Ethosomes can provide better skin permeation. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcomed by ethosomes to significant extent. Application of ethosomes provides the advantages such as improved permeation through skin and targeting to deeper skin layers for various skin diseases. Its suitability as a topical dosage form can be easily judged by considering entrapement efficiency and release rate which makes ethosomes a suitable carrier for topical drug delivery.

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