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FORMULATION AND EVALUATION OF LIPOSOMAL GEL OF ETODOLAC FOR BETTER ANTI INFLAMMATORY ACTIVITY

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ABSTRACT

The main factor that limits use of NSAIDs is concern over the development of gastrointestinal (GI) side effects. Topical dosage forms provide relatively consistent drug levels for prolonged periods and avoid gastric irritation, as well as the other typical side effects of oral NSAID administration. In this research work liposomal gel of Etodolac was developed for topical application. Preformulation studies (organoleptic studies, melting point, partition coefficient, absorption maxima, FTIR study) were performed for identification of drug. Prepared liposomes of Etodolac were incorporated into a gel using Carbopol 940. Rheological and texture properties of prepared gel formulation showed suitability of the gel for topical application. The developed formulation was evaluated for drug entrapment efficiency, pH measurement, in vitro drug release, drug content, and skin irritation studies etc.

Keyword: Etodolac, Liposomes, Gel.

1. INTRODUCTION

1.1 Arthritis

Arthritis is generally a class of mucoskeletal disorders that involves inflammation of one or more joints. There are over 100 different forms of arthritis. Rheumatic diseases are characterized by inflammation of connective tissue which leads to chronic systemic inflammatory disorder that may affect many tissue and organs like skin, blood vessels, heart, lungs and muscles but principally attacks the joints producing proliferative synovitis that often leads to articular cartilages and ankylosis of joints. When primary involvement is in the soft tissue, the term non- articular rheumatism is employed. The study of all conditions embraced by the term arthritis and rheumatism is called rheumatology (Harsh Mohan, 2000).

1.2 Epidemiology

Arthritis is predominantly a disease of the elderly, but children can also be affected by the disease. More than 70% of individuals in North America affected by arthritis are over the age of 65years. Arthritis is more common in women than men at all ages and affects all races ethnic groups and cultures. But the incidence and severity seems to be less in rural areas (Becker and Michael A, 2005).

1.3 Types of arthritis (Beckar *et al,*2005)

- **Osteoarthritis** - cartilage loses its elasticity. If the cartilage is stiff it becomes damaged more easily. The cartilage, which acts as a shock absorber, will gradually wear away in some areas. As the cartilage becomes damaged tendons and ligaments become stretched, causing pain. Eventually the bones may rub against each other causing very severe pain.
- **Rheumatoid arthritis** - this is an inflammatory form of arthritis. The synovial membrane (synovium) is attacked, resulting in swelling and pain. If left untreated the arthritis can lead to deformity.
- **Infectious arthritis (septic arthritic)** - an infection in the synovial fluid and tissues of a joint. It is usually caused by bacteria, but could also be caused by fungi or viruses. Bacteria, fungi or viruses may spread through the bloodstream from infected tissue nearby, and infect a joint.

1.4 Symptoms

RA usually affects joints on both sides of the body equally. Wrists, fingers, knees, feet, and ankles are the most commonly affected. The disease often begins slowly, usually with only minor joint pain, stiffness, and fatigue. Joint symptoms may include:

- Morning stiffness, which lasts more than 1 hour, is common. Joints may feel warm, tender, and stiff when not used for an hour.
- Joint pain is often felt on the same joint on both sides of the body.
- Over time, joints may lose their range of motion and may become deformed.

Other symptoms include:

- Chest pain when taking a breath (pleurisy)
- Dry eyes and mouth (Sjogren syndrome)
- Eye burning, itching, and discharge
- Nodules under the skin (usually a sign of more severe disease)
- Numbness, tingling, or burning in the hands and feet
- Sleep difficulties

2. PREFORMULATION AND STANDARDIZATION

2.1 Identification of drug

The drug (ETODOLAC) was received as a gift sample from Dr.Reddy's Ltd (Hyderabad) and was identified for its physical appearance, melting point, UV absorption maxima and IR spectra.

Table 2.1: Physicochemical and Spectral Characteristics of Etodolac

S.No.	Parameter	Standard	Observation
1.	Physical Appearance	White crystalline powder.	Fully complied
2.	Melting point	145-148°C	146°C
3.	Partition coefficient	11.4	9.8
4.	UV spectroscopy	The UV absorption maxima of Etodolac in methanol exhibits maximum at 279nm.	Fully complied
5.	IR spectroscopy	prominent peak between 3500-3200cm-1 shows presence of non bonded aromatic immino gp	Fully complied

2.2 Solubility profile

The solubility of the Etodolac was determined in different polar and non-polar solvents. The results are reported in Table.

Table 2.2 : Solubility of Etodolac in various solvents

S. No.	Solvent	Status of solubility
1	Methanol	+++
2	Ethanol	+++
3	Water	-
4	Ether	-
5	Di methyl sulfoxide	+++
6	Chloroform	++
7	Dichloromethane	-
8	Acetone	++

+++: Freely soluble, ++: Slightly soluble, +: Very slightly soluble, -: Insoluble

2.3 Analytical evaluations

Preparation of Standard Curve of Etodolac in Phosphate Buffer Solution (pH 7.4) at λ_{max} 279 nm

The absorbance was taken at wavelength maxima (λ_{max}) 295 nm against blank. The standard curve was plotted between absorbance and concentration (Fig 4.5, Table 4.5).

Table 2.3: Standard Curve of Etodolac in Phosphate Buffer Solution (pH 7.4) at λ_{max} 279 nm

S. No.	Concentration (µg/ml)	Absorbance	Statistical Parameter
1	2	0.123	**Correlation coefficient** $R^2 = 0.999$ **Straight line equation** y = 0.136x+0.019
2	4	0.255	
3	6	0.380	
4	8	0.505	
5	10	0.675	
6	12	0.794	
7	14	0.949	
8	16	1.059	
9	18	1.2	
10	20	1.343	

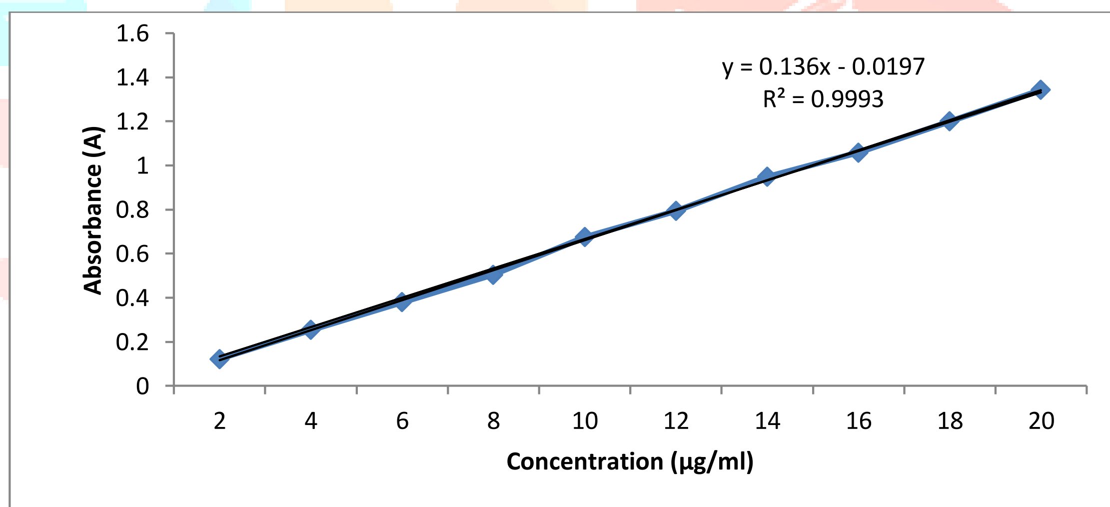

Figure 2.1 : Standard curve of Etodolac in Phosphate Buffer Solution (pH 7.4) at λmax 279 nm

3. MATERIALS AND METHOD

3.1 Materials:

Etodolac was obtained as a gift sample from Dr. Reddys Laboratory, Hyderabad. Soya lecithin, cholesterol and carbapol 940 were obtained from Hi Media, Pvt. Ltd. Mumbai. All other chemicals and reagents used were of analytical grade.

3.2 Preparation of drug loaded liposomes:

Aqueous liposomal dispersion was prepared by conventional lipid film hydration method reported by Sudhakar *et al,* 2012. with slight modification. Different ratios of phospholipid (Soya lecithin), cholesterol and drug were dissolved in10 ml solvent system consisting of chloroform and methanol mixture in the ratio of 2:1(v/v) in 250 ml round bottom flask. A thin film was formed on evaporating organic solvent. Subsequently the flask was kept overnight under vacuum to ensure complete removal of organic solvent. The dried lipid film formed was hydrated with 10 ml of phosphate buffer solution (pH 7.4). The resultant suspension was left undistributed at room temperature for 2-3 hour to allow complete swelling of the lipid film. Then the suspension was bath sonicated for 5 minutes.

3.2.1 Optimization of drug loaded liposomes

Preparation of liposomes involves various process variables of which the following were considered for the optimization of the formulation:

1. Effect of varying soya lecithin: cholesterol ratio.
2. Effect of varying drug: lipid ratio.
3. Effect of varying sonication time.

3.2.1.1 Effect of varying Soya lecithin : Cholesterol ratio

Liposomes were prepared varying only the lipid (soya lecithin) and cholesterol ratio while keeping the amount of the drug constant. The effect of varying lipid: cholesterol ratio on the size and amount of drug entrapped are reported in Table5.1 and shown in Figure5.1 respectively.

Table 3.1: Effect of varying Lipid(soya lecithin): cholesterol ratio

S. No.	Formulation code	Lipid: CH ratio (%w/w)	Vesicle Size (nm)*	Percentage Entrapment efficiency*
1	EL_1	10:0	85±0.5	66.07±0.94
2	EL_2	9:1	88±0.6	68.04±0.95
3	EL_3	8:2	89±0.2	72.03±0.97
4	**EL_4**	**7:3**	**92±0.3**	**76.02±1.12**
5	EL_5	6:4	87±0.4	74.05±0.92

***Values represent mean ± S.D. (n=3)**

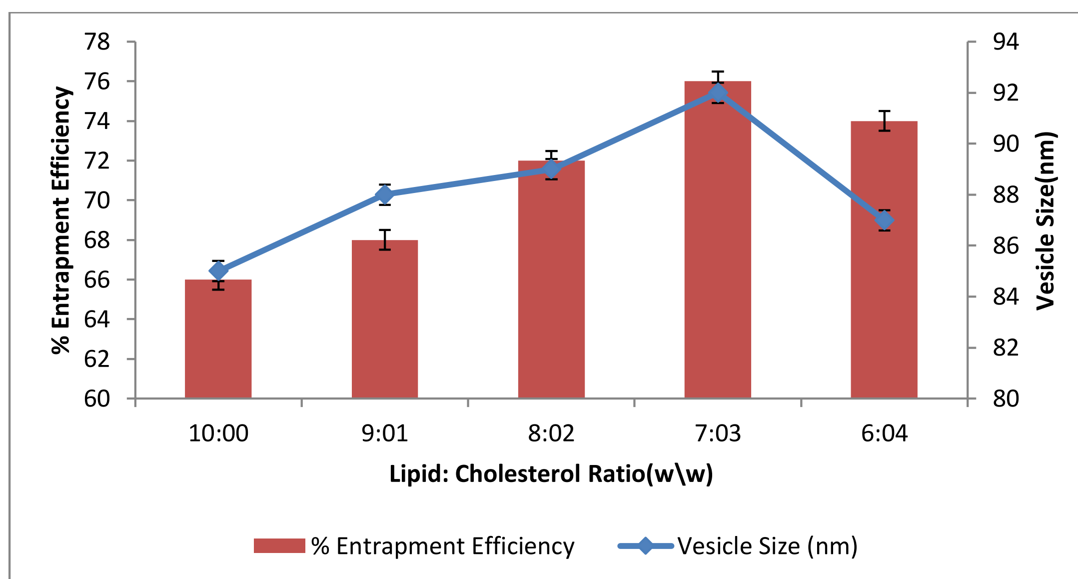

Figure 3.1: Effect of varying Lipid (soya lecithin):cholesterol ratio on Particle Size and % EE.

3.2.1.2 Effect of varying Drug: Lipid ratio

The amount of the drug and lipid taken also poses effect on the particle size and % entrapment efficiency of the liposomes. In order to determine the effect of amount of drug liposomes were prepared varying only the amount of drug while keeping the amount of the lipid constant.

Table 3.2 : Effect of varying drug: lipid ratio

S. No.	Formulation code	Lipid: drug ratio	Vesicle Size (nm)*	Percentage Entrapment efficiency*
1	EL_1	10:0.5	82±0.2	68±0.93
2	EL_2	10:01	83±0.5	69±0.92
3	EL_3	10:02	85±0.2	72±0.95
4	**EL_4**	**10:03**	**87±0.3**	**74±1.1**
5	EL_5	10:04	84±0.1	71±1.3

***Values represent mean ± S.D. (n=3)**

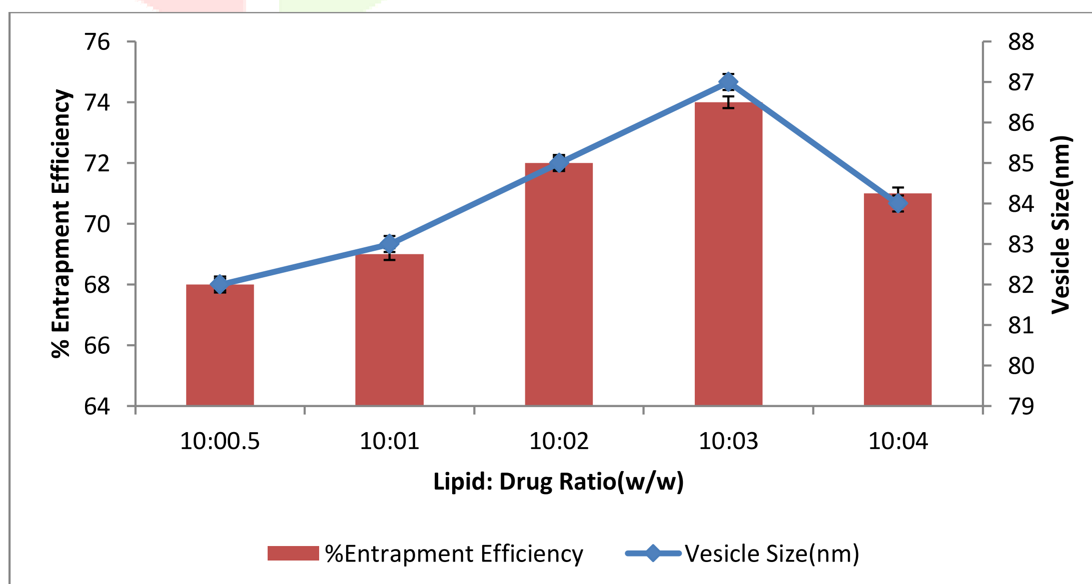

Figure 3.2: Effect of varying Drug: Lipid ratio on Particle Size and % EE.

3.2.1.3 Effect of varying sonication time.

Sonication is done in order to reduce vesicle size of the liposomes. In order to investigate the effect of sonication time on vesicle size and entrapment efficiency prepared liposomes were sonicated for different time. The effect of varying sonication time on the size and amount of drug entrapped are reported in Table.

Table 3.3: Effect of varying sonication time

S. No.	Formulation code	Sonication time(min)	Vesicle Size (nm)*	Percentage Entrapment efficiency*
1	EL_1	0	92±0.1	76.42±0.96
2	EL_2	5	88±0.3	74.23±0.89
3	EL_3	10	87±0.2	72.03±1.11
4	**EL_4**	**15**	**82±0.6**	**73.53±0.82**
5	EL_5	20	84±0.5	69.12±0.32

***Values represent mean ± S.D. (n=3)**

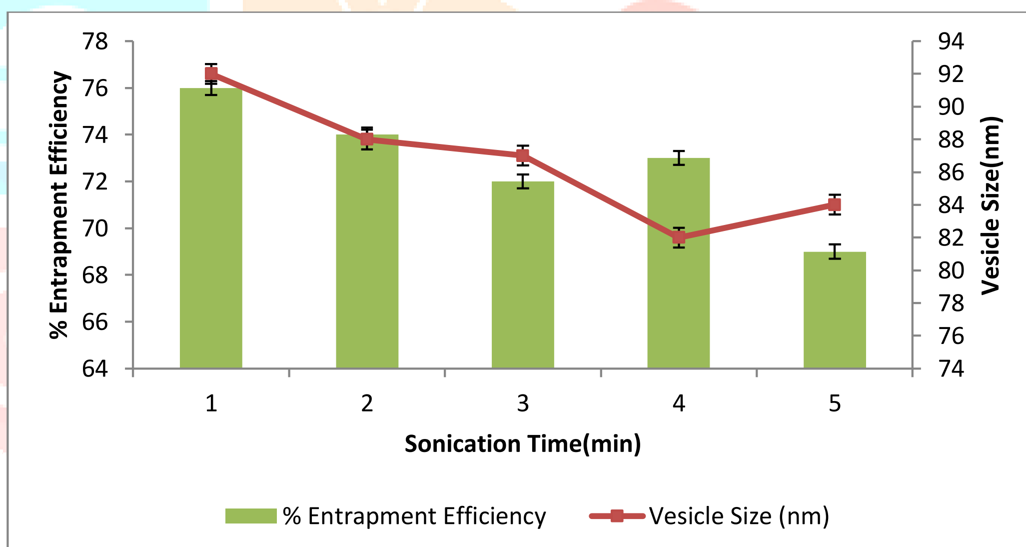

Figure 3.3: Effect of varying sonication time on Particle Size and % EE.

3.3 Characterization of etodolac loaded liposomes

3.3.1 Shape and surface morphology

Scanning electron microscopy (Jeol JSM-6390A scanning microscope, Japan) was employed to determine the shape and surface morphology of produced liposomes. A small amount of liposome was stucked on double sided tapped metallic coated under vaccum (10^{-6}torr) with a thin layer of gold before scanning. Structure of liposomes is shown in the fig.

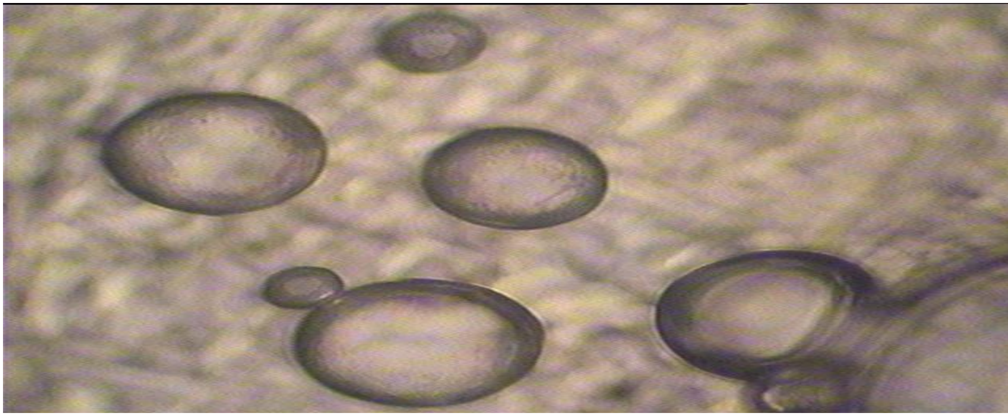

Figure 3.4: Microscopic view of Etodolac loaded liposomes

3.3.2 Particle size analysis

All sizing were made on a Zetasizer Nano ZS at 25°C. Sizing measurements were made on the neat liposome samples. The Nano ZS incorporates non-invasive backscatter (NIBS™) optics for sizing measurements. The detection angle of 173°enables size measurements of concentrated,turbid samples to be made. The photomicrograph is shown in the figure 5.5.

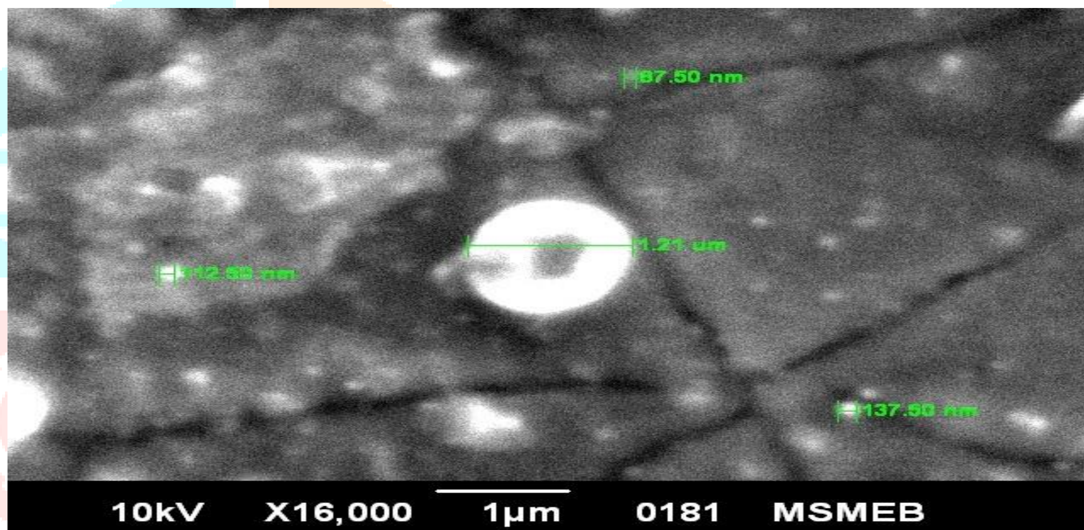

Figure 3.5: Photomicrograph of Etodolac loaded liposomes

3.3.3 Entrapment efficiency determination

Drug associated with liposome was separated from unentrapped drug using centrifugation method. Liposomes were centrifuged at 2000rpm for 1 h at controlled temperature of 4 C. Supernatant containing unentrapped drug was withdrawn and measured under UV spectrophotometrically against phosphate buffer saline (pH 7.4). The amount of drug entrapped in liposome was determined as follow:

$$EE\ (\%) = [(C_d – C_f)/C_d]\ 100$$

Where C_d is concentration detected of total drug and C_f is concentration of free drug.

The entrapment efficiency was obtained by repeating the experiment in triplicate and the values were expressed as mean standard deviation.

3.3.4 *In-vitro* drug release study

In vitro release studies were performed using modified Franz diffusion cell.

Table 3.4: % Cumulative drug release from Etodolac Loaded liposomes

S.No.	Time(hrs)	% Cumulative drug release*
1	0.5	9.85±0.34
2	1	16.26±0.12
3	2	27.58±0.32
4	4	42.33±0.38
5	6	58.70±0.30
6	8	64.02±0.41
7	12	70.20±0.46

***Values represent mean ± S.D. (n=3)**

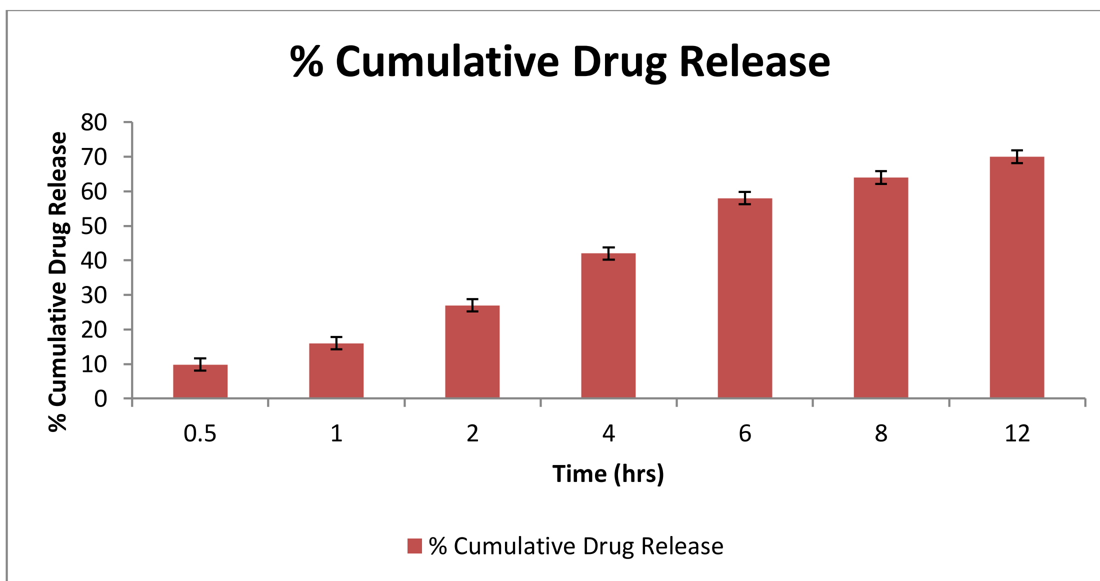

Figure 3.6 :% Cumulative drug release from Etodolac loaded liposomes

3.4 Preparation of carbopol gel and incorporation of drug containing liposomes into carbopol gel

3.4.1 Preparation of carbopol gel

As a vehicle for incorporation of liposomes for topical delivery, carbopol gel was prepared by following the method given below reported by Anis *et al*, 2007. Carbopol 940 (1 g) was dispersed in distilled water (20ml) by stirring at 800 rpm for 60 minutes. Then, propylene glycol (5ml) was added and the mixture was neutralised by dropwise addition of triethanolamine. Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 6.5.

3.4.2 Optimization of carbopol gel

In order to achieve proper optimization gel preparation was done by the same method as given above by varying only the concentration of polymer (carbapol940) viz. 1,2,3and 4g respectively being used as gelling agent. The effect of varying polymer concentration on the viscosity and drug content are reported in the table 5.4 and shown in the figure.

Table 3.5: Effect of varying polymer concentration

S. No.	Formulations	Polymer (carbopol940) concentration(g)	Viscosity(Cp)*	Drug content*
1	ELG1	1	1450	96.94
2	ELG2	2	2050	99.30
3	**ELG3**	**3**	**5450**	**102.10**
4	ELG4	4	8932	98.42

***Values represent mean ± S.D. (n=3)**

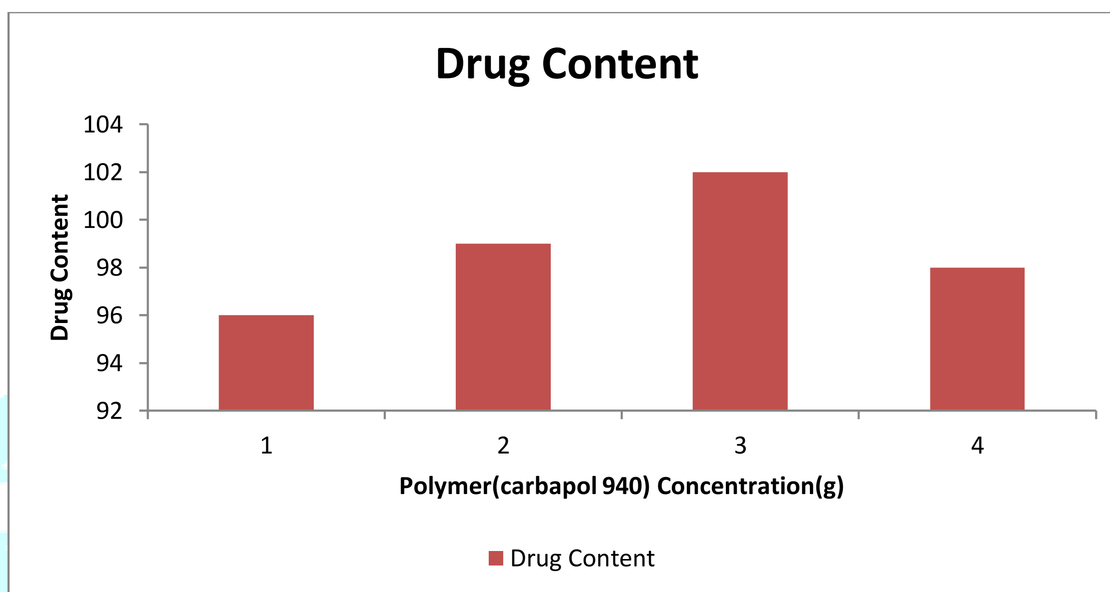

Figure 3.7: Effect of varying polymer concentration on drug content

3.4.3 Incorporation of liposomes of optimized batch into carbopol gel

Etodolac liposomes were prepared by thin film hydration technique. Liposomes containing etodolac (separated from the unentrapped drug) were mixed into the 3% (w/w) carbopol gel with an electrical mixer (25 rpm, 2 min) to get liposomes incorporated gel.

3.5 CHARACTERIZATION OF ETODOLAC LOADED LIPOSOMAL GEL (ELG)

Table 3.6: Evaluation results of optimized Etodolac liposomal gel

Formulation	Homogeni ty	pH	Viscosity(cP)	Spreadability (gm.cm/sec) (mean ± SD)*	Drug content*
ELLG	Good	6.8	2619	3.81 ± 0.165	96±0.3

***Values represent mean ± S.D. (n=3)**

3.5.1 In-vitro drug release study

Table 3.7: % Cumulative drug release from Etodolac Loaded liposomal gel

S.No.	Time(hrs)	% Cumulative drug release
1	0.5	5.65±0.56
2	1	12.20±0.78
3	2	24.78±0.68
4	4	35.30±0.30
5	6	49.24±0.47
6	8	57.72±0.39
7	12	65.02±0.54

*** Values represents mean ± S.D (n=3)**

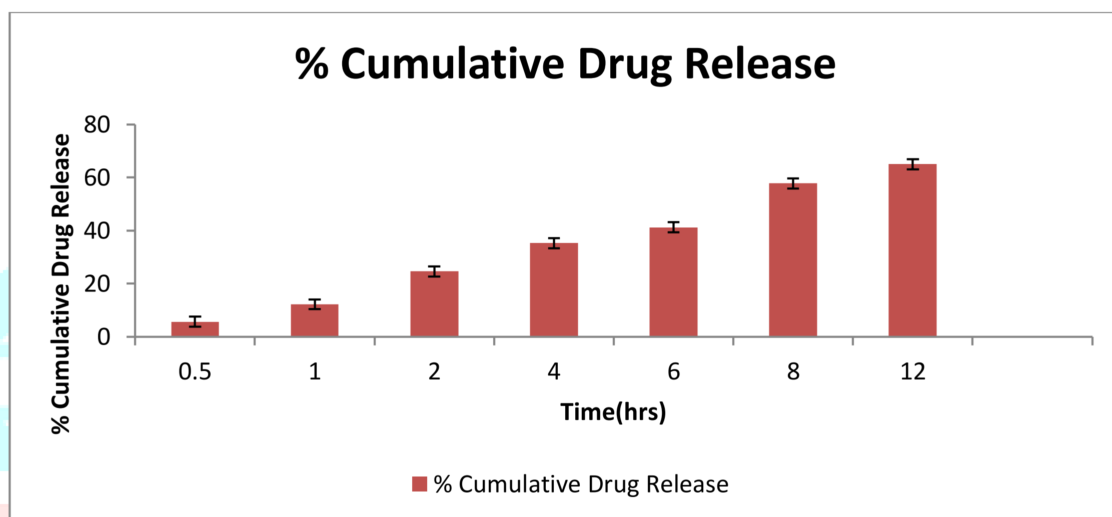

Figure 3.8: % Cumulative drug release from Etodolac loaded liposomal gel

4. RESULTS AND DISCUSSION

Liposomes were prepared by employing film hydration method. Several batches of liposomes were prepared to study the effect of varying drug : lipid ratio, effect of cholesterol and sonication time. In order to find out the influence of cholesterol which is a commom component of liposomes providing rigidity to the membrane, controlling permeability and improving plasma stability five formulations were prepared using different lipid/cholesterol ratio. Lipid\cholesterol ratio of 7:3 was observed to be optimum since it increased the % EE (entrapment effiency) from 66±0.07% (formulation with no cholesterol) to 76.02±1.12%.. This may be attributed to the fact that incorporation of cholesterol increases the hydrophobicity in the membrane bilayer that favours encapsulation of hydrophobic drugs. In addition further increase in the amount of cholesterol % EE decreases might be due to alignment of both the molecules within hydrophobic region and competition between them. Secondly in the same manner five batches of varying Drug /Lipid ratio were prepared to find out the influence on % EE. It was found that drug\lipid ratio of 10:3 has given greater % EE of 74±1.1 as compared to others. % EE increased on increasing drug concentration upto 10:3 then further increase in amount of drug reduced the % EE as reported in table. This may be due to saturation of lipid bilayer with the drug. Thus %EE was found to be dependent on drug/lipid ratio.

As a third part of study five batches of liposomes were prepared and sonicated for different time in order to find its influence on the vesicle size and % EE, sonication time of 5 minutes is sufficient to reduce liposomes

upto size range optimum for skin penetration and optimum drug entrapping efficiency. Optimized batch of liposomes where photographed using scanning electron microscope. Vesicles were found to be spherical in shape.Vesicle size was found to be varied from 82±0.2 nm to 92 ±0.3nm. Size was found to be reproducible reported. The encapsulation efficiency of the drug in the liposomes is from 66.07± 76%. The % cumulative drug release from Etodolac loaded liposomes was found to be 61.20±0.70 after 24 hrs as given in table.

Carbapol gel was prepared by the method reported by Anis *et al.,* and optimized by varying the concentration of carbapol 940 which was used as gelling agent its influence on drug content and viscosity of the gel was determined and reported. As the concentration of carbapol 940 increased viscosity of the gel also increased due to dense cross linking of the constituting entities but drug content increased only upto a suitable concentration further increase in carbapol concentration decreased the drug content may be due to change in the relative orientation of constituting molecules and surface properties of the gel.

Etodolac loaded liposomes were incorporated into 3% w/v of carbapol 940 gel by simple mixing. Etodolac liposomal gel was further characterized and physical examination of gel showed good homogeneity with absence of lumps. The prepared Etodolac loaded liposomal gel was much clear and transparent. The pH value of Etodolac loaded liposomal gel was found to be 6.8 and viscosity of 2619cP with drug content of 102.10. The spreadability was found to be 3.81±0.165 g.cm/sec . The results of pH ,viscosity, drug content and spreadability.

The % cumulative drug release was found to be 65.02±0.54 after 12 hrs. On the basis of results obtained from % Cumulative drug release of Etodolac loaded liposomes and Etodolac loaded liposomal gel it was found that release of Etodolac from liposomal gel is more rapid and advantageous as compared to Etodolac loaded liposomes.

Stability studies

Table 4.1: Storage stability data of Etodolac loaded liposomal suspension Stored at 4°C

Weeks	PARAMETERS	
	Particle Size(nm)*	**Drug Content***
1	87±0.28	96.85
2	87±0.27	96.78
3	87±0.24	96.54
4	86±0.21	96.31

*** Values represents mean ± S.D (n=3)**

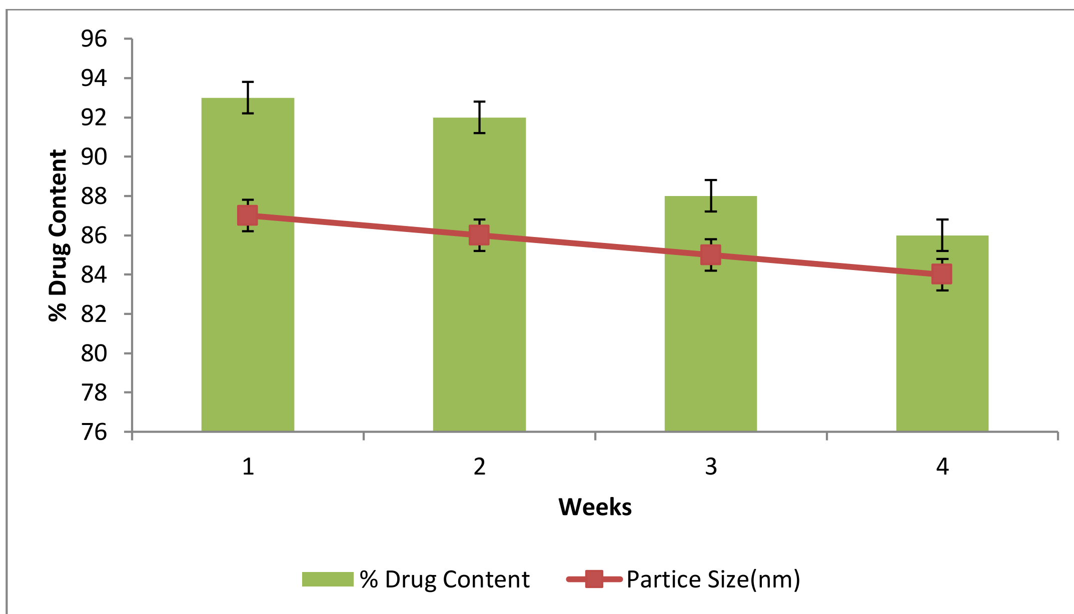

Figure 4.1: Effect of temperature on Particle Size and Drug Content of Etodolac loaded liposomal suspension

Table 4.2 : Storage stability data of Etodolac loaded liposomal suspension Stored at 25±2°C

Weeks	PARAMETERS	
	Particle Size(nm)*	Drug Content*
1	87±0.42	94.90
2	86±0.87	94.78
3	86±0.76	94.54
4	86±0.64	94.31`

* Values represents mean ± S.D (n=3)

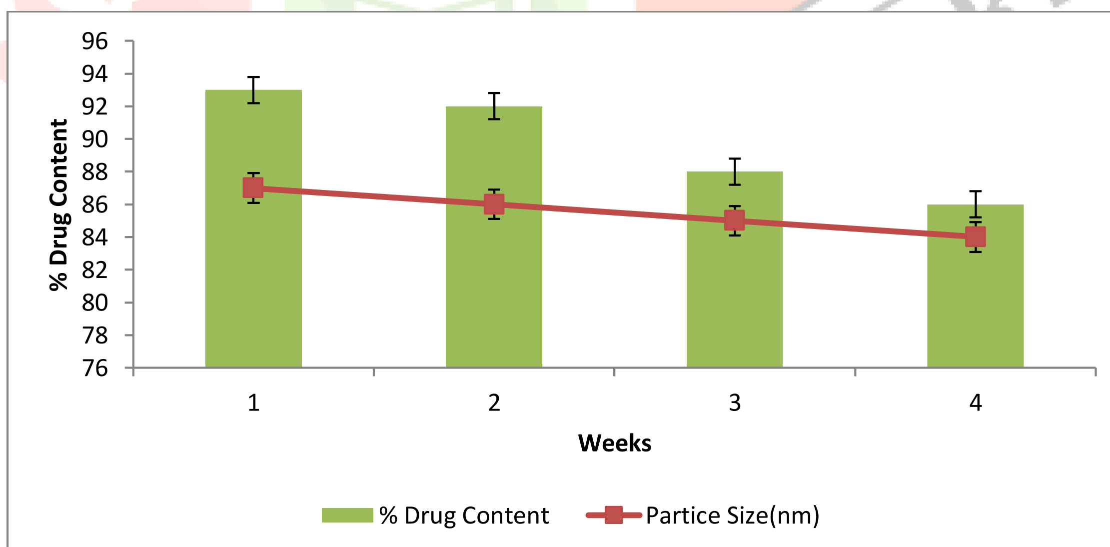

Figure 4.2: Effect of temperature on Particle Size and Drug Content of Etodolac loaded liposomal suspension

Table 4.3: Storage stability data of Etodolac loaded liposomal suspension Stored at 40±2ºC

Weeks	PARAMETERS	
	Particle Size(nm)*	Drug Content*
1	87±0.21	93.34
2	86±0.54	92.02
3	85±0.32	88.21
4	84±0.42	86.14

*** Values represents mean ± S.D (n=3)**

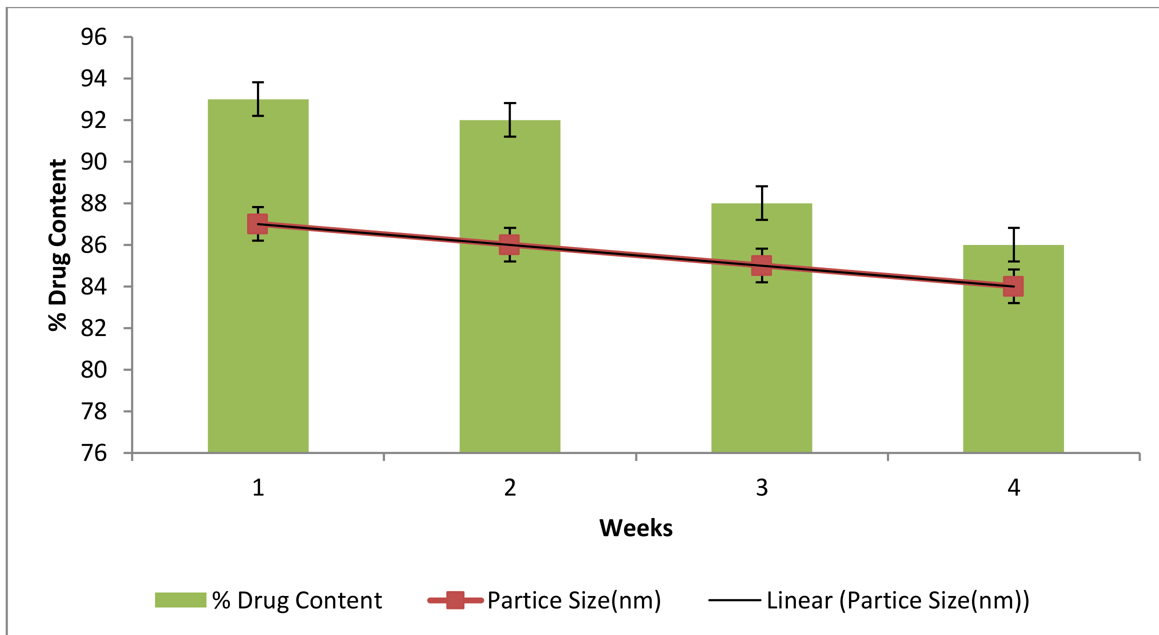

Figure 4.3: Effect of temperature on Particle Size and Drug Content of Etodolac loaded liposomal suspension

5. ANTI ARTHRITIC ACTIVITY

Anti- arthritic *In Vivo* Studies

The animals used for in-vivo experiment were adult wistar rats weighing 150 ±170gm.They were kept under Standard laboratory conditions.Carrageenan induced paw edema method was used to study the in-vivo performance of the prepared drug delivery system. Anti-inflammatory activity was determined by measuring the change in the volume of inflamed paw , produced by infection of carrageenan (0.1 ml of w/v) using plethysmometer.

Rat right hind paw was marked and everytime and it was dipped in plethysmograph (Mercury displacement Method) upto fixed mark to ensure constant paw volume. Wistar rat were divided into three groups including one controlled with each group consisting of three animals . The paw volume was measured at 0,1,2,4,6 upto 10 hours

Etodolac liposomal gel was applied transdermally to rat of respective groups, excluding the animals of controlled groups. The controlled group animals were injected with saline (0.9% NaCl) containing no drug. After 30 min of application of formulation, 0.1ml of 1% w/v carragenen (in 0.9% normal saline) was injected in the sub planter region of right hind paw of rat. The initial readings just after injection and subsequent paw volume was measured upto 10 hours.

The percent inhibitor of edema induced by carrageenan was calculated for each group using the following equation :-

% Inhibiton of edema = 100 [1-a-x/b-y]

Where,

a= Mean paw volume of treated animals after carageenan injection.

X= mean paw volume of treated animals before carragenan injection.

b= Mean paw volume of control animals after carageenan injection.

Y= mean paw volume of control animals before carageenan injection.

Table 5.1: Anti- inflammatory activity of Etodolac liposomal gel

Formulation	% Swelling of induced edema after				
	2h	4h	6h	8h	10h
Control	100±0.5*	99±0.5	100±0.5	98±0.5	100±0.5
ELG43	70.0±0.5 (30.3)	50.2±1.5 (49.8)	30.3±0.9 (69.5)	43±0.5 (54.3)	55.1±0.4 (45.9)

*Mean±SD

The values between paranthesis indicate the % inhibition of edema

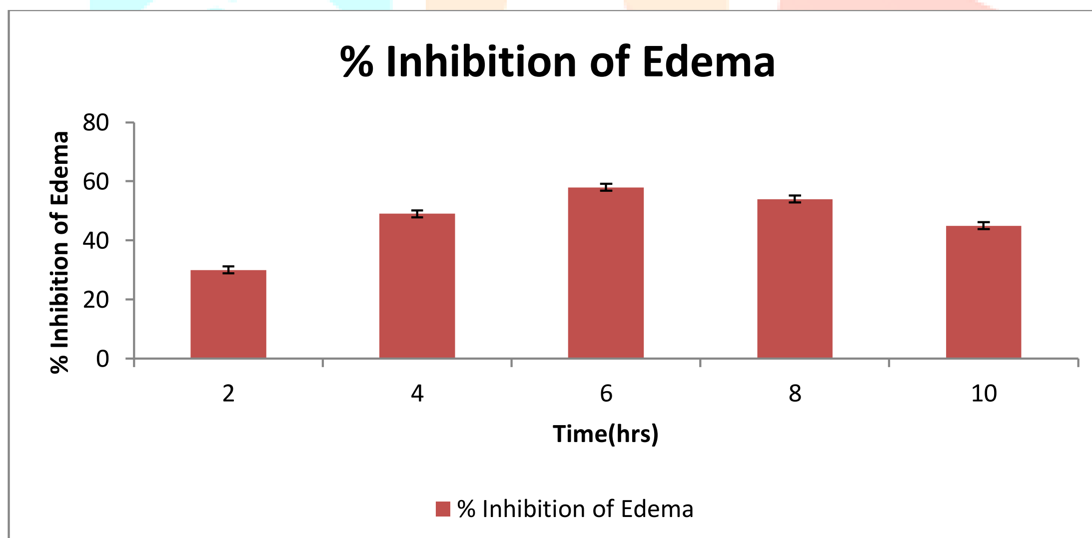

Figure 5.1: % Inhibition of edema with time (hrs)

SUMMARY AND CONCLUSION

Liposomes are spherical microscopic vesicles composed of one or more concentric lipid bilayers separated by water or aqueous buffer compartments with a diameter ranging from 25 nm-10000 nm. Liposomal formulations are widely used in the pharmaceutical field as drug delivery systems due to their versatility and clinical efficacy and they have been used to administer drugs by several routes such as the oral, parenteral, and topical. Among these, topical delivery of drugs carried by liposomes exhibits interesting applications, not only for promoting dermal delivery of drugs, but also for enhancing transdermal delivery of drugs intended for systemic use, thus more effectively exploiting this non-invasive alternative route to oral administration. Due to the forementioned advantages, in this study liquid-state liposomes were chosen to serve as the drug

delivery system. Although liposomes demonstrated promise for Transdermal drug delivery, the practical application of these formulations onto the skin is less. However, these can be incorporated into the gels than can applied onto the skin. It has been found that liposomes incorporate into the hydrogels are more stable . In this study such as application for Etodolac has been investigated.

Etodolac a member of the pyrano carboxylic acid group of non steroidal anti inflammatory drug (NSAIDs), exhibit anti inflammatory, analgesic and antipyretic properties. It is indicated for relief of sign and symptoms of rheumatoid arthritis and osteoarthritis. The anti-inflammatory effect of etodolac mainly result from inhibition of enzyme Cyclo oxygenase (COX). This decreses the synthesis of peripheral prostaglandins involved in mediating inflammation. It is extensively metabolized in the liver and because of its short biological half-life, frequent administration is required. Gastrointestinal bleeding and ulceration are quite common due to oral therapy of Etodolac. Clinical evidences suggest that topically applied nonsteroidal anti inflammatory drugs (NSAIDs) are safer and at least as efficacious as oral NSAIDs in the treatment of rheumatic diseases. Gastrointestinal adverse drug reactions after topical NSAIDs use are rare when compared to the 15% incidence of serious GI events associated to orally administered NSAIDs.

Identification studies of drug Etodolac indicated that the supplied drug was matching with standards as prescribed in I.P. procedures. An UV absorption maxima of drug was found to be 279 nm. Purity of drug was checked by FTIR result showed that drug was available in the pure form. Solubility Profile of Etodolac in different solvents indicated that the drug was freely soluble in methanol, ethanol and Di methyl sulphoxide , very slightly soluble in chloroform and acetone and insoluble in water and ether.

Partition coefficient of the drug was found to be 9.8 in n-octanol :Phosphate buffer solution (pH 7.4) suggesting the drug to be lipophilic in nature. Standard curves of Etodolac were prepared in different fluids i.e. PBS (pH 7.4) . The R^2 value was 0.999 and a straight line was obtained in the range of 2-20µg/ml. This was in consideration with Beer-Lambert's law, in the used range in UV Spectroscopy.

Liposomes were prepared by employing film hydration method. Several batches of liposomes were prepared to study the effect of varying drug : lipid ratio, effect of cholesterol and sonication time. In order to find out the influence of cholesterol which is a commom component of liposomes providing rigidity to the membrane, five formulations were prepared using different lipid\ cholesterol ratio. Incorporation of cholesterol increases the hydrophobicity in the membrane bilayer that favours encapsulation of hydrophobic drugs. Increase in the amount of cholesterol beyond a suitable saturation concentration% EE decreases might be due to alignment of both the molecules within hydrophobic region and competition between them. Secondly in the same manner five batches of varying Drug \Lipid ratio were prepared to find out the influence on % EE.. % EE increased on increasing drug concentration . This may be due to saturation of lipid bilayer with the drug. Thus %EE was found to be dependent on drug\lipid ratio. Optimized batch of liposomes where photographed using scanning electron microscope to study shape and surface morphology. Particle size analysis have been done by using Nano ZS zetasizer at 25°C.

Carbapol gel was prepared and optimized by varying concentration of carbapol 940 used as gelling agent and considering its effect on drug content and viscosity. As the concentration of carbapol 940 increased viscosity of the gel also increased due to dense cross linking of the constituting entities but drug content increased only

upto a suitable concentration further increase in carbapol concentration decreased the drug content, may be due to change in the relative orientation of constituting molecules and surface properties of the gel. Etodolac loaded liposomes were incorporated into 3%w/v of carbapol 940 gel by simple mixing. Etodolac liposomal gel was further characterized and physical examination of gel showed good homogeneity with absence of lumps. The prepared Etodolac loaded liposomal gel was much clear and transparent. The pH of various gel formulations was determined by using digital pH meter. Viscosity of prepared gels were measured by Brookfield Viscometer.

Spreadabality was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slides. Drug content was determined using methanol and in vitro drug release using Franz diffusion cell. On the basis of results obtained from % Cumulative drug release of Etodolac loaded liposomes and Etodolac loaded liposomal gel it was found that release of Etodolac from liposomal gel is more rapid and advantageous as compared to Etodolac loaded liposomes.Stability studies was done by keeping the Etodolac loaded liposomal suspension at three different temperature conditions, i.e., 4-8°C (Refrigerator; RF), 25±2°C (Room temperature; RT), and 40±2°C for a period of 4 weeks. Drug leakage, particle size growth and the chemical stability of drugs were studied at different temperatures.

The results obtained have shown that prepared vesicles were physically and chemically stable at 4ºC and 25ºC, no significant changes were observed during the course of study at 4ºC and 25ºC,but the Etodolac loaded liposomal suspension stored at 40ºC showed a slight increase in the particle size due to aggregation or swelling of liposomes. Slight decrease in the drug content was also observed that was may be due to leakage of drug.

The *in vivo* performance of Etodolac loaded liposomal gel was carried out using Carrageenan induced paw edema method. Etodolac loaded liposomal gel not only reduced the inflammation to an larger extent but also gave an sustained effect.

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