CHAPTER 5

PREPARATION, CHARACTERIZATION AND EVALUATION OF FORMULATIONS FOR NASAL DELIVERY

Much attention has been paid to the use of the nasal route for the systemic delivery of drugs that are conventionally administered by injection The nose has many advantages as a potential site for drug delivery, being readily accessible facilitates self-medication, which may improve patient compliance compared to parenteral routes. The nasal mucosa has a relatively large absorptive surface area and is highly vascularised Furthermore, the blood is drained directly from the nose into the systemic circulation, thus, avoiding first pass metabolism predominantly by the liver However, many drugs, particularly polar drugs such as peptides and proteins, are not well absorbed from the nasal cavity when administered as simple solutions, with bioavailabilities in the order of 1% or less (Illum, 2000).

The major factors limiting the bioavailability of nasally administered drugs are the poor ability of polar compounds, especially the large molecular size peptides and proteins, to cross mucosal membranes, and the mucociliary clearance mechanism in the nasal cavity that rapidly removes nonbioadhesive solutions and powders from the absorption site and down the throat

Several formulation factors like pH, viscosity and mucoadhesive property, affect the drug bioavailability from nasal cavity need to be monitored to have similar in vivo batch to batch performance. Recently, the mucoadhesive function has received much attention for prolonging the residence time of dosage forms at the absorption site (Guputa et al . 1990) By combining chitosan and liposomal characteristics, specific, prolonged, and controlled release may be achieved (Takeuchi et al., 1996) Carbopol has been also shown to be a useful mucoadhesive polymer (Ishida M et al , 1982, Akiyama Y et al , 1995)

Any developed formulations needed to be exploited commercially, they should be prepared using standardized and validated technique, characterized and studied for stability. The diffusion of the drug from the developed formulations should be studied and finally in vivo studies/clinical evaluation should be conducted. In this investigation, nasal formulations of LN and LEU were prepared and characterized for the pH, viscosity and mucoadhesive performance At present, in vitro test systems have not been developed which can accurately predict the rate of drug release from

liposomes in vivo Therefore an in vitro diffusion technique is proposed, validated and utilized for drug diffusion studies from potential liposomal formulations The in vitro studies were followed by in vivo studies in rats

5.1 REAGENTS

(i) Water Double distilled water

(ii) Acetate buffer (ionic strength, 0.261), pH 5 2: It was prepared as per the procedure given in the Indian Pharmacopoeia

(iii) Simulating nasal fluid 7 45 gm Sodium chloride, 1 29 gm Potassium chloride and 0 315 gm Calcium chloride were dissolved in 1000 ml of double distilled water (Melon, 1968)

(iv) Phosphate buffer saline, pH 7 4 (PBS). As described in Indian Pharmacopoeia

(v) 20% methanolic PBS. Accurately measured 800 ml of pH 7 4 Phosphate buffer saline was transferred to a clean, dry 1000 ml volumetric flask and to it was added 200 ml of Methanol.

5.2 PREPARATION OF FORMULATIONS

The optimized liposomal batches, LLN; LLEU, LLEUn were used to prepare nasal formulations.

5.2.1 PREPARATION OF CARBOPOL DISPERSION (1%W/V):

The polymer powder was weighed accurately and dispersed in double distilled water The dispersions were then stirred using magnetic stirrer till the clear solution results The dispersion formed was kept overnight in freeze to remove entrapped air The final

polymer concentration was adjusted to 1% w/v Prepared solutions were use to incorporate the drug (LN)

5.2.2 PREPARATION OF CHITOSAN SOLUTION (1%W/V):

The polymer powder was weighed accurately and dispersed either in 0 01% acetic acid (for LN) or acetate buffer pH 5 2 (for LEU) The dispersions were then stirred using magnetic stirrer till the clear solution results The solution formed was kept overnight in freeze to remove entrapped air The final polymer concentration was adjusted to 1% w/v Prepared solutions were use to incorporate either drug or prepared liposomes

5.2.3 PREPARATION OF LN WITH CARBOPOL DISPERSION (LN+CP)

(1 MG/ML):

The LN was weighed accurately and dispersed in double distilled water and sonicated for approximately 1 hr to get particle size in range of 10-15 micron The suspension was diluted to get final drug concentration of 2 mg/ml The resulting suspension was further diluted with the equal volume of Carbopol dispersion The resulting mixture was mixed well and stored in glass vial in refrigerator till use

5.2.4 PREPARATION OF LN WITH CHITOSAN SOLUTION (LN+CS) (1MG/ML):

The LN was weighed accurately and dispersed in double distilled water and sonicated for approximately 1 hr to get particle size in range of 10-15 micron The suspension was diluted to get final drug concentration of 2 mg/ml The resulting suspension was further diluted with the equal volume of Chitosan solution The resulting mixture was mixed well and stored in glass vial in refrigerator till use

5.2.5 PREPARATION OF LEU WITH CHITOSAN SOLUTION (LEU+CS) (0.1MG/ML):

500 µg of drug was weighed accurately and transfer to 10 mL volumetric flask Acetate buffer pH 5 2 (5 mL) was added and volume was made up to the mark with 1% chitosan solution in acetate buffer pH 5.2 (5 mL). The resulting solution was mixed well and stored in amber colored glass vial in refrigerator till use

5.2.6 PREPARATION OF LLEU WITH CHITOSAN (LLEU+CS) (0.1MG/ML):

5 ml of LLEU containing 500 µg of drug was diluted with equal volume of chitosan solution The resulting suspension was mixed well and stored at refrigerator till required

Along with above formulations, LN suspension in water (particle size- 10-20 µm), LN physical mixture with liposomal constituents (LN PM) and optimized LLN, LLEU, LLEUn formulations in similar concentrations were also used in further studies

5.3 CHARACTERIZATION OF FORMULATIONS

The developed formulations were characterized for pH, viscosity and mucoadhesion test as given below

5.3.1 pH DETERMINATION

pH of the formulations were determined using pH meter and results are recorded in Table 5.1.

5.3.2 VISCOSITY

Viscosities of the formulations were determined using Oswald viscometer and results are recorded in Table 5.1

5.3.3 ASSAY

Samples were withdrawn and assay was performed using method described in Chapter 3 (Section 3 4 5 and 3 5 5) and results are recorded in Table 5 1

5.3.4 MUCOADHESION

The mucoadhesive property of the developed formulations was evaluated by an in vitro adhesion testing method known as the wash-off method (Lehr et al , 1990) The mucoadhesiveness of developed formulations were evaluated on freshly excised bovine nasal mucosa (1 × 1 cm) mounted on glass slides (3 × 1 inch) with cyanoacrylate glue The glass slides were connected with a suitable support 0 1 ml formulation was placed on nasal mucosa The tissue was then placed in the dessicator to maintain at > 80% relative humidity at room temperature for 15 min to allow the formulation to interact with the nasal mucosa and also to prevent drying of the mucus

Tissues were seen under microscope, and the numbers of particles/liposomes attached to the particular area were counted The support was hung onto the arm of a USP tablet disintegrating test machine When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up-and-down movement in the simulating nasal fluid at 37°C contained in a 1 L vessel of the machine At the end of 30 minutes, at the end of 1 hr, and at the end of 2 hr. the machine was stopped and the number of particles/liposomes still adhering to the same portion of the tissue was counted under microscope

The adhesion number or percentage of mucoadhesion was calculated and results obtained are recorded in Table 5 2

5.4 DRUG RETENTION STUDIES

The prepared formulations LLN, LLEU, LLEUn and LLEU+CS were subjected to drug retention studies for a period of 6 months Prepared batches were sealed in 30 ml

glass vial and stored at refrigeration temperature (2-8°C) and room temperature (25°C ± 2°C) The percent drug retained in liposomes was determined at specific time intervals using the method described in Chapter 3 (Section 3 4 7 and 3 5 7) The results of this study are recorded in Table 5 3-5 4 and shown graphically in Figure 5 2-5.3

The increase in vesicle size of liposomes was determined from changes in vesicle diameter for liposomes at refrigeration temperature (2-8°C) after 3 months and 6 months storage period as described in Chapter 4 (Section 4 4 1) The results are recorded in Table 5.3-5 4

5.5 IN VITRO DIFFUSION STUDIES

The in vitro methods are valuable screening procedures for deducing phyico-chemical parameters such as fluxes, partition coefficients and diffusion coefficients Though according to Gemmell and Morison, 1957, in vitro methods may be of limited predictive value but they are the means of accessing the ability of a vehicle or base to liberate medicament under the conditions of the test A theoretical disadvantage of such a technique is that the method does not exactly duplicate the behavior of living tissue in situ, particularly with respect to capricious blood supply and metabolism However, since performing bio studies on every manufacturing batch is impractical and costly, formulators must rely on in vitro testing to ensure batch-to-batch uniformity and consistency in bioavailability.

5.5.1 EXPERIMENTAL SETUP

5.5.11 Preparation of the membrane:

Dialysis membrane (250-9U, molecular weight cut off. 12000 Dalton, Sigma. Hyderabad, India), 200µm in thickness, pH 5.8 to 8, breaking strength 2 75 kg f/cm and porosity 0 45 µm was used as a membrane for in vitro diffusion studies because

of simplicity, homogeneity, and uniformity This membrane was pretreated with ethanol (95%) followed by hydration in PBS for 24 hr prior to permeation runs

5.5.12 Design of diffusion Cell:

For the present study a vertical type of membrane diffusion system was designed (Figure 5 1) The system consists of a hollow glass tube open at both ends with inner diameter of 18 mm and length of 6 cm The diffusion membrane was tied to one end of the tube with a nylon string, serving the purpose of a donor compartment The tube was immersed in 20 ml diffusion medium (20% methanolic PBS for LN formulations and PBS for LEU formulations), maintained at 37 ± 0 5°C under continuous stirring at a rate of 50 rpm, in a way that the membrane just flushes to the surface of the diffusion fluid

5.5.13 Validation of Diffusion Cell:

The hydrodynamic characteristics of the diffusion cell were established using the benzoic acid disc method (Chein and Valia, 1984, Shahiwala, 1999)

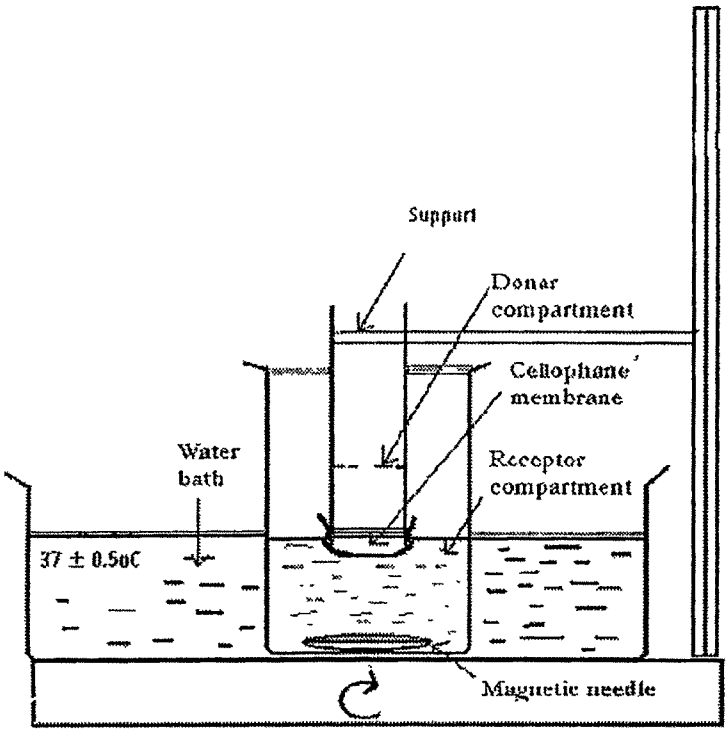

Figure 5.1: Design of Diffusion cell

5.5.2 METHOD

Diffusion studies were carried out for plain drug and liposomal formulations of LN and LEU Plain drug suspension/solution and liposomal formulations containing either1 mg LN or 500 µg LEU were transferred to the donor compartment One ml of sample was withdrawn from the receptor compartment at definite time intervals and equivalent amount of fresh medium was replaced to the receptor compartment The estimation of the drugs in sample was carried out using the procedure described in Chapter 3 (Section 3 6 8 and 3 7 8) All diffusion runs and sample analysis were carried out in triplicate on three consecutive days and mean values along with standard error of mean are recorded in Table 5 5 and Table 5 8 and shown graphically as Q vs t (hours) in Figure 5 4 and Figure 5 6 Regression coefficients by different release kinetic models were calculated and recorded in Table 5 6 and 5 9 The mean flux values, J (µg/min), and diffusion coefficients were also calculated for all the formulations and recorded in Table 5 7 and Table 5 10 and shown graphically in Figure 5 5 and Figure 5 7

5.6 IN VIVO STUDIES

5.6.1 LN FORMULATIONS

5.6.11 Animals

Six female albino rats (200 ± 50 g) for each group were used Three male Albino rats (250 ± 20 g) for each group were used for mating studies. Only animals with proven fertility record were selected for the studies All animals were housed in polypropylene cages with free access to palletized chow and tap water The animals were exposed to alternate cycles of 12 hr light and darkness Animal experiments were approved by social justice and empowerment committee, ministry of government of India, New Delhi, India

5.6.12 Methodology

Rats were divided into 6 groups of 6 animals each Five groups were administered intranasally with LN suspension, LN PM, LLN, LN+CS and LN+CP formulations respectively and one group administered orally with LN suspension

Nasal administration

At the time of administration, animals were partially anesthetized with anesthetic ether and 10 µl of formulations containing 10 µg drug were placed along the nasal wall with a micropipette.

Oral administration

For oral administration, 10-µg drug suspension (LN) was instilled through month using 28 gauge long blunt needle.

200µl blood samples were withdrawn from tail vein at specific time points and estimation of the drugs in sample was determined using the procedure described in Chapter 3 (Section 3.6 9) The data of drug plasma concentration are shown in Figure 5 8 Various pharmacokinetic parameters. (Tmax, Cmax, and $t_{1/2}$) were calculated from the Figure 5 8 and recorded in Table 5 11 AUC and bioavailability (F*) were also calculated and are recorded in Table 5 11. Each set of result represents the mean values of six experimental determinations along with its standard error mean

Fertility performance studies were also carried out for LN formulations Various LN formulations were administered for a maximal period of four consecutive estrous cycles. Formulations LN suspension (oral), and LN suspension, LN PM and LLN formulations (intranasal) were administered daily, while LN+CS, LN+CP formulations were administered once in a two days. Each female was inspected every morning for evidence of mating (the presence of vaginal plugs or sperm) During the LN formulations administration period, if any animal had mated, it was removed from

the mating cage immediately after observing one or both coital signs (day 1) and was killed on ninth day of postcoitum to count the number of implantations The remaining animals, in which coital signs were not observed, were also killed ninth day after the last night's cohabitation and inspected for any sign of implantation

5.6.2 LEU FORMULATIONS

5.6.21 Animal Selection

White albino rats of either sex (equal in numbers) weighing 170± 20 g (120~140 days of age) were used. Male and female rats were caged separately during the study unless otherwise required All the animals used for the study were of proven fertility record All animals were housed in polypropylene cages with free access to palletized chow and tap water. The animals were exposed to alternate cycles of 12 hr light and darkness. Animal experiments were approved by Social Justice and Empowerment Committee, Ministry of Government of India, New Delhi, India

5.6.22 Methodology:

Rats of either sex were divided into 8 groups of 6 animals each. One group was kept as control Control group was treated with the composition containing HSPC, CHOL and CS of the concentration used in formulations Other groups of animals were treated with different formulations containing LEU

Nasal administration

At the time of administration, animals were held from the back in horizontal position and 10 µl of formulations containing 5 µg drug were placed along the nasal wall with a micropipette.

Subcutaneous administration

For subcutaneous administration, 5 µg drug solution (LEU) was injected into the nape of the neck

Blood was sampled from tail vein at different time points Serum were separated and stored at -20°C. till the serum LH concentrations determined by hormone radioimmuno-assay as described in Chapter 3 (Section 3 5 9)

Fertility performance of male rat

Fertility performance studies were also carried out for selected LEU formulations Selected formulations were administered in male rats for 26 days After the completion of drug schedule the male rats (placebo/treated) were paired overnight with normal cyclic female rats (1 : 1) on their proestrous phase The presence of spermatozoa in the vaginal smear following exposure was taken as an index of the first day of gestation The male rats were sacrificed, blood collected and plasma separated Sperms were collected from the left caudal epididymis immediately after sacrifice for sperm count. Mated normal female rats were autopsied on the tenth day of their gestation The fertility test was considered positive when implantation sites were present

Sperm count

The epididymal sperm count was done by the method of Linder et al. The epididymal sperm were collected by cutting epididymis into small pieces and flushing the sperm in normal saline The sperm collected was centrifuged at 2000 rpm for 10 min The pellet was resuspended in 2 ml of normal saline. An aliquot (0.5 ml) of sperm suspension was homogenized for few seconds, centrifuged at 6,000 rpm for 10 min and again rehydrated with 5 ml of normal saline An aliquot of this solution was placed in haemocytometer and motile sperm were counted by using microscope. To minimize the error, count was repeated at least five times for each rat

Fertility performance of female rat

Selected formulations were administered in female rats for two estrous cycles to see their effect on the normal cyclicity of the rats

5.7 STATISTICAL ANALYSIS

Mucoadhesion test

The adhesion number or percentage of mucoadhesion was calculated by the following equation

$Na = (N/N_0) \times 100$

Where, Na = adhesion number

N_0 = total number of applied particles

N= number of particles attached to the substrate

Stability Studies

For drug retention studies, the data of percent drug retained in liposomes at 3 different storage conditions were compared using ANOVA and the differences were considered significant at $P<0.05$.

The vesicle size of liposomes (LLN, LLEU, LLEU+CS and LLEUn) was measured immediately after preparation and after 3 month and 6 months storage at refrigeration temperature (2-8°C) and room temperature (25±2°C). The mean vesicle diameter obtained immediately after preparation and after 3 months storage was statistically evaluated using student's t-test and the differences were considered significant at 5% level.

Diffusion Studies

Six batches were evaluated for in vitro diffusion and the results are expressed as mean± SEM

(a) Percent Drug Diffused

The percent drug diffused across artificial membrane at each sampling points was determined by the formula given below

$$\text{Pecent Drug Diffused (R)} = \frac{CrVr}{CdVd} \times 100$$

Cr = Concentration of drug in receptor compartment

Vr = Volume of the receptor compartment

Cd = Initial concentration of drug in donor compartment

Vd = Initial volume of donor compartment

(b) Kinetics of Release

To study the mechanism of drug release from the formulations, the release date were fitted to the following equations.

Zero-order equation $Q = Q_0 - k_0 t$

Where Q is the amount of drug release at time t, and k_0 is the release rate;

Higuchi's equation. $Q = k_2 t_{1/2}$

Where Q is the amount of drug release at time t, and k_2 is the diffusion rate constant

First-order equation $\ln Q = \ln Q0 - k_1 t$

Where k_1 is the release rate constant,

The order of drug release was determined by performing regression over the mean values of percent drug diffusion vs t and percent drug diffusion vs Root t

(c) Mean Steady state flux,

The flux across the membrane was calculated using the following formula

$J = V (dc/dt)$

Where J = flux of the drug across the membrane

V = Volume of receptor compartment

(dc/dt) = Rate of change of concentration

Mean steady state flux is the mean of individual flux values at all sampling points

(d) Diffusion Coefficient

The diffusion coefficient of the drug at every sampling point was calculated using the following equation:

$R= 200 \text{ Sq rt } Dt/\pi h^2$

Where, R = Percent drug diffused

h = thickness of the membrane (0.02 cm)

t = time (sec)

D = diffusion coefficient (cm^2/sec)

The diffusion coefficient used for the discussion is the mean of the value (D) obtained at each sampling point

The mean flux values (n=3), J, and mean percent drug diffused (n=3), Q, obtained for LLN-DPI were compared with that of LN by applying student's t-test and the differences were considered significant at 5% level. Similarly, the mean flux values (n=3), J, and mean percent drug released (n=3), Q, obtained for LLEU-DPI and LLEUn-DPI were compared with that of LEU by applying ANOVA and the differences were considered significant at $P < 0.05$.

(v) In vivo studies:

Each experiment was repeated six times and the results obtained are expressed as mean± SEM

The drug plasma concentrations at each sampling time point were plotted against time in hr. Maximum plasma concentration (Cmax), time in hr to achieve Cmax (Tmax) and drug plasma half-life ($t_{1/2}$) were determined from drug plasma concentration-time curve from best fit curve using major and minor gridlines with ±0.2 unit accuracy. The area under the plasma level curve was calculated by the trapezoidal rule. Data were compared using ANOVA and difference at $p<0.05$ were considered significant.

The relative percent pulmonary bioavailability/bioactivity (F*) with respect to orally/subcutaneously administered LN was calculated by,

$$I^{*} = \frac{\text{AUC Intratracheal Route} \times \text{Subcutaneous Dose}}{\text{AUC Subcutaneous Route} \times \text{Intratracheal Dose}} \times 100$$

5.8 RESULTS & DISCUSSION

In this investigation, formulations of plain and liposomal drugs were prepared for intranasal application using composition as shown in Table 5.1. Developed formulations were evaluated for parameters like assay, pH, viscosity, mucoadhesion, in vitro diffusion studies across dialysis membrane and in vivo performance in rats. Liposomal formulations were also subjected to drug leakage studies. Assay values for all the formulations were 99-101%. pH determination was performed because of the possible influence of pH on the irritancy for mucosa, while viscosity determination was performed because of the possible influence of the viscosity on the nasal mucociliary clearance and pourability of the dosage form. Preparations having pH above 3.5 and below 8 suggest that they are not irritant in delivered volume. Also, viscosities of different formulations suggest that they are pourable.

The results of the mucoadhesion test are shown in Table 5.2. Liposomes and/or drug in CP and/or CS exhibited good mucoadhesive properties in the *in vitro* wash-off test (Table 5.2). Formulations with CS showed better mucoadhesion property compared to formulation with CP. This may be due to the strong interactions between the positively charged CS and negatively charged nasal mucosa. When mucoadhesion of LLEU was compared to that of LLN, higher mucoadhesion was observed. This may be due to the ionic interactions between LEU induced positive charges in liposomes and negatively charged nasal mucosa.

Drug retention studies of LN liposome (LLN) formulations:

The drug retention studies were carried out at refrigerated temperature (2-8°C) and room temperature (25°C±2°C) for the LLN stored in sealed glass vials. LLN batches were evaluated for PDE in liposomes and the results are recorded in Table 5.3 and shown graphically in Figure 5.2.

Table 5.1. Plain and liposomal Formulations

Formulation	Content	Assay	pH	Viscosity
LN+CP	Plain LN in 0 5% CP solution (Distilled water)	99 8	3 9	210 6 cps
LN+CS	Plain LN in 0 5% CS solution (Distilled water)	100 1	3 8	157 5 cps
LEU+CS	Plain LEU in 0 5% CS solution (Acetate buffer, pH 5 2)	100 3	5 2	159 8 cps
LLEU+CS	Liposomal LEU in 0.5% CS solution (Acetate buffer, pH 5 2)	99.9	5.2	158 4 cps

Table 5.2: Mucoadhesion Test for Different Formulations

Formulation	Percentage of particles/liposomes adhering to tissue at different time points		
	30 min	1 hour	2 hour
LN	--	--	--
LLN	--	--	--
LLEU	19±1.7	--	--
LLEUn	--		
LN+CP	82±1.7	77±2.2	62±1.4
LN+CS	89±2 1	83±2.5	71±1.8
LLEU+CS	83±1.8	79±1.9	68±2.0

Drug retention studies at 25±2°C showed about 10% of drug was leaked after storage for the three months from LLN and therefore were discontinued afterwards at this temperature LLN stored at refrigerated condition was found to be stable with regard to percent drug retention (96 4%±0 34%) during 6 months stability period The size of liposomes were also determined immediately and after 3 and 6 months storage at refrigerated conditions and results are recorded in Table 5 3 The D [4,3] was increased insignificantly (p>0 05) after storage at refrigeration temperature upto 6 months

Drug retention studies of LEU liposomal formulations:

Similarly, LLEU. LLEUn and LLEU+CS were also subjected to drug retention studies at refrigerated temperature (2-8°C) and room temperature (25°C±2°C)

Stability studies at 25±2°C resulted in 6-7% drug leakage after storage for the three months from LLEU and LLEUn, therefore stability studies were discontinued afterwards for these products at this temperature. However, LLEU and LLEUn batches were found to be stable over 6 months stability period with regard to percent drug retention (more than 95%) under refrigerated conditions The size of liposomes were also determined immediately and after 3 and 6 months storage at refrigerated temperature and results are recorded in Table 5 4 The D [4,3] was increased insignificantly (p>0 05) after storage at refrigeration temperature during the stability period of 6 months

After incorporation of CS in LLEU formulations were found to result into significant improvement with regards to percent drug retention as shown in Table 5 4 and Figure 5 3 LLEU+CS formulation was found to be stable for 3 months at accelerated conditions compared to two months stability of LLEU and LLEUn formulations The values of percent drug retention were significantly higher at all sampling points at both the conditions for LLEU+CS formulations compared to LLEU formulations. The increase in stability of liposomes after CS incorporation may be due to the repulsive forces between positively charged CS and positive charged LEU may prevent the drug

leakage from liposomes The increase in viscosity also contributed to increase in liposomal stability

As obvious, when compared between the batches stored at lower temperature (refrigerator) compared with the one stored at higher temperature (controlled room temperature), batches at lower temperatures showed higher preservation

IN VITRO DRUG DIFFUSION STUDIES

LN FORMULATIONS

Comparative diffusion studies were carried out between various LN formulations for a period of 24 hr to 72 hr using dialysis membrane in self designed and validated diffusion cell. As the drug was soluble in 20% methanolic PBS, sink conditions were maintained when 50 ml of the medium used as a diffusion medium and hence zero-order flux conditions not violated The results obtained are recorded in Table 5 5 and shown graphically in Figure 5 4 ANOVA was performed over all the mean percent drug diffused values and differences larger at $p<0.05$ were considered as significant.

From the results of mean cumulative amount of drug released Q ($\mu g/cm^2$), (n=3), at each sampling time point shown in Table 5 5 reveals that cumulative permeation of LN was significantly higher ($p<0.05$) from LLN at all time points. This shows liposomal encapsulation of drug significantly prolongs the drug diffusion. When LN and LN+CS formulations were compared, no significant differences were observed However, significant differences ($p<0.05$) were observed when LN and LN+CP formulations were compared. This may be due to formation of gel at diffusion medium pH at the contact surface to the membrane with CP.

A fair amount of attention has been given in the literature to gain a mechanistic understanding of drug release from the preparations and the factors affecting it. The percent drug release is plotted against time (T) in Figure 5 4. The non linearity of the graph suggests that the release pattern does not follow zero order kinetics. An attempt was made to understand the diffusion kinetics; kinetic parameters were calculated and

are shown in Table 5.6. The drug diffusion data from various formulations fit a Higuchi's equation as the highest correlation (R^2 = 0.98-1.00) obtained. Higuchi obtained a mathematical relationship for cases where the drug particles are dispersed in homogeneous uniform matrix which acts as the diffusion medium and where the drug particles are incorporated in an essentially granulated matrix and released by the leaking action of the permeating solvent.

In case of liposomal preparations, the efflux rate of drug is generally calculated and the mean flux values are reported (Masini et al., 1993, Rolland, 1993). Mean flux values of various LN formulations were calculated and recorded in Table 5.7 and shown graphically in Figure 5.5. The diffusion co-efficient of different LN formulations were also calculated and recorded in Table 5.7 and its graphical presentation is given in Figure 5.5. The mean flux values of the LN formulation were found to be two to five times higher than those of LLN formulation, indicating that liposomal formulation prolong the drug diffusion. Similarly the diffusion coefficient of the LN is much higher to that of the LLN formulation confirming a prolong drug diffusion following liposomal encapsulation of drug.

Lower mean diffusion flux and diffusion coefficients values for LN+CS and LN+CP compared to that of plain drug formulation of LN may be due to the increase in viscosity of the formulations. Gel formation takes place at the contact points of CP containing formulation with the membrane diffusion medium pH of 7.4 contributing to further lowering of mean flux and diffusion coefficient values.

Table 5.3: Percent Drug Retention (PDR) in Batch LLN at different storage conditions

Time in months	PDR±SEM* at 2-8°C	PDR±SEM* at 25±2°C
0 5	99 8±0 14	98 1±0.24
1	99 8±0 23	96 8±0 53
2	99 5±0 19	93 4±0 63
3	98.2±0 47/12 9±0 2#	89 6±0 35
6	96 4±0 34/13 1±0 2#	--

*(n=3), # D[4,3]

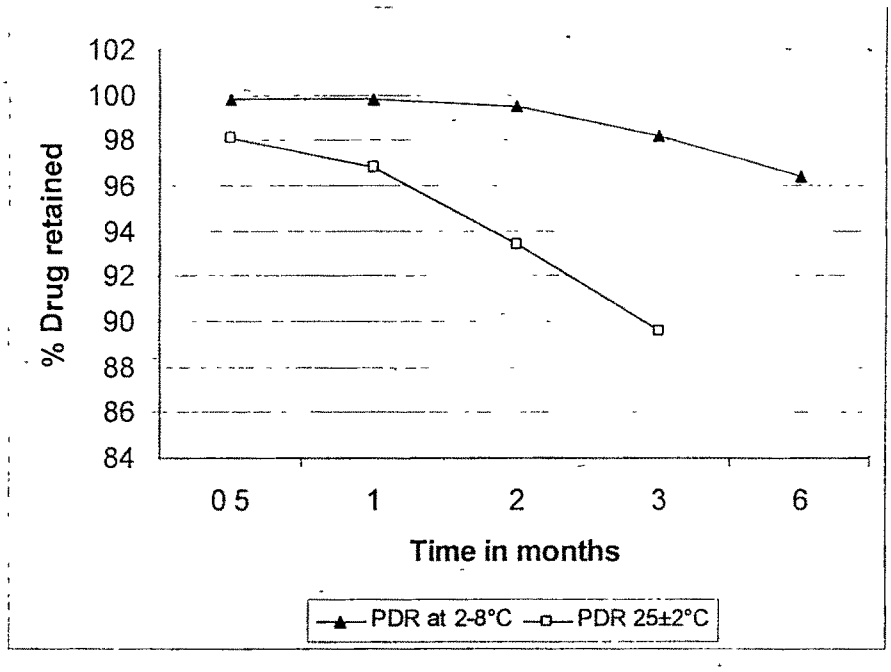

Figure 5.2: Drug Retention Studies of LLN formulation

Table 5.4: Percent Drug Retention (PDR) in liposomal formulations of LEU at different storage conditions

Time in month	PDR±SEM* at 2-8°C			PDR±SEM* at 25±2°C		
	LLEU	LLEUn	LLEU+CS	LLEU	LLEUn	LLEU+CS
0 5	99 8±0 35	99 9±0 26	99 9±0 48	98 7±0 24	98 2±0 32	99 1±0 41
1	99 2±0 31	99 5±0 21	99 7±0 28	97 3±0 53	97 6±0 45	98 2±0 55
2	98 5±0 34	99 0±0 30	99 1±0 31	95 9±0 63	96 7±0 52	97 4±0 75
3	97 6±0 47 12 11±0 03[=]	98 0±0 27/ 11 06±0 01[#]	98 7±0 36/ 11 01±0 02[#]	92 6±0 35	94 1±0 40	95 1±0 36/ 11 78±0 01[#]
6	96 7±0 34 12 10±0 02[=]	97 4±0 31/ 11 15±0 01[#]	98 2±0 33/ 11 10±0 02[#]	--		92 3±0 85

*(n=3), [#] D[4.3]

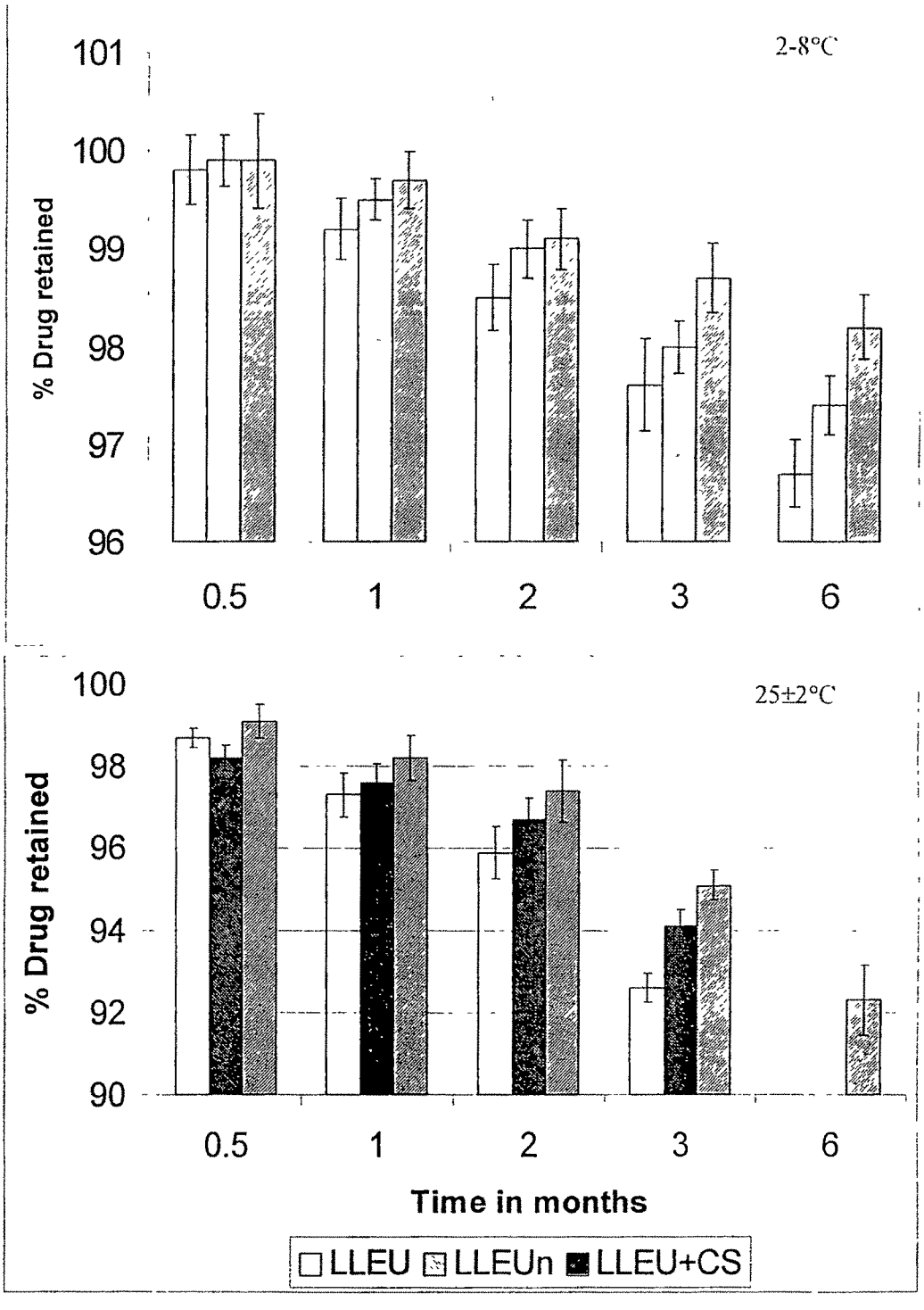

Figure 5.3: Drug Retention Studies of LLEU formulations

LEU FORMULATIONS

Comparative diffusion studies were carried out between various LEU formulations for a period up to 36 hrs using dialysis membrane in self designed and validated diffusion cell. As the drug was soluble in PBS, sink conditions were maintained when 50 ml of the medium used as the diffusion medium and hence zero-order flux conditions not violated The results obtained are recorded in Table 5 8 and shown graphically in Figure 5 6

Figure 5 6 clearly shows that encapsulating LEU in liposomes substantially slowed down its release Further delay in release was observed when these liposomes were incorporated in to CS solution Also, when individual formulations compared, significant differences ($p<0.05$) between plain drug and plain drug with CS were observed The repulsive forces between the positively charged CS and positively charged LEU may responsible for this effect

The non-linearity of the percent drug diffused vs time graph suggests that the release pattern does not follow zero order kinetics However, when correlation coefficients for different kinetic models were compared (Table 5 9), highest correlation (R^2 = 0 98-1 00) by Higuchi's equation obtained suggesting that the release obeys Higuchi's diffusion controlled model

Mean flux values of various LEU formulations were calculated and recorded in Table 5 10 and shown graphically in Figure 5 7 The diffusion co-efficient of different LEU formulations were also calculated and recorded in Table 5 10 and its graphical presentation is given in Figure 5 7 The mean flux values of the LEU formulation are found to be two to three times higher than those of liposomal formulations, indicating that liposomal formulations are potentially sustaining the drug release.

Table 5.5: In vitro diffusion studies of LN formulations

Time	LN	LLN	LN+CS	LN+CP
01	13 00 ±0 98		11 15 ± 0 69	9 5 ± 0 76
02	31 24 ± 1 01	14 3 ±0 78	33 69 ± 0 95	34 1 ± 1 05
04	51 1 ± 1 12	30.2 ± 0 97	47 26 ± 1 10	50 8 ± 1 36
06	70 79 ± 1.17	35 41 ± 1 36	65 03 ± 1 16	62 7 ± 1 59
08	83.06 ± 2 24	54 1 ± 1 21	78 11 ± 1 01	74 6± 1 42
10	92.21 ± 2.10	59 32 ± 1 65	92 95 ± 1 68	85 ± 1 99
12		65.1 ± 1.83		95 2 ± 2 00
24		91 4 ± 2 03		

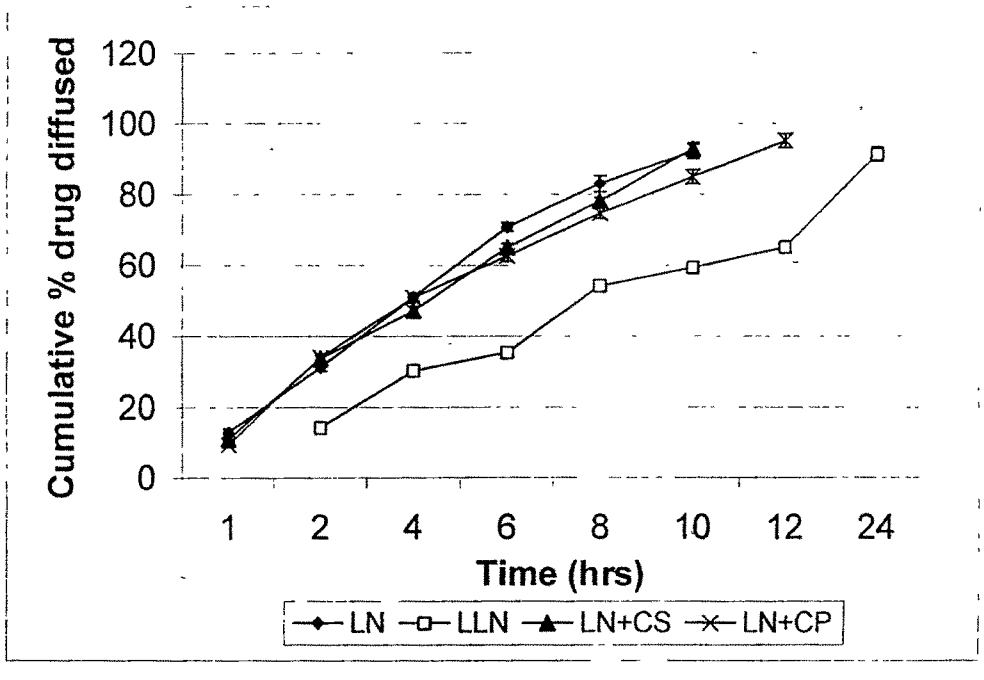

Figure 5.4: Cumulative % LN diffused during In vitro diffusion studies

Table 5.6: Regression Coefficients of LN Formulations by different models

Formulations	Zero-order equation	Higuchi's equation	First-order equation
	R^2	R^2	R^2
LN	0 9636	0 9963	0 8207
LLN	0 9083	0 9777	0 7125
LN+CS	0 972	0 9905	0 7957
LN+CP	0 9448	0 9868	0 7199

Table 5.7: Mean Flux and Diffusion Coefficient values of LN formulations

	LN	LLN	LN+CS	LN+CP
Mean Flux	2 64	1.31	2 58	2 31
Diffusion Coefficient	5 61E-09	2 44E-09	5 19E-09	5 17E-09

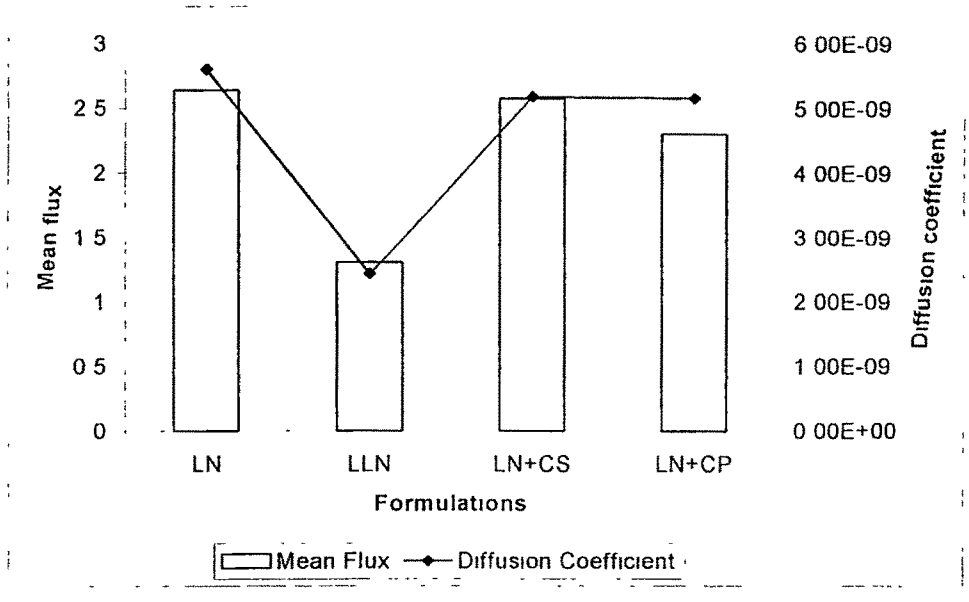

Figure 5.5: Mean Flux and Diffusion Coefficient values of LN formulations

Table 5.8: In vitro diffusion studies of LEU formulations

Time	LEU	LEU+CS	LLEU	LLEUn	LLEU+CS
01	17 6 ± 1.08	15 6 ±1 16	2 06±0 58		3±0 44
02	44 0± 1 15	35 48±1 09	12 5±0 69	8 11±0 64	13 6±0 59
04	64 72± 99	59.3±1 11	30 3±0 71	21 7±0 88	27 2±0 53
06	77 3±1 24	70.2±1 54	38 5±0 98	29 5±0 84	34 3±0 81
08	94 0±1 30	82.7±1 29	46 6±1 24	36 7±0 90	40 8±0 94
10		94.4±1 87	52 9±1 00	44 09±1.06	48±1 11
12			58 7±1 09	53 63±1 01	54 5±1 23
24			90 4±1 63	72 44±1 54	86 4±1 69
36				92 01±1 46	98±1 33

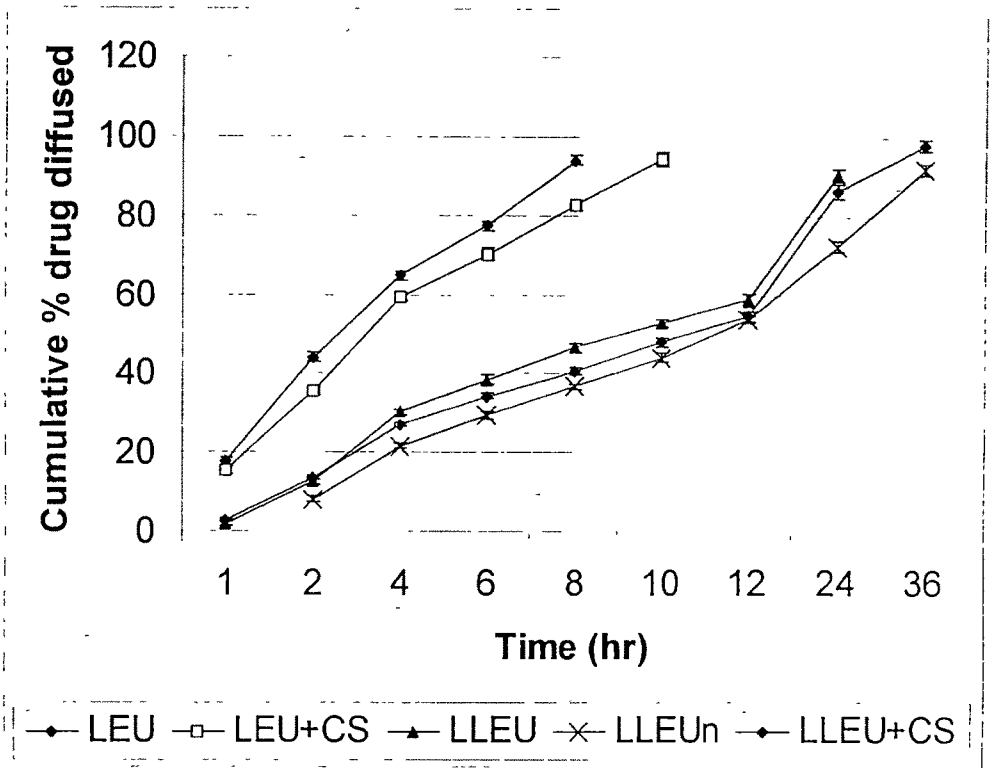

Figure 5.6: Cumulative % LEU diffused during in vitro diffusion studies

Table 5.9 : Regression Coefficients of LEU Formulations by different models

Formulations	Zero-order equation	Higuchi's equation	First-order equation
	R^2	R^2	R^2
LEU	0 9421	0 9818	0 7916
LEU+CS	0 949	0 9906	0 8007
LLEU	0 926	0 9952	0 5279
LLEUn	0 9342	0 991	0 6679
LLEU+CS	0 9222	0 9922	0 5445

Table 5.10: Mean Flux and Diffusion Coefficient values of LEU formulations

	LEU	LEU+CS	LLEU	LLEUn	LLEU+CS
Mean Flux	3 45	1 96	1 19	1 01	1 15
Diffusion Coefficient	7 72E-09	6 28E-09	1 89441--09	1 4739E-09	1 6924E-09

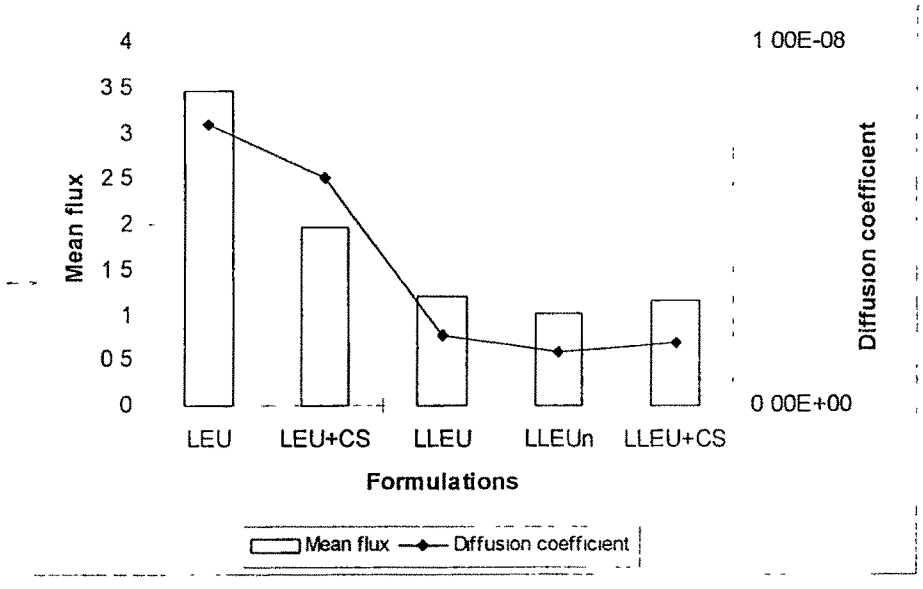

Figure 5.7: Mean Flux and Diffusion Coefficient values of LEU formulations

On comparing the individual formulations it was found that the flux values were depend on the charge of liposomes as shown in Figure 5 7 The reduction in mean flux and diffusion coefficient values of negatively charged liposomes were may be due to the attractive forces between negatively charged lipid (DCP) and positively charged LEU The increase in viscosity after addition of CS into LEU liposomes (neutral) and also repulsive forces between positive charge of CS molecule and positively charged LEU may reduce the diffusion of the drug from the liposomes This may be the reason for the significantly lower ($p<0.05$) mean flux and diffusion co-efficient values observed for CS containing formulations with respect to their plain counter parts

Assuming that the permeability of artificial membrane remains constant, the diffusion coefficient is ultimately governed by the concentration of free drug in donor compartment, which intern depends upon the rate of drug diffusion from liposomes. Thus there are two rate controlling barriers acting on the drug diffusion to the receptor compartment, one is the liposomal membrane and other is the artificial membrane The artificial membrane acts as a physical barrier preventing liposomes to enter into the sampling port and is not regulating the drug diffusion to receptor compartment

IN VIVO STUDIES

Rats were used as a model animal for screening of anti-fertility drugs (Ghosh, M,N. 1984) Size of the drug particles and liposomes in all the formulations was kept between 10-15 μm, as the particles with 10-20 μm are all deposited in the nasal cavity, whereas particles smaller than 1 μm pass with inspired air into the lungs (Jones et al. 1997) LLN, LN PM or LN suspension containing 10-μg LN were administered nasally in three different group of rats. Similarly, 10-μg of LN suspension was administered orally. Blood samples were collected at regular time points and plasma LN concentrations were estimated by spectrofluorimetric method.

The data of drug plasma concentration are shown in Figure 5.8. Various pharmacokinetic parameters, (Tmax, Cmax, and $t_{1/2}$) were calculated from the Figure and recorded in Table 5.11. AUC and bioavailability (F*) were also calculated and are recorded in Table 5.11.

TABLE 5.11: Pharmacokinetics of different formulations following Oral and Nasal Administration of LN in rats

Formulation	AUC (ng-hr/mL)	Bioavailability	T_{max} (hours)	C_{max} (ng/mL)	$T_{1/2}$ (hours)
LN (oral)	261.408±12.36	--	2.1±0.2	14.4±0.2	16.9±0.2
LN (nasal)	78.245±8.31	29.9321±1.87	4.2±0.2	7.13±0.2	7.0±0.2
LN PM (nasal)	84.007±9.00	32.1364±1.98	4.6±0.2	6.1±0.2	11.9±0.2
LLN (nasal)	67.901±8.51	25.9751±2.11	4.6±0.2	5.24±0.2	9.40±0.2
LN+CS (nasal)	265.862±13.12	101.7038±3.42	4.4±0.2	4.73±0.3	55.7±0.4
LN+CP (nasal)	259.888±14.20	99.4185±2.14	5.0±0.3	4.70±0.3	52.9±0.4

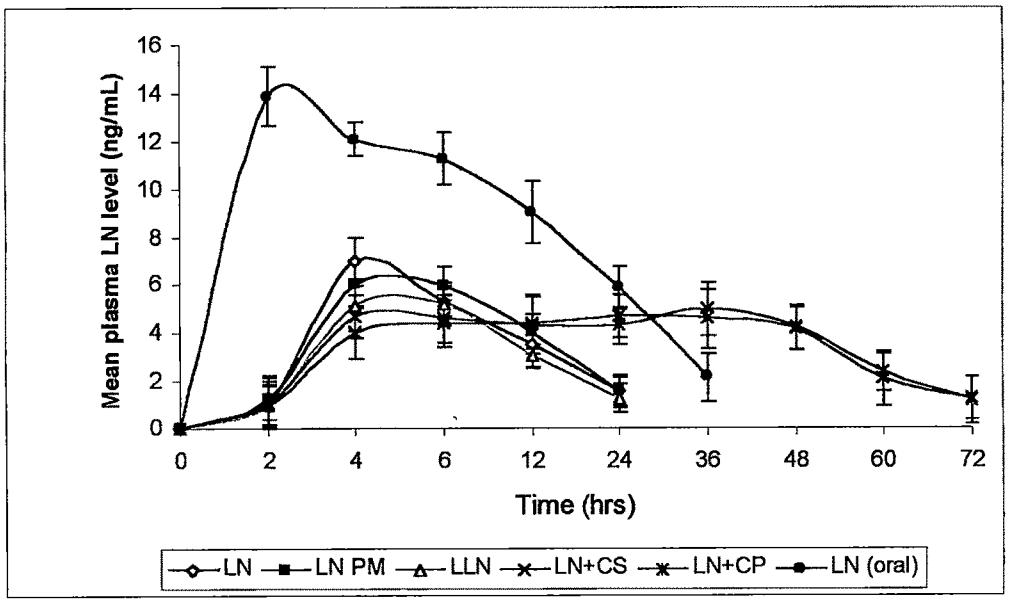

Figure 5.8: Plot of mean plasma level of Levonorgestrel Vs time following oral and nasal administration

TABLE 5.12: Antifertility effect of Levonorgestrel treated with different formulations

Formulation	No of rats mated	No. of rats pregnant	No. of Implantations
Control	4/4	4/4	1-10, 2-9, 3-9, 4-8
LN (oral)	4/4	0/4	-
LN (nasal)	4/4	1/4	1-4
LN Physical mixture (nasal)	4/4	1/4	1-3
LLN (nasal)	4/4	2/4	1-1, 2-3
LN+CS (nasal)	4/4	0/4	-
LN+CP (nasal)	4/4	0/4	-

Plasma levels of LN after a single oral dose showed considerably higher level of LN (C_{max} 14 4 ng/mL) with T_{max} of 2 1 hr as compared to LN suspension, LN PM and LLN formulations given intranasally (C_{max} 7 13 ng/mL, 6 1 ng/mL, 5 24 ng/mL) at T_{max} of 4 2 hr, 4 6 hr and 4 6 hr respectively Levels of LN fall precipitously to levels below 1 ng/mL in all the cases Also, when AUCs of nasally administered LN suspension, LN PM and LLN formulations were compared to that of orally administered LN suspension, significant difference ($p<0$ 05) was observed F* of these formulations were found to be significantly less i e , 29 93%, 32 14%, and 25 97% The large number of fenestrated capillaries just below the surface epithelium may well contribute to absorption (Fisher, 1990) However, the mucociliary clearance under normal conditions rapidly clears the applied material and hence there is a little time of contact between the drug and the nasal mucosa This is what we observed in case of LN suspension, LN PM and LLN formulations delivered intranasally and hence resulted in to significantly low ($p<0$ 05) F* of the drug Therefore, it was thought worthwhile to incorporate mucoadhesive agents which can prolong the contact time of the drug with the absorptive surfaces (nasal mucosa). When the drug was formulated with mucoadhesive agents, CS (LN+CS) and CP (LN+CP), significant improvement in F* of the drug were observed (101.70% and 99 42% respectively) Plasma half lives ($t_{1/2}$) were also significantly increased from 7 0 hr to 55 7 hr and 52 9 hr The T_{max} values were 4 4 hr and 5 0 hr with C_{max} of 4 73 ng/mL and 4 70 ng/mL respectively for LN+CS and LN+CP formulations. The clearance of administered drug was delayed by using mucoadhesive polymers such as CS and CP and hence resulted into significantly improved F* and $t_{1/2}$ CS acts by opening tight junction between epithelial cells (20) It may also enhance the absorption of drugs by being a useful bioadhesive and slowing mucociliary transport (Aspden et al , 1995) Carbopol hydrogel is a thin liquid at acidic pH but it gels at physiological pH and thus has great potential for nasal delivery of drugs (Morimoto et al , 1985)

When the $t_{1/2}$ value of orally administered formulation was compared to nasally administered mucoadhesive formulations, significant increases in $t_{1/2}$ were observed (16.9 hr to 52.9 hr-55 7 hr). The results clearly indicate that the dosing interval can be changed to once in two days from daily oral administration with out changing the dose. The reduction in the drug dose and maintenance of therapeutic concentration in

plasma of the drug for at least up to the 48 h is expected to reduce the reported side effects in humans and probably the cost of the therapy due to lower dose

Results are dependent upon the deposition of the instilled dose within the nose The deposition and absorption of sprays in the nose are different from those of drops The nasal spray deposits anteriorly in the nasal atrium while the drops are dispersed throughout the length of the nasal cavity Nasal sprays deposit more anteriorly, resulting in slower clearance of sprays than of drops (Hardy et al , 1985) However, due to impracticality of giving nasal sprays in rats, experiments were only performed with the drops There are chances that studies in larger animals with spray form of these formulations may results in different deposition and absorption pattern which ultimately helps in deciding type of dosage form chosen for human use

Pharmacokinetic studies were followed by pharmacodynamic studies, where the animals were administered with different formulations intranasally for four weeks and allowed for mating during the treatment period Numbers of implantations in mated female rats are recorded in Table 5 12 Animals when treated nasally with LN suspension, LN+PM and LLN were failed to show contraceptive efficacy, may be due to short plasma half lives of the drug However, in case of LN+CS and LN+CP cent percent anti-fertility was observed even formulations were administered on alternate days. These results are in agreement with pharmacokinetics, which further confirms the contraceptive efficacy of proposed formulations for prolonged period of time.

Levonorgestrel. an orally active progestronic derivative, is associated with various side effects may be due to initial very high plasma concentration (Cmax) achieved which is significantly higher than the therapeutic window of the drug (Active therapeutic window- 4-6 ng/mL) Nasal delivery with mucoadhesive agents, gives an extended release of the drug over prolong period of time without resulting into initial higher plasma concentrations. This study demonstrates prolonged LN absorption following closely zero-order kinetics in rats after nasal administration. Maintenance of effective drug concentration in blood for prolonged period of time is expected to reduce dose and/or frequency of drug administration and probably the side effects provided similar findings are demonstrated in humans.

LEU Formulations

To investigate the contraceptive activity, LEU solution, LEU PM, LLEU and LLEUn containing 5 µg LEU were administered intranasally Similarly, 5 µg LEU solution was administered through s c route Blood samples were collected at specific time intervals and serum LH concentrations were estimated by specific radioimmunoassay The results of mean serum LH concentrations vs time (hr) were plotted in Figure 5 9 Various parameters for LH release in serum (Cmax, Tmax, $T_{1/2}$, and F*) were calculated from the Figure 5 9 and recorded in Table 5 13

In a group of animals, control formulation was administered and serum LH level was monitored LH levels were found to be very low (around 2 mIU/ml) In LEU treated animals, regardless of the route of administration and formulations of LEU, serum LH concentrations transiently rose to peak at 1 hr-2 1 hr then decreased gradually to the pretreatment level within 24 h The highest Cmax value of 263 ± 1 8 mIU/mL was obtained after s c administration Lower Cmax values of 27 ± 1 0 mIU/ml, 27 ± 1 1 mIU/ml, 59 ± 1 0 mIU/ml and 47 ± 1 2 mIU/ml for LEU solution, LEU PM, LLEU and LLEUn formulations were obtained after intranasal administration Due to the mucociliary clearance of nasal cavity, nasally delivered formulations clear rapidly from site of absorption resulting into little contact time between the drug and the nasal mucosa and hence, poor drug absorption When relative bioactivities of nasally administered formulations were compared, LLEU and LLEUn showed higher relative percent bioactivity (F* 27 83% and 21 3% respectively) compared to LEU solution and LEU PM (F* of 10 89% and 10 96% respectively) The relatively higher bioactivity of liposomal formulations compared to plain formulations may be due to their action on nasal mucosa by incorporating phospholipids in the membrane and opening "new pore" in the paracellular tight junction (Illum, 1997). Liposomal formulations were also showed significantly higher ($p<0.05$) $t_{1/2}$ (4 2 hr to 4.5 hr) compared to plain LEU formulations (2.3 hr to 2.6 hr). This may be due to the surface viscosity of liposomes

The prevalence of the repellent forces between negatively charged liposomes and negatively charged nasal mucosa may be responsible for low bioactivity of negatively

charged liposomes compared to plain liposomes This may also responsible for less $t_{1/2}$ of negatively charged liposomes

To enhance the residence time of the formulation and to impart mucoadhesion, CS was incorperated in selected formulations LLEU was selected as it showed significantly higher ($p < 0.05$) F* compared to other formulations LEU solution was also selected to see the effect of CS on bioactivity of plain drug For both LEU solution and LLEU formulations with 0 5% CS, marked increase ($p< 0.05$) in F* were observed (i e , 10 89 to 49 13% for LLU+CS and 27 83 to 88 90% for LLEU+CS) Significantly higher $t_{1/2}$ of 8 8-9 0 h was also observed in both the cases Prolonging the contact time of the drug with the absorptive surfaces by means of CS was contributed to increase in the F* of intranasally administered formulations CS also acts by opening tight junction between epithelial cells (Arthurson et al , 1994) The F* determines ultimate fate of the formulation in the body while, lower Cmax followed by plateau for prolonged period of time for LLEU+CS formulation may decrease the chances of concentration related side effects of the drug Intranasal administration of LLEU+CS showing comparable bioactivity to that of LEU solution administered subcutaneously (s c) was used for further studies

In male rats, sperm count and fertility performance studies were carried out for LEU solution administered s c and LLEU+CS formulation administered intranasally Spermatozoa were collected from left cauda epididymis and were counted under a microscope and recorded in Table 5.14 It was found that complete azoospermia was achieved in case of LLEU+CS formulation administered nasally and LEU solution subcutaneously after 26 days treatment Duration of treatment was kept to 26 days to cover two seminiferous cycles (13 2 x 2 days) in rats. Females were mated with treated males and no implantation sites were observed in case of nasal administration of LLEU+CS formulation and s c. administration of LEU solution due to the azoospermic potential of both the formulations

Table 5.13: Pharmacodynamic parameters of LEU formulations following s.c. and nasal administration

Formulation	AUC (ng-h/mL)	F* (%)	Tmax (hours)	Cmax (ng/mL)	T1/2 (hours)
LEU (s c)	720 5±78 21	--	1 2 ± 0.2	263 ± 1 8	3 1± 0 2
LEU	78 5±11 23	10 89± 1 3	1 0± 0 2	27 ± 1 0	2 3± 0 2
LEU PM	79 0±13 24	10 96±1 1	1 0±0 2	27 ± 1 1	2 6± 0 2
LLEU	200 5±18 75	27 83±1 8	2 0±0 2	59 ± 1 0	4 5± 0 2
LLEUn	153.5±16 03	21 30±1.6	2 0±0 2	47 ± 1 2	4 2± 0 2
LEU+CS	354±41 21	39 13±2 5	1 2±02	45 ± 1 4	8 8± 0 2
LLEU+CS	640 5±35 96	78 90±2 1	2 1±0 2	80 ± 1.2	9 0± 0 2

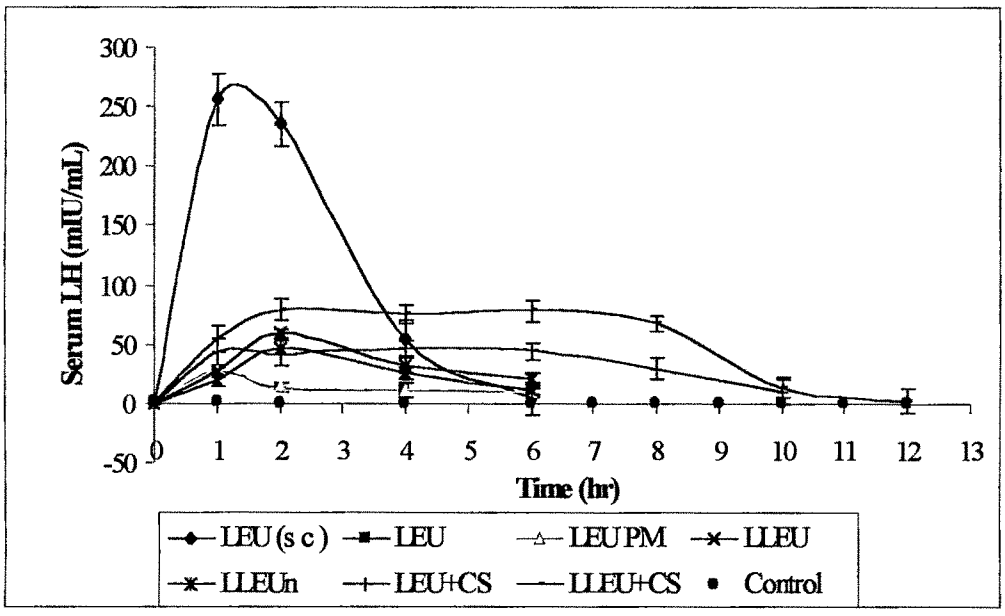

Figure 5.9: Plot of mean serum level of LH Vs time following s.c. and nasal administration of Leuprolide acetate

Table 5.14: Sperm count after 26 days treatment with selected formulations

Group	Sperm count (10^6/cauda epididymis)
Control	135.75±8.39
LEU1	--
LEU4C	--

In case of female rats, cyclicity was observed to evaluate fertility performance and ceasation of estrous cycles was observed from the first treatment cycle in female rats treated with LEU solution and LLEU+CS through s c and nasal routes. Animals were return to normal cyclicity after the cessation of the treatment

Preliminary experiments conducted on rats have demonstrated the use of nasal administration of LLEU+CS developed in this investigation in producing contraception by treating male and female rats The results of the developed formulation were found to comparable to available parenteral dosage of LEU in producing contraception on rats However, its role in clinical practice can only be settled after extensive experiments on rats and one more species of animal followed by clinical trials

Comparisions:

When mucoadhesion of LLEU was compared to that of LN, higher mucoadhesion was observed This may be due to the ionic interactions between LEU induced positive charges in liposomes and negatively charged nasal mucosa

When the results of drug retention study for LN and LEU formulations were compared, LEU formulations showed higher drug retention at all the sampling time points This may be due to the Tg of the phospholipids used for the preparation of these liposomes The higher Tg of HSPC used in the preparation of LEU liposomes compared lower Tg of EggPC resulted into higher stability of LEU liposomes

In vitro drug diffusion studies were also carried out for LN and LEU formulations. Different diffusion medium were used as LN and LEU being lipophilic and hydrophilic in nature. Hydro-alcoholic medium was used for LN and PBS was used for LEU to maintain the flux and sink conditions.

In vivo studies were carried out in rats for LN and LEU formulations Developed formulations were given intranasally in comparison to their presently available route of administration (oral for LN and s c. for LEU). Developed liposomal LN

formulation showed low but equal bioavailability to that of plain LN formulation Similar lipophilicity of the drug and the liposomally encapsulated drug may be responsible for similar pharmacokinetic/pharmacodynamic behavior of the formulations of plain and liposomally encapsulated LN formulations However, liposomal LEU formulation showed significantly higher bioactivity compared to that of plain LEU formulation This might be due the fact that LEU being a hydrophilic in nature having low partition coefficient and mucosal permeability Incorporation into carrier such as liposomes renders the drug as lipophilic and opening of new pore into nasal mucosa (Colombo, 1997) might responsible for the improvement in LEU bioactivity As mucociliary clearance under normal conditions rapidly clears the applied material, there is a little time of contact between the drug and the mucosa may be the reason for low F* of formulations of both the drugs Hence, it was thought worthwhile to incorporate mucoadhesive agents into the formulations LN+CS and LEU+CS when compared, LN+CS showed almost 100% bioavailability while LEU+CS only resulted into 50% bioactivity This is may be due to the effect of drug as the LN being lipophilic in nature it has higher partition coefficient compared to that of LEU (hydrophilic)

In conclusion, Stable liposomal formulations of both LN and LEU for the nasal administration were developed In vivo studies including pharmacokinetics/pharmacodynamics in rats were carried out followed by in vitro diffusion studies to create in vitro testing procedures Maintenance of lower but effective LN plasma concentrations for extended period of time after administration of LN formulations given intranasally developed in this investigation are expected to reduce frequency of dosing of the oral route and likely to reduce systemic side effects associated with oral administration of the drug. The bioactivity/fertility performance studies in both male and the female rats after intranasal administration of the liposomal formulation of LEU with CS was found to comparable to available parenteral dosage of LEU in producing contraception on rats, leads to patient compliance, self medication and avoiding the complications related to injection procedure

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