The drug loaded carrier systems of nonsteroidal anti-inflammatory medication mefenamic acid and indomethacin

Bhavesh Sen[1], Firoz Khan[2], Kailash Kumar Pathak[3], Nida Khan[4], Deepak Verma[5]

College of Pharmacy, SSSUTMS sehore

Abstract:

The drug loaded carrier systems of nonsteroidal anti-inflammatory drug mefenamic acid and indomethacin were prepared and further incorporated in transdermal gel formulation. The formulation of liposome and transferosomes were optimized based on drug entrapment efficiency and in-vitro drug permeation.

The optimized formula of liposome contained phospholipid 109.9 mg (0.14 mM) and cholesterol 27.68 mg (0.071 mM) along with 100 mg (0.28 mM) mefenamic acid, whereas the optimized formula of transferosomes contained phospholipid 91.41 mg (0.12 mM), surfactant 24 mg (0.06 mM) and cholesterol 34.60 mg (0.09 mM) in formulation with 100 mg (0.28 mM) mefenamic acid.

The % drug entrapment of optimized formulation of liposomes was 41.02 % and that of transferosomes was found to be 47.44 % which were very close to the target responses fixed in response surface methodology. Also, permeation flux of optimized liposome and transferosome transdermal gel formulations were 26.88 and 28.69 µgcm-2 hr-1 respectively

Keywords: Mefenamic Acid, Indomethacin, Transdermal Gel.

Introduction

Rheumatoid arthritis (RA) is a prolonged and the inflammation occurs throughout the body and the cause of the disease is not known. The disease was associated with severe effect on psychosocial and economical. The symptoms of rheumatoid arthritis was synovitis and whole body inflammation., at this stage if it was not treated it leads to severe life threatening effects like cartilage damage and disability also the quality of life was reduced 1.

The primary symptoms are unclear because the environmental factors, hormones, genetics plays a major role. If the immune response was triggered, the immune system produces auto antibodies and cytokines which cause inflammation thus pannus was formed. This pannus destroys the bone and cartilage. As the bones and joints got damaged it initiates the release of inflammatory mediators which leads to complex process and causes the systemic complications 2 .

RA risk was more twice with person who has first degree RA. Also there is a relation with harmones. It was more in females compared to men and the rate of onset of disease increases in pregnancy. Environment steer like exposure to chemical and smoking increases the chances of autoimmunity which leads to inflammation. Now-a-days improvement in diagnosis and treatment leads to use of the biological agents, which causes target release of specific drugs. The treatment with a combination of conventional disease modifying anti-rheumatic drugs with biological agent reduces drastically the symptoms, changes in erosions and finally reduction of disease 3.

Material and Methods

Preformulation studies:

Melting Point:

The melting points of the drugs were determined by an open capillary method using the melting point apparatus.

Solubility Study of Drug[4]

Solubility studies of the drug were disbursed in numerous varieties of solvents which are used for further study.

Fourier Transform Infrared Spectroscopy of Drug:

The infrared spectra of the pure drug were recorded by PerkinElmer FT -IR spectrometer. Samples were prepared by KBr disc method (2 mg sample in 100 mg KBr) and examined within the transmission mode. Each spectrum was at frequency range of 4000 - 400 cm^{-1}.

Ultraviolet Spectroscopy:[5]

1. Determination of Maximum Wavelength < A max):

7. Differential Scanning Calorimetry (DSC) Study of Drug [6:]

DSC analysis was performed using Hitachi 7020 thermal analysis system on 2-4 mg samples. The sample was heated in an open nitrogen pan at a rate of 10 °C/min conducted over a temperature range of 30 to 240 °C for Mefanamic acid under a nitrogen flow of two bar pressure. Thermogram was represented.

8. Partition Coefficient (Kp) [7,8] : The partition coefficient of the drug decided by shaking equal volumes of oil and aqueous solution and therefore it was introduced into a separating funnel. A drug solution of 1 mg/ml was prepared in water and 40 ml of this solution was taken during a separating funnel and shaken with an equal volume of octanol for 10 min and allowed to face for seventy-four hours with intermittent shaking. Using a UV spectrophotometer get the partition coefficient values.

Rotary vacuum evaporation process variables: - The process variables, temperature, RPM and time of operation were varied based on 3 factors, 3 level general factorial designs and based on the quality of film produced, the process was optimized.

Table: 1 Process variables in rotary vacuum evaporation

Parameter	Temperature	RPM	Variables
Level		High (90)	High (20)
		Medium (80)	Medium (14)
		Low (60)	Low (10)

Probe sonication process variables:

Sonication was performed to downsize the vesicles as per the methods mentioned in literature.[7]

Table 2. Process optimization of probe sonication

Probe	Amplitude	Time and Pulse	Temperature
13 mm standard	60 %	2 minutes 2 sec on,2 sec off	4^{o} C

Process of synthesis of liposome as drug carriers by film hydration method:-

1. The excipients Phospholipids (0.067 mMol to 0.133 mMol) & cholesterol(0.064 to
2. 0.129 mMol) were solubilized in 10 ml chloroform-methanol (9:1) mixture. Drug: Lipid molar ratio (2.12 to 4.20 mMol).The quantities were converted into mg for formulation. Hydration volume was kept as 20 ml.
3. Thin film formation was done by vacuum rotary evaporator at temperature of 40 ^{o}C, 90 rpm for 20 minutes. Thin film was evaporated under vacuum for removal of even trace amount of organic solvent and then kept in desiccators overnight for removal of even trace amount of organic solvent.
4. Dried thin film was hydrated by 20 ml phosphate buffer saline pH 7.4 containing 100 mg of drug to prepare drug loaded multilamellar vesicles.
5. The vesicular dispersion was sonicated by probe sonicator (Vibra cell ,Sonics) for 4 cycles of 2 minutes

at pulse of 2 s using standard 13 mm probe at amplitude of 60% to obtain small unilamellar vesicles form large multilamellar vesicles. The vesicles were observed under Carl Zeiss trinocular microscope at 40 X and 100 X magnification.

Preparation of trial batches of liposomes based on factorial design:

The quantity of phospholipid was varied as 0.067 mMol to 0.133 mMol (40 -100 mg) &cholesterol as 0.064 to 0.129 mMol (24-40 mg) for preparation of factorial batches of liposomes. The quantities were converted into mg. The batches were named as L1 to L4.The drug to lipid molar ratio was taken as 2.12 to 4.20 mMol and the hydration volume was kept as 20 ml. The quantities of drug and excipients were converted into milligrams for preparation of batches.

Table 3. Factorial design for factors screening in liposome preparation

Formulation	Quantity of phospholipid (mg)	Quantity of cholesterol (mg)
L1	100	40
L2	100	24
L3	40	40
L4	40	24

Analysis of factorial batches of liposomes of mefenamic acid:

The factorial batches were analyzed by pareto chart, main effect plot, interaction plot and cube plot using statistical software Minitab 16 to determine the effect and influence of factors on responses of % drug entrapment and size of liposomes.

Process of Synthesis of trial batch of transferosomes as Drug carriers by thin film hydration Method:

The transferosomes were prepared as per the methods described in literatures with modifications based on requirements of formulation.[8,9,10]

Selection of surfactants for preparation of transferosomes:-

The surfactant was selected based on preliminary evaluation of trial batches of transferosomes. The phospholipid1, 2-disteroyl–sn-glycero-3-Phospho-ethanolamine, Na salt selected in the earlier studies on liposomes was used for preparation of transferosomes also.

Preparation of trial batches for selection of surfactants:-

The quantity of surfactants was varied as 40 mg and 100 mg and cholesterol were varied as 24 mg and 40 mg for preparation of factorial batches of transferosomes. Transferosomes were prepared using the surfactants span 40, span 60, span 20 and sodium cholate which are reported earlier for transferosome preparation.[11,12,13,14,15]

The quantity of phospholipid was kept 100 mg for all the trial batches and the batches were named as T1 to T16. Selection was performed based on evaluation data of % drug entrapment and size of transferosomes formed.

Table 4. Formulation of transferosomes using different surfactants

Formulation	Drug(mg)	Phospholipid (mg)	Span40(mg)	Cholesterol(mg)
T1	100	100	40	24
T2	100	100	100	40
T3	100	100	100	24
T4	100	100	40	40

Formulation	Drug(mg)	Phospholipid (mg)	Span60(mg)	Cholesterol(mg)
T4	100	100	100	40
T6	100	100	100	24
T7	100	100	40	24
T8	100	100	40	40

Formulation	Drug(mg)	Phospholipid (mg)	Span20(mg)	Cholesterol(mg)
T9	100	100	40	40
T10	100	100	100	24
T11	100	100	40	24
T12	100	100	100	40

Formulation	Drug(mg)	Phospholipid (mg)	Na.Cholate(mg)	Cholesterol(mg)
T13	100	100	100	24
T14	100	100	40	40
T14	100	100	40	24
T16	100	100	100	40

Drug release study of transferosome prepared using span 40 and span 60:-

As the drug entrapment and size were found to be nearly same for the transferosomes prepared using span 40 and span 60, the transferosomes were incorporated into gel and further evaluated for % drug release for 6 hrs.

Preparation and evaluation of trial batches of transferosomes based on factorial design:-

For preparation of factorial design bathes of transferosomes, the quantity of phospholipid was varied as 40 mg and 100 mg (0.067-0.133 mMol), the quantity of selected surfactant span 60 was varied as 24 mg and 40 mg (0.06-0.12 mMol) and quantity of cholesterol was varied as 24 mg and 40 mg(0.064-0.129 mMol). Batches were named as T1 to T8 and evaluated for size and entrapment efficiency.

Table 3. Factorial design for factors screening in transferosome preparation

Formulation	Quantity of phospholipid (mg)	Quantity of surfactant (mg)	Quantity of cholesterol (mg)
T1	40	40	24
T2	40	24	24
T3	100	24	24
T4	100	40	24
T4	40	40	40
T6	100	24	40
T7	40	24	40
T8	100	40	40

Evaluation of liposomal gel and transferosomal gel based on gel characteristics:-
The drug loaded liposomes and transferosomes incorporated gels along with plain drug gel were evaluated for pH, spreadability, gel strength , extrudability and drug release and permeation studies based on the methods in literatures.[10]

Determination of pH:
The pH of gels was checked by using a digital pH meter at room temperature. Initially, the pH meter was calibrated using standard buffers of pH 7 and then 10 gm of gel was weighed and dispersed in 24 ml of distilled water and then electrode of pH meter was dipped in the dispersion and the pH was noted.

Spreadability:
A quantity of 0.4 g gel was placed within a circle of 1 cm diameter on a premarked glass slide over which a second glass slide was placed. A weight of 2 g was allowed to rest on the upper glass slide for 1min. The increase in the diameter due to spreading of the gel was noted. Spreadability was calculated by using the following formula:
$S = M.L / T$

Where, S = Spreadability, M = weight attached to upper slide, L = length of spread, T = time taken.

Gel strength:
The apparatus for measuring gel strength consisted of a plunger having a pan to hold weights at one end whereas the other end was immersed into the gel. Formulated gels were placed in glass bottle where marking was done at 1cm below the filling mark. The weight required for the plunger to sink to a depth of 1 cm through the prepared gel was measured for each formulation.

Extrudability:-
The prepared gel was filled in tube and sealed. 3 Markings were made at intervals of 1.4 cm from bottom of tube. The tube was pressed at marking using Pfizer hardness tester with a pressure of 1 kg/cm^2, the weight of gel expelled in form of continuous ribbon was measured for each formulation and uniformity of release of gel from the tube was determined.

Results
Melting Point: According to the Indian Pharmacopoeia , the temperature /melting range of a substance is defined as the temperature points in which /the point at which the substance begins to coalesce and is completely melted, as defined differently for certain substances. 6 The melting point of the drug is consistent with reported literature values. The melting point of drug was observed to be in the range of 230-231 °C with decomposition , that is, substance is characterized when it begins to melt, as shown in the Table 5.

Table 5: comparison of the result of the melting point of drug sample with the reported standards

Sr. no.	Identification Test	Observed Result	Reported standard
1.	Melting Point	230 °C	230-231 °C

Solubility Study of Drug: A solubility test becomes a purity test only when a special quantitative test is given in the individual monograph and is an official requirement. Mefenamic acid has poor solubility in water and on other hand it has high solubility in water- miscible organic solvent26 The solubility study of the drug sample was studied in different kinds of solvents, and the data shows that the drug was slightly soluble in water, soluble in phosphate buffer (pH 7.4) and freely soluble in the rest of another solvent which is shown in Table 6.

TABLE 6: SOLUBILITY OF DRUG IN A DIFFERENT SOLVENT

Sr. no.	Solvent	Solubility (mg/ml)
1	Isopropyl Myristate	67.04
2	Oleic acid	0.284
3	PG	0.417
4	PEG 400	4.341
4	Tweens 80	0.284
6	Methanol	1.441
7	MethanolicHCl	0.448
8	Phosphate Buffer pH 7.4	2.32
9	Water	10

Fourier Transform Infrared Spectroscopy of Drug: As we know, the infrared spectroscopy is mostly used for the identification of organic compound whose spectra are complex and as it provides numerous maxima and minima that are useful for comparison purpose. Infrared spectroscopy finds application in qualitative and quantitative analysis as no hvo compounds give similar absorption spectra in the IR region. The spectrum was recorded by scanning in the wavelength region of 4000- 400cm-1 using FTIR spectrophotometer.

The FTIR spectra of Mefanamic acid were taken which is shown in Fig. 2. The IR peak should be compared with Literature (IP 2014 vol. I) Fig. 3. The principal peak for IR of drug sample matched with the standard spectrum for Mefanamic acid which is shown in Table 4.

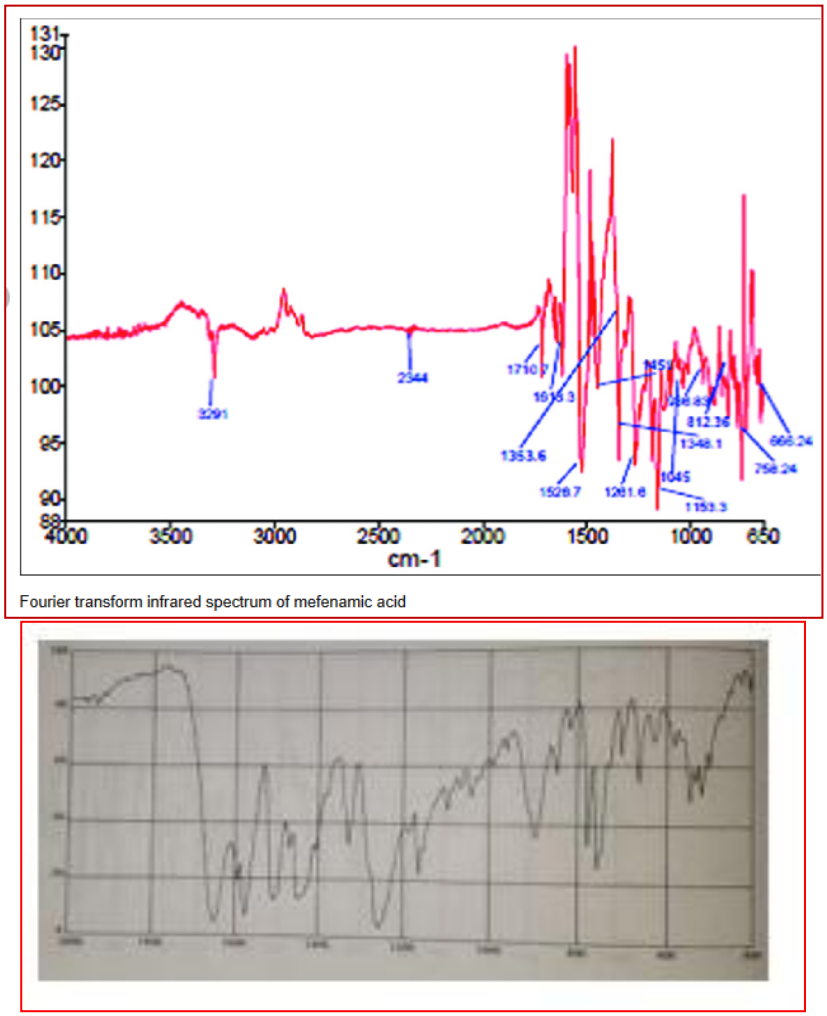

Figure 1: IR SPECTRA IN IP 2014

Ultraviolet Spectroscopy:

Determination of Maximum Wavelength (λ max): Maximum wavelength is different and specific for each drug substances, and it is also helps in identification criteria. The maximum absorbance for drug is taken in Methanol, MethanolicHCl and Phosphate Buffer (pH- 7.4).Observed peak and reported standard peak are shown in Table 4.

Table 6: maximum wavelength ((λ max) of the drug in methanol, methanolic hcl and phosphate buffer

Solvent	ʎmax (nm) Observed Peak
Methanol	284
MethanolicHCl	280
Phosphate buffer (pH-7.4)	286

ʎmax for the drug in methanol, methanolic HCl , and phosphate buffer (pH-7.4) was found, and it is shown in Fig. 2.

Spectra for the drug in methanol is observed in the range of 200 nm to 400 nm which it shows absorption maxima at about 284nm and minimum at about 247 nm which is shown in Fig. 4.

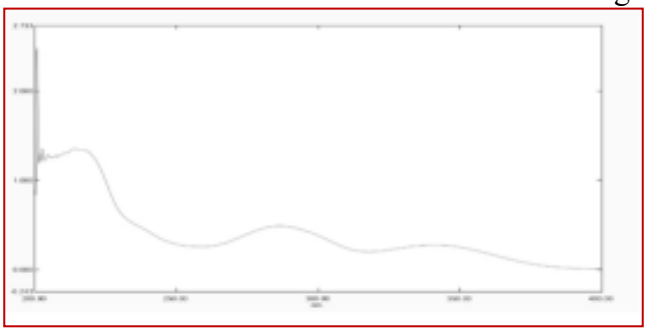

Figure 2: ʎmax FOR THE DRUG IN METHANOL

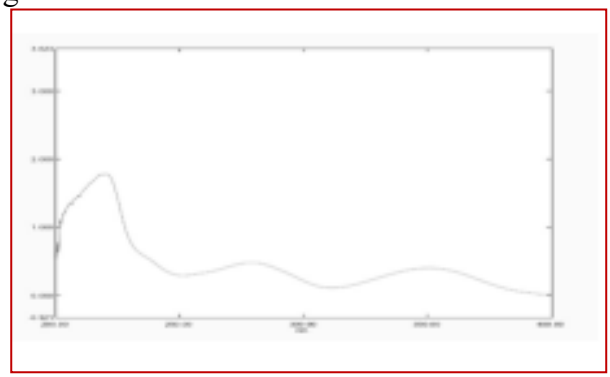

Figure 3: ʎmax FOR THE DRUG IN METHANOLIC HCL

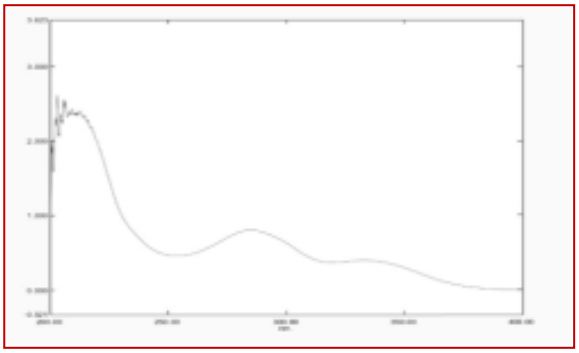

Figure 5: ʎ max for the drug in phosphate buffer

Differential Scanning Calorimetry (D.S.C.) Study of Drug: The endotherm of melting corresponds to the portion of the DSC curve that's far from the baseline and later returns to it. Melting is a process that change a substance from solid to liquid. This happens once the internal energy of the solid increase, generally by the

application of heat that will increase the substance's temperature to the melting point. In DSC, as the temperature increases, the sample reaches its melting temperature (Tm). The melting process leads to an endothermic peak in the DSC curve. DSC studies were performed for drug sample. The DSC thermogram drug sample is presented in Fig. 10 and interpretation is shown in Table 9. Thermogram of DSC of the drug shows melting in the range between 230 - 234 ºC, and also the sharp peak was seen at 234.9 ºC. So, it shows endothermic reaction. The following figure shows the endothermic peak of the drug with height -34.63 mW

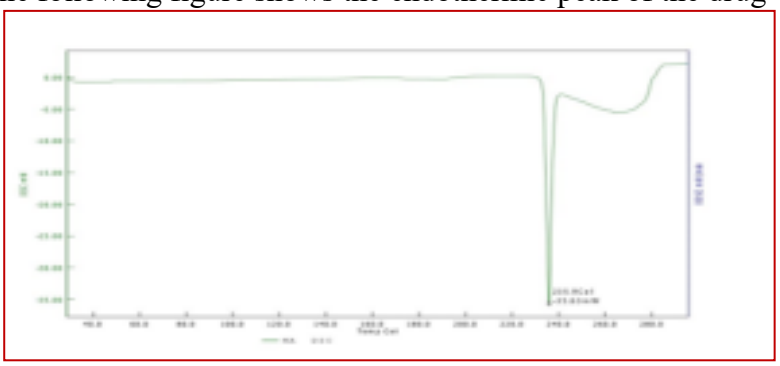

Figure 5: DSC THERMOGRAM OF DRUG

TABLE 8: INTERPRETATION OF DSC OF DRUG

Name of substance	Melting Point	Inference
Mefenamic Acid	234.9 C	Sharp endothermic peak obtained and it matches with official standard

Rotary vacuum evaporation –

The process variables, temperature, RPM and time of operation were varied based on 3 factors, 3 level general factorial design and based on the quality of film produced, the process was optimized.

Table 9. Process variables of rotary vacuum evaporation

Parameter	**Temperature**	**RPM**	**Variables**
Level	High (70^{o} C)	High (90)	High (20)
	Medium (60^{o} C)	Medium (80)	Medium (14)
	Low (40^{o} C)	Low (60)	Low (10)

Table10. Effect of process parameters

Process Parameters		**Observation**	
Temperature ^{o} C	**RPM**	**Time (min)**	**Quality of film**
60	**90**	**20**	**Thin film formed- uniform and translucent in appearance**
60	80	10	Dry thin film not formed
40	80	20	Thin film formed- uniform in appearance
60	90	14	Thin film formed- not uniform in appearance
70	80	14	Thin film formed- not uniform in appearance

40	**90**	**20**	**Thin film formed- uniform and translucent in appearance**
70	80	20	Thin film formed- uniform in appearance
60	80	20	Thin film formed- uniform in appearance
40	90	14	Thin film formed- not uniform in appearance
70	60	10	Dry thin film not formed
40	90	10	Dry thin film not formed
70	60	20	Thin film formed- uniform in appearance
70	90	20	Thin film formed- uniform in appearance
70	80	10	Dry thin film not formed
60	60	20	Thin film formed- uniform in appearance

Then, hydration of thin film was performed with solvent Phosphate buffer saline pH 7.4 at 40° C temperature and 90 rpm for 20 minutes. Similar studies of screening of process parameters were performed for transferosomes also using the same combinations of process conditions and it was observed that thin film formation was possible at 40° C temperature and 90 rpm, when film formation was allowed for 20 minutes in rotary vacuum evaporator. The hydration of thin film of transferosomes was done using the similar method as applied for liposomes.

Then prepared liposomes and transferosomes were reduced in size by probe sonication.

Preliminary screening of surfactants for preparation of transferosomes:- The surfactant was selected based on preliminary evaluation of trial batches of transferosomes. The phospholipid, 1, 2-disteroyl-sn-glycero-3-phospho-ethanolamine, Na salt selected in the earlier studies on liposomes was used for preparation of transferosomes also.

Table 11. Selection of surfactant for transferosome preparation

Surfactant	**Observation**
Sodium cholate	Transferosome dispersion was homogenous. On observation under trinocular microscope vesicles were visible.
Tween 80	Frothing observed in dispersion.
Span 40	Transferosome dispersion was homogenous. On observation under trinocular microscope, vesicles were visible.
Span 60	Transferosome dispersion was homogenous. On observation under trinocular microscope, vesicles were visible.
Span 20	Transferosome dispersion was homogenous. On observation under trinocular microscope, vesicles were visible.

- Surfactants span 40, span 60, sodium cholate and span 20 were found to produce transferosome vesicles. Tween 80 was not found suitable for preparing transferosomes as frothing was observed.

Screening of surfactant by formulation of transferosomes using different types and quantities of

surfactants:-

The quantity of surfactants was varied as 40 mg and 100 mg and cholesterol were varied as 24 mg and 40 mg for preparation of factorial batches of transferosomes. The surfactants used for preparation of trial batches were span 40, span 60, span 20 and sodium cholate. The batches were named as T1 to T16. Quantity of drug taken was 100 mg and selection of surfactant was performed based on evaluation data of % drug entrapment and size.

Table 11. Formulation of transferosomes using different types and quantities of surfactants in combinations with cholesterol in varying quantities.

Formulation	Drug (mg)	Phospholipid (mg)	Span 40 (mg)	Cholesterol (mg)	Avg. size (nm)	% Drug entrapment
T1	100	100	40	24	400	47.47
T2	100	100	100	40	440	61.69
T3	100	100	100	24	400	49.08
T4	100	100	40	40	400	42.63

Formulation	Drug (mg)	Phospholipid (mg)	Span 60 (mg)	Cholesterol (mg)	Avg. size (nm)	% Drug entrapment
T4	100	100	100	40	340	69.48
T6	100	100	100	24	300	42.64
T7	100	100	40	24	340	60.24
T8	100	100	40	40	400	64.44

Formulation	Drug (mg)	Phospholipid (mg)	Span 20 (mg)	Cholesterol (mg)	Avg. size (nm)	% Drug entrapment
T9	100	100	40	40	600	21.14
T10	100	100	100	24	740	29.34
T11	100	100	40	24	640	24.22
T12	100	100	100	40	700	28.12

Formulatio	Drug (mg)	Phospholipid (mg)	Na. Cholate (mg)	Cholesterol (mg)	Avg.size (nm)	% Drug entrapment
T13	100	100	100	24	600	34.10
T14	100	100	40	40	400	30.33
T14	100	100	40	24	440	30.24
T16	100	100	100	40	400	38.43

Drug release study of transferosome prepared using span 40 and span 60:-

As the drug entrapment and size were found to be nearly same for the transferosomes prepared using span 40 and span 60, the transferosomes batches T1 to T8 were incorporated into gel and further evaluated for % drug release for 6 hrs.

Table 13. % Drug release of transferosomal gel formulations

Formulation	Surfactant used	% Cumulative drug release
T1	span 40	17.24
T2	span 40	12.27
T3	span 40	19.47
T4	span 40	22.11
T4	span 60	84.63
T6	span 60	76.78
T7	span 60	71.64
T8	span 60	82.88

➢ From in-vitro % drug release studies, span 60 was found to give better drug release after 6 hrs as compared to span 40, therefore nonionic surfactant span 60 and cholesterol were selected as factors and their quantities varied in experimental design.

Formulation batches of drug carriers (liposomes) incorporated gel based

The experimental batches based on central composite design were further prepared by varying the level of phospholipids (0.067 mM to 0.133 mM) and cholesterol (0.064 to 1.129 mM). Drug: Lipid molar ratio= 2.12 mMol to 4.20 mMol .Hydration volume kept was 20 ml. The responses measured for batches were % drug entrapment and in-vitro permeation flux and the effect of factors on the responses were studied.

Table 14. Liposome batches based on central composite design

Formulation	Quantity of Phospholipid (mg)	Quantity of Cholesterol (mg)
M1	74.000	19.8223
M2	74.000	37.4000
M3	100.000	24.0000
M4	74.000	37.4000
M4	39.644	37.4000
M6	40.000	24.0000
M7	40.000	40.0000
M8	74.000	37.4000
M9	74.000	37.4000
M10	74.000	37.4000

M11	74.000	44.1777
M12	100.000	40.0000
M13	110.344	37.4000

Evaluation of transferosomal gel on the basis of gel characteristics:-

Table 15. Evaluation and characterization of transferosomes incorporated gel

Formulation	pH	Refractive index	Spread ability (gm cm/sec)	Gel strength (gm)	Extrudability (gm)		
					Press 1	Press 2	Press 3
F1	6	1.38	1.4284	21.48	3.208	2.133	1.118
F2	6.4	1.33	1.3990	23.02	3.323	2.024	1.236
F3	6.7	1.36	1.3428	22.62	3.442	2.046	1.289
F4	6	1.40	1.3494	24.72	2.988	2.012	1.208
F4	4.9	1.37	1.431	24.12	3.181	2.068	1.970
F6	6.2	1.38	1.4416	22.32	3.889	2.018	1.411
F7	6.4	1.41	1.2962	22.32	3.244	2.049	1.218
F8	6.3	1.33	1.4603	22.20	3.494	2.112	1.424
F9	6.2	1.39	1.4244	20.32	3.986	2.017	1.398
F10	6	1.34	1.3428	21.44	2.828	2.049	1.778
F11	6.7	1.38	1.2962	22.43	3.234	2.133	1.890
F12	6.8	1.38	1.3990	24.16	3.643	2.477	1.769
F13	6.6	1.34	1.4603	23.42	3.324	2.412	1.363
F14	6.4	1.36	1.3494	22.89	3.188	2.768	1.444
F14	6.4	1.37	1.4244	23.44	3.743	2.478	1.689
Plain drug gel	6	1.34	1.3494	17.23	2.880	1.996	1.234

In-vitro and ex-vivo membrane permeation studies and determination of permeation flux for liposomal gel and transferosomal gel:-

In-vitro and ex-vivo permeation studies were performed as per the ethical guidelines approved by institutional animal ethics committee. The in-vitro permeation studies were carried out for all the experimental design batches whereas ex-vivo studies were performed for optimized batch. The permeation flux for experimental batches of liposomal gel, transferosomal gel and plain drug gel were determined.

❖ **In-vitro permeation studies of liposomes**: - Studies were carried out for all the experimental batches of liposomes and the cumulative drug release as well as the permeation flux was determined.

Table 16.Cumulative drug release (µg) in 6 hrs by experimental batches

Time (hr)	M1	M2	M3	M4	M4	M6	M7	M8	M9	M10	M11	M12	M13	Plain drug gel
0	0	0	**0**	0	0	0	0	0	0	0	0	0	0	0
1	313.1	364.4	**488.1**	376.1	396.9	396.1	380.4	316.1	381.1	337.1	398.7	347.9	364.1	314.9
1	474.4	469.0	**697.1**	414.7	481.7	431.3	484.3	466.8	467.4	444.8	430.7	468.3	487.1	468.7
3	644.8	643.3	**761.8**	674.4	638.4	690.1	667.4	639.0	616.3	613.4	649.1	616.4	639.6	614.1
4	876.1	761.9	**831.9**	863.1	881.1	846.1	846.3	816.8	787.1	731.6	996.3	748.1	813.4	746.8
4	998.4	874.4	**1071**	997.8	991.8	873.8	869.7	1171	819.3	864.1	1048	886.3	997.1	831.7
6	1186.4	1108	**1136.1**	1180	1101	1149	1184	1140	1190	1146	1148	1176	1134	1078
14	1741	1377	**1680**	1404	1468	1397	1634	1614	1411	1434	1601	1413	1487	1133

Permeation flux is the slope of percentage drug release v/s time. It is expressed as $\mu g.cm^{-1}/hr^{-1}$

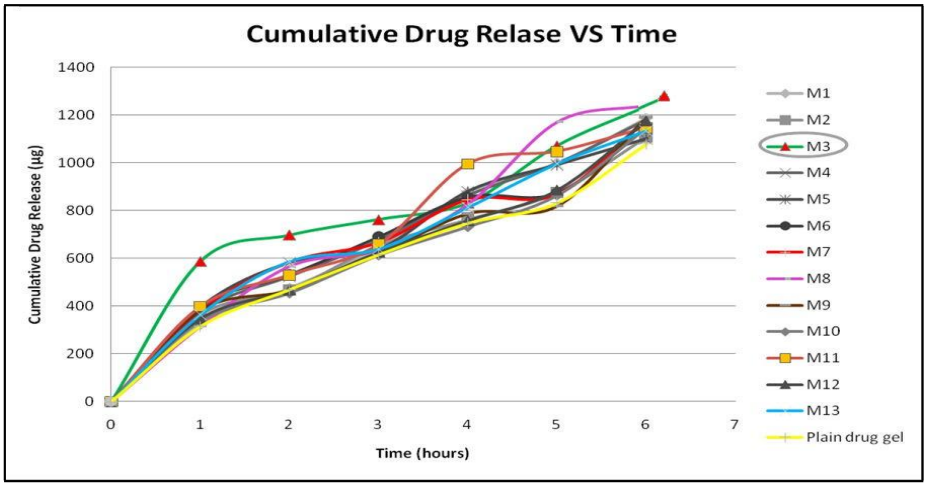

Figure 4A.16 Graph of in-vitro permeation studies of liposomal gel.

Table 17. % Drug entrapment and in-vitro permeation flux ($\mu g cm^{-1}$ hr^{-1}) of liposomal gel batches

Formulation	Liposomes		Liposomal gel
	Size	% Drug entrapment	Permeation Flux ($\mu g.cm^{-1}$ hr^{-}
M1	131	47±1.8	14.18
M1	119	31±1.6	11.06
M3	**163**	**49±1.6**	**16.08**
M4	118	44±1.9	13.11
M4	178	43±1.1	14.04
M6	116	37±1.1	11.34
M7	198	44±1.6	13.74
M8	111	47±1.7	14.33

M9	301	41±1.1	13.14
M10	184	38±1.8	11.31
M11	317	47±1.7	14.86
M11	104	41±1.6	11.91
M13	111	44±1.9	13.14

Conclusion

Both the gel formulation showed sustained release of drug for more than 6 hrs As the results of transferosomal gel of mefenamic acid was found to me more promising as compared to liposomal gel, the transferosomal gel was prepared for another drug indomethacin and tested. It was observed that transferosomal gel of mefenamic acid and indomethacin, both were found to have better permeation characteristics as compared to their plain drug gel for arthritis, whereas the permeation flux of mefenamic acid transferosomal gel was also found to be slightly higher than indomethacin transferosomal gel.

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