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Development and Evaluation of Aceclofenac Liposomes

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Abstract

This review gives concise information about the application of dendrimers as drug delivery carrier in the field of drug delivery. Due to their unique architecture these have improved physical and chemical properties. Due to their terminal groups these show high solubility, miscibility and reactivity. Dendrimers have well defined size, shape, molecular weight and monodispersity. These properties make the dendrimers a suitable carrier in drug delivery application. Dendrimers are unimolecular miceller in nature and due to this enhances the solubility of poorly soluble drugs. Their compatibility with DNA, heparin and polyanions make them more versatile. Dendrimers, also referred as modern day polymers, they offer much more good properties than the conventional polymers. Due to their multivalent and mono disperse character dendrimers have stimulated wide interest in the field of chemistry biology, drug delivery, gene therapy and chemotherapy. Self-assembly produces a faster means of generating nanoscopic functional and structural systems. But their actual utility in drug delivery can be assessed only after deep understanding of factors affecting their properties and their behaviour in vivo.

Keywords: Dendrimers, Drug targeting, nanoscale carriers.

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Introduction:

The Liposomes are specifically targeted drug delivery system which helps to carry the drug at a specific site and shows its therapeutic effect. Over the past few decades, liposomes have received widespread attention as a carrier system for therapeutically active compounds, due to having a specialized characteristic such as the capability to incorporate both hydrophilic as well as in hydrophobic drugs, low toxicity, good compatibility, lack of immune system activation and targeted delivery of a bioactive compound to the site of action.[1] Liposomes are colloidal vesicular structures composed of one and more than one lipid bilayer surrounding an equal number of aqueous compartments. Generally, liposomes are simple, small microscopic vesicle structures that incorporate both the type of drug either it is hydrophilic and lipophilic.[2] The main aim of any drug delivery system is to minimize toxicity and increase its effectiveness, safety, target specificity, and target ability at a particular site. The liposomes are so formed to targeting and site-specific delivery of a drug, to increase the circulation, time of drug and release slowly for the extended action of a drug, drug protective from degradative enzymes. The liposomes are directly delivered to the drug at a targeted site of action and provide maximum therapeutic efficacy and help to prevent the drug from any degradation and protect the body from any inappropriate and adverse drug reaction.[3,4]

Liposomes provide a wide range of attention, it provides a targeted carrier for many drugs such as anticancer, anti-depressant, anti-asthmatic, anti-fungal and also helps to deliver the drug at a targeted site. Due to their phospholipid bilayer structure, liposomes can easily cross the drug from the blood-brain barrier (BBB) in the case of the hydrophilic nature of anti-depressant drugs.[5,6]

The structure of phospholipid is amphipathic in nature, due to this it helps to incorporate water-soluble drug as well as a lipid-soluble drug as well as lipid-soluble drug. The tail of lipophilic is repelled by the water and the head of hydrophilic is repelled by the lipid.[7]

Liposomes play a major role in the pharmaceutical industry, cosmetic and dermatologist and carry both hydrophilic and lipophilic drugs and entrap the drug by liposomes and target the drug at a specific-organs. Due to their structure, liposomes also help to prevent the drug from oxidation. Liposome helps to penetrate the dermatological preparation into the deeper skin.[8] The objective of this delivery system is to target the drug at specific site during the time period of treatment as to produce the stable, efficacious and safe delivery system of Aceclofenac to overcome complications related to oral route by formulating the liposomes of Aceclofenac for topical use. Aceclofenac used in the pain induced by rheumatoid arthritis and osteoporosis, which reduce the level of PGE2 in synovial fluid & suppresses the production from blood polymorphonuclear leukocytes (mononuclear leukocytes). This delivery system is used in

study because of their specialized characteristics and helps to incorporate the Aceclofenac efficiently and used as topically and ultimately reduce the oral side effects of Aceclofenac.[9]

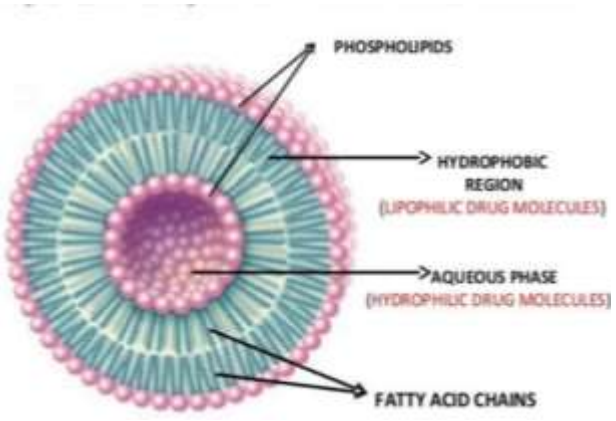


Figure 1: Liposome[10]

Materials and Method:

Materials

Aceclofenac was received as a gift sample from S. P Pharma, Chandigarh India. Lecithin, Methanol, Cholesterol, Mannitol, and Chloroform were taken from the Global Institute of pharmaceutical education and research institute Kashipur Laboratory. All other materials and chemicals used were of either pharmaceutical or analytical grade.

Method:

Preparation of topical drug-loaded Aceclofenac liposomes was prepared by the thin-film hydration method. In this method, 3gm of mannitol powder and cholesterol in 3%, 2%, 4%, and 5% were placed in 250ml RBF and held at a temperature of 70-80°C and also flask rotated at a speed of 85±5 rpm for 25-30 min in a rotatory evaporator.[11]

Aceclofenac (50mg) and lecithin with a ratio of 0.1:1, 0.1:2, 0.1:3 and 0.1:4 was dissolved in methanol and chloroform in the ratio of 1:4 v/v and add 0.5ml aliquot of the above organic solution were introduced in RBF containing mannitol and cholesterol at 37◦C. After drying a second aliquot (0.5ml) of the solution was added and then dried, a thin film is formed on the surface of RBF and placed in a desiccator overnight and the sieved with 100 mesh. The Aceclofenac-loaded liposomes were prepared and mentioned as f1, f2, f3, and f4.[12]

Composition of different formulation:

Formulation code	Drug (mg)	Chloroform (ml)	Methanol(ml)	Lecithin(gm)	Mannitol(gm)	Cholesterol (%)
F1	50	4	1	1	3	3%
F2	50	4	1	2	3	2%
F3	50	4	1	3	3	4%
F4	50	4	1	4	3	5%

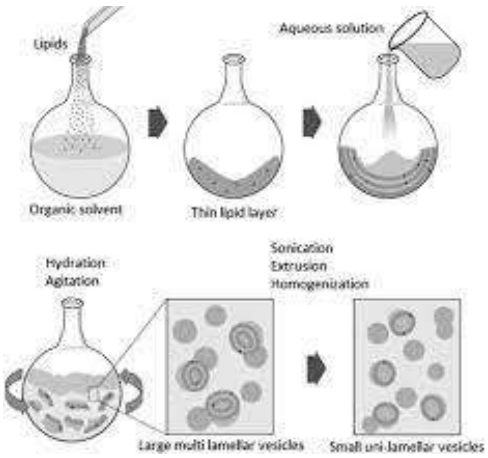


Figure 2: Lipid Film Hydration Method[13]

Pre-formulation studies

Pre-formulation study related to drug is necessary to develop the effective and safe dosage form. It is the first step to form any dosage form. It is also helping to shows the compatibility between excipients and drug and also finds out the physical and chemical characteristic.

Pre-formulation study is necessary to develop the: -

- Safe and effective use of drug.
- Compatibility study of drug with different excipients.
- To find the release kinetics.[14]

Physical appearance of drug is also observed, its color, odour, taste.

Organoleptic properties: -

- **Color: -** Powder of white crystalline
- **Odour** - Odourless
- **Taste: -** tasteless
- **Solubility: -** Water insoluble, in acetone soluble freely and solubilise in alcohol.

Angle of repose

It is done to check the flow property of powder. It is range from 0 to 90 degree. In this method, glass funnel is used.[15]

$$\text{Tan } \theta - h/r$$

Whereas,

θ =Angle of repose

h- heap height

r- Heap Radius

Relation b/w flowability and angle of repose

Angle of repose	Flowability
<20	Excellence
20 to 30	good
30 to 40	Passable
>40	Very poorly

Bulk density

It depends on size of particle, its shape and adhering tendency. For this, a powder mass is taken in 10ml measuring cylinder. And then, filling was done; cylinders dropped at the surface from one inch height in 2sec interval.

The bulk density determination calculated by: -

$$Pb = M/Vb$$

Whereas,

Pb- Bulk dens.
M- Powder wt.

Tapped density

In this, sample is taken in measuring cylinder and tapped and then calculated by following method.

$$Pt = M/Vt$$

Whereas,

Pt = Tapped density
M= powder weight
Vt = Tapped density

Carr's Compressibility Index: This method is used for determining weight uniformity.

$$\text{Car's Index} = \text{Bulk density} - \text{Tapped density}/\text{Tapped density} \times 100$$

Car's compressibility Index

Percentage Compressibility	Description of flow
5 to15	Excellence
12 to 16	good
18 to 21	Fairly
23 to 28	Poorly
28 to 35	Poorly
35 to 38	very poorly
Greater than 40	Extreme poorly

Solubility analysis

The solubility of Aceclofenac was done by using a different solvent. In this method, an amount of solvent taken in a test-tube after that drug also added in it and left overnight for the complete solubilization. On the next, solution was sonicated for some time after that a small amount of solution is pipette out around 0.1ml and further dilutions were prepared by using this. After a several dilutions were prepared, absorbance was determined with different concentration by using UV spectrophotometer with the blank solution. By using the calibration curve, amount of dissolve drug was calculated.[16]

Determination of melting point

In this method, the melting point of Aceclofenac was determined to check the purity of drug. At which temperature, a substance starts melts known as melting point. It is carried out by M.P apparatus, in this drug filled in capillary and one ended of the capillary sealed with the help of flame and attached with thermometer. Note the time at which drug starts melts.

Moisture content Determination

Formulation was allowed to content of moisture study by Infra-red moisture balance by placing liposomes for 10 minutes in 105◦C.

Calibration curve preparation: -

Calibration curve of Aceclofenac prepared in $PO4^{3-}$ buffer 6.8.

Calibration curve preparation in phosphate buffer 6.8

In this method, 10mg of Aceclofenac powder were dissolved in 10ml of $PO4^{3-}$ buffer having 6.8 PH to produce 1000ug/ml. After that several dilutions were prepared by using the above solution having 0.5ml, 1ml, 2ml, 3ml, 4ml was taken and diluted with phosphate buffer 6.8 up to 100ml. The prepared dilutions were analysed by UV- spectrophotometer at 273nm. [17]

Compatibility studies of drug- excipients: FTIR study

FTIR studies were done for determining compatibility b/w drug and excipients. This study was performed by using the saturated potassium bromide. Drug sample were prepared with KBr pellets i.e., 2mg sample in 200mg KBr with a hydrostatic force for 5.2N cm-2 for 3 minutes.

Development of Aceclofenac liposomes by thin film hydration tech.

In this, a specified lipid &drug amount were dissolved with chloroform in an RBF. After that, evaporation of solvents takes place to produce the thin film by reducing the pressure. Trace's solvent was removed by using vacuum by storing the flask overnight and then film was hydrated with phosphate buffer 6.8.[18]

RESULT:

Flow property of Aceclofenac powder

S.NO	Properties of powder	F1	F2	F3	F4
1	Angle of repose	31.5±0.02	31.2±0.10	30.1±0.07	31.4±0.04
2	Bulk density(gm/ml)	0.65±0.05	0.66±0.05	0.68±0.03	0.62±0.01
3	Tapped density	0.72±0.09	0.74±0.11	0.78±0.07	0.73±0.05
4	Carr's index	9.76±0.06	9.75±0.08	9.72±0.06	9.8±0.06

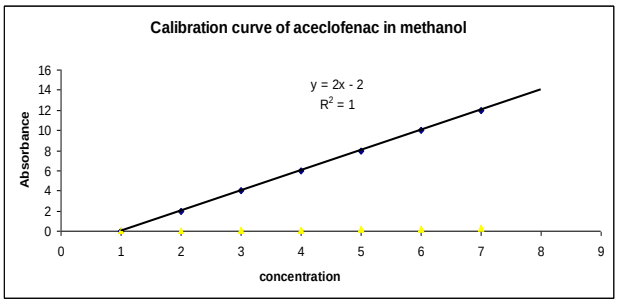
Organoleptic properties of Aceclofenac

S.NO	Properties	Results
1.	Physical appearance	White crystalline powder
2.	Odor	Odorless
3.	Taste	Tasteless
4.	Solubility	Practically Insoluble in water, freely soluble in acetone, soluble in alcohol (95%)
5.	Melting point	149-153◦C
6	Moisture content	0.6%

Solubility: - The solubility study of Aceclofenac performed and result observed in the form of calibration curve.

Calibration of Aceclofenac in methanol

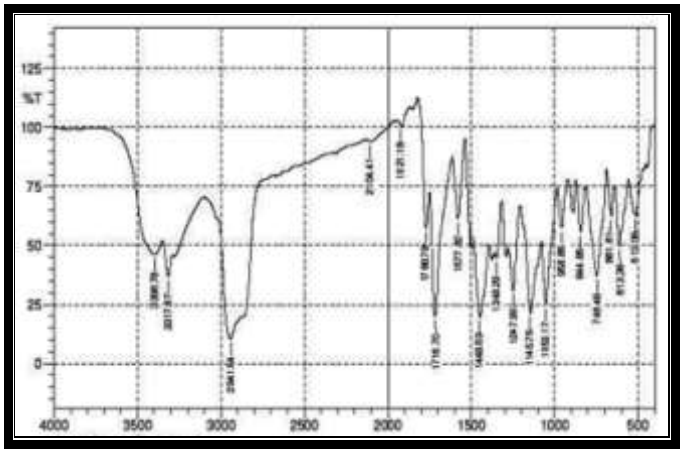
S.no	Concentration(µg/ml)	Absorbance(nm)
1.	2	0.049±0.02
2.	4	0.086±0.04
3.	6	0.0139±0.012
4.	8	0.198±0.010
5.	10	0.246±0.14
6.	12	0.298±0.016



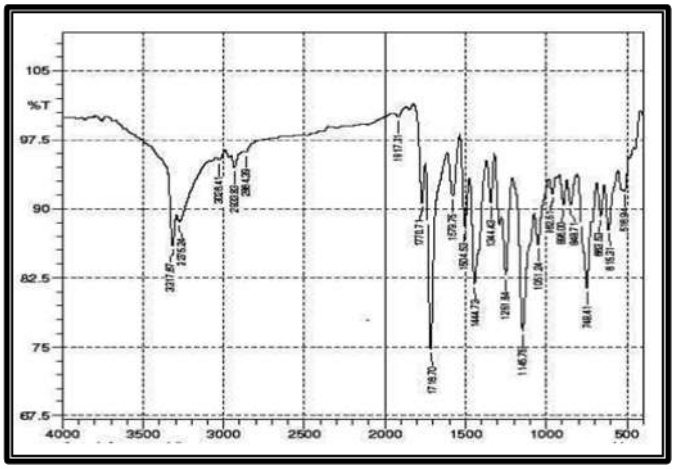
Calibration Curve of Aceclofenac in Methanol

Compatibility study: - Compatibility study was done to determine the interaction of drug with excipients. The peak of Aceclofenac and peak of Aceclofenac and mannitol is almost similar. So, the sample of Aceclofenac properties matched with the standard value.[13]

FTIR Spectra of API Aceclofenac



FTIR Spectra of Aceclofenac and Mannitol

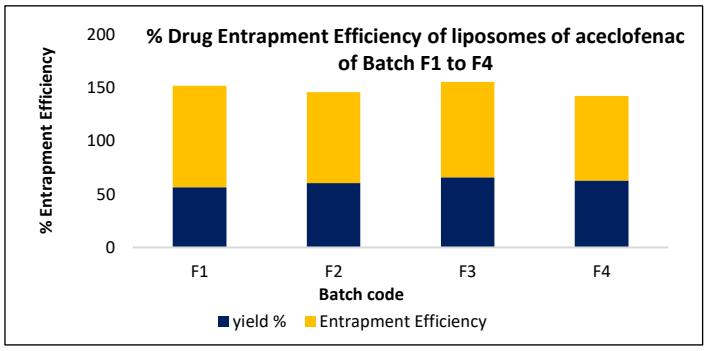


FTIR Spectra Interpretation

	FUNCTIONAL GROUP (wave number cm)					
	O-H	C-H	C=O	NH2	P=O	S-OR
Aceclofenac	3399.78 3317.18	2941.64 613.08	1788.78 1718.70	1577.92	1247.99 1146.76	844.85 881.81
Aceclofenac +Mannitol	3276.24 2833.83	3028.41 2994.23	1770.71 1718.70	1579.75	1145.75	896.00 848.71

Characterization of liposomes of Aceclofenac

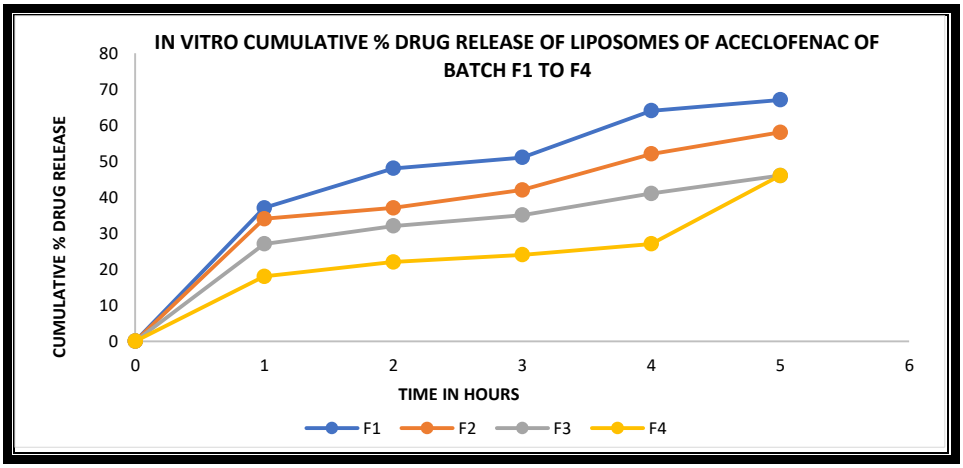
Batch code	Yield (%)	Entrapment Efficiency (%)
F1	56.37±0.003	95±0.05
F2	60.23±0.06	85±0.03
F3	65.46±0.04	89.45±0.07
F4	62.45±0.07	79.15±0.02



Comparison of Entrapment Efficiency of Different Liposomes of Aceclofenac

***In vitro* cumulative percent drug release profile of Aceclofenac of batch F1 to F4**

Time(hour)	F1	F2	F3	F4
0	0	0	0	0
1	37±0.007	34±0.06	27±0.05	18±0.02
2	48±0.002	37±0.07	32±0.003	22±0.019
3	51±0.03	42±0.05	35±0.01	24±0.002
4	64±0.014	52±0.02	41±0.012	27±0.005
5	67±0.020	58±0.04	46±0.008	46±0.09



Percentage of Drug Released From Liposomes of Aceclofenac of Batch F1 to F4

Release kinetic study

Kinetic study of formulation F1

Time (hour)	Square root of time	Log time	Cumulative percent drug release	Log Cumulative percent drug release	% ARA	Log cumulative % drug remaining
1	1	0	37	1.56	63	1.79
2	1.4	0.30	48	1.68	52	1.71
3	1.7	0.47	51	1.70	49	1.69
4	2	0.60	64	1.80	36	1.55
5	2.2	0.69	67	1.82	33	1.51

Kinetic study of formulation F2

Time (hour)	Square root of time	Log time	Cumulative percent drug release	Log Cumulative percent drug release	% ARA	Log cumulative % drug remaining
1	1	0	34	1.53	66	1.81
2	1.4	0.30	37	1.56	63	1.79
3	1.7	0.47	42	1.62	58	1.76
4	2	0.60	52	1.71	48	1.68
5	2.2	0.69	58	1.76	42	1.62

Kinetic study of formulation F3

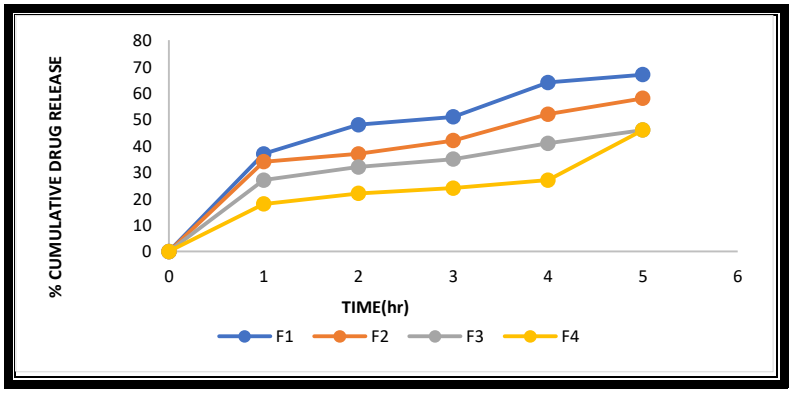
Time (hour)	Square root of time	Log time	Cumulative percent drug release	Log Cumulative percent drug release	% ARA	Log cumulative % drug remaining
1	1	0	27	1.43	73	1.86
2	1.4	0.30	32	1.50	68	1.83
3	1.7	0.47	35	1.54	65	1.81
4	2	0.60	41	1.61	59	1.77
5	2.2	0.69	46	1.66	54	1.73

Kinetic study of formulation F4

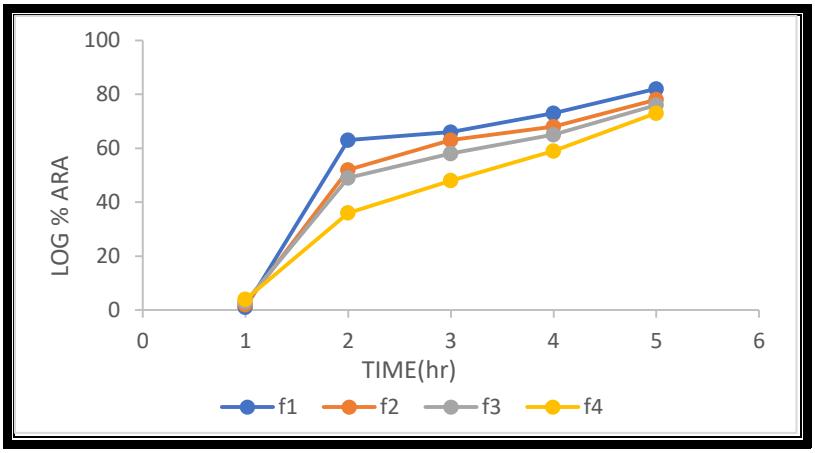
Time (hour)	Square root of time	Log time	Cumulative percent drug release	Log Cumulative percent drug release	% ARA	Log cumulative % drug remaining
1	1	0	18	1.25	82	1.91
2	1.4	0.30	22	1.34	78	1.89
3	1.7	0.47	24	1.38	76	1.88
4	2	0.60	27	1.43	73	1.86
5	2.2	0.69	46	1.66	54	1.73

Drug release kinetic with model fitting

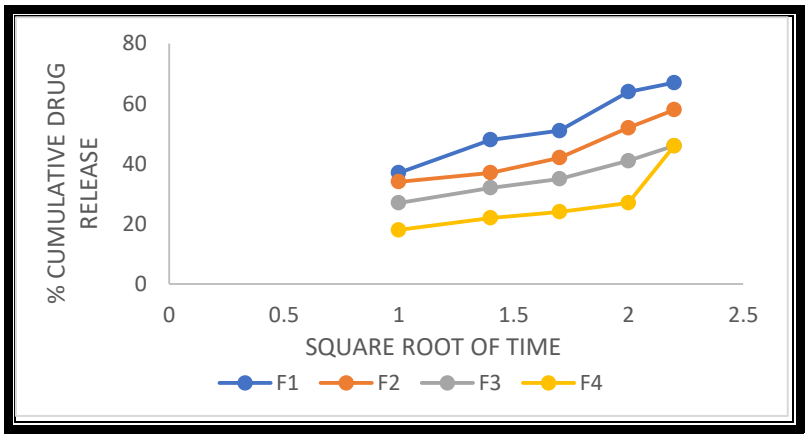
Formulation code	R2			n value	Best fit model	Mechanism of release
	Zero order	First order	Higuchi matrix			
F1	0.987	0.9875	0.9676	0.4407	First order	Non-Fickian diffusion
F2	0.9811	0.888	0.9703	0.3836	First order	Non-Fickian diffusion
F3	0.9886	0.9301	0.8807	0.3923	First order	Non-Fickian diffusion
F4	0.9298	0.9341	0.8929	0.5859	Zero order	Fickian diffusion



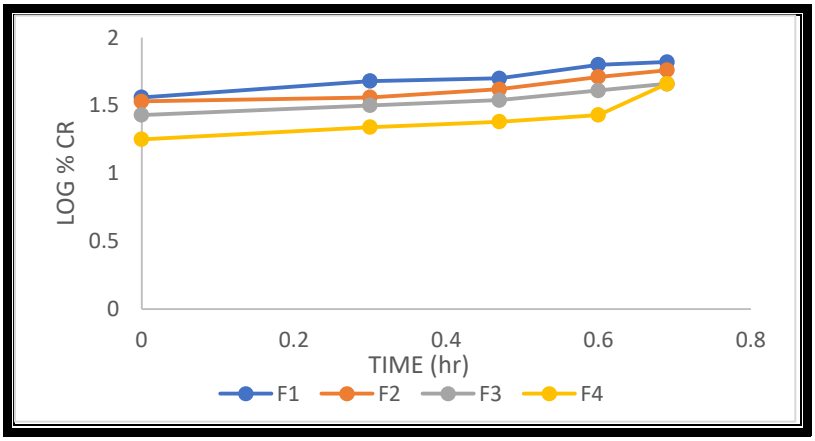
Kinetic release model of zero order release



Kinetic release model of first order release



Kinetic release model of Higuchi release



Kinetic release model of Korsmeyer peppas release

DISCUSSION:

Preformulating studies: Preformulating study of Aceclofenac was done by placing the following test: -i.e., melting point, solubility, flow property was done according to I.P.

Physical appearance: - The organoleptic properties of liposome were observed by physical and visual method, properties were matched with the standard drug and the prepared Aceclofenac liposomes were sticky in appearance.

Solubility: - Aceclofenac is freely soluble in acetone, soluble in alcohol (95%) and practically insoluble in water. Aceclofenac liposome solubility matched with the standard drug.

Melting point: - The melting point of Aceclofenac liposomes was found to be 150◦C and the standard range is 149-153◦C.

Flow property of Aceclofenac

- **Angle of repose: -** It was found to be 31.5, it indicates the powder is passable.
- **Bulk density: -** It was found to be 0.65 having good flow property.
- **Tapped density: -** It was found to be 0.72.

Preparation of calibration curve

The calibration curve of Aceclofenac was plotted in phosphate buffer having pH 6.8 and the graph was plotted between concentration (x-axis) and absorbance (y-axis). The results of calibration curve of Aceclofenac were shown in Figure 16. Table 10 shows the absorbance of Aceclofenac standard

solution containing 10-50 µg/ml of drug in phosphate buffer pH 6.8.

***In vitro* drug release studies of liposome**

In vitro- drug release study was carried out in USP XIII dissolution test apparatus type II.

In this, a temperature was set at 37◦C ±5◦C and set at 50rpm.

phosphate buffer of 1000 ml and set for 12 hours. Release of drug at different time interval has been analyzed by UV spectrophotometer at 274 nm.

Compatibility study

Compatibility study of drug and excipient was done by FTIR method. The peak of alone Aceclofenac and peak of Aceclofenac with excipients was almost same but a little different due to presence of excipients. There was no appearance or disappearance of peaks found in the drug-lipid mixture which confirms the absence of any chemical interaction between the drug and excipients.

Release kinetics: -

Drug release kinetic model are used to illustrate the drug release mechanism. For this various model are used like zero order, first order, Higuchi, korsmeyer peppas model to obtain the value of R2 and n-value for the determination of best fit model. R2 value was compared for all the formulation which shows the best fit model and by noticing n value which is from korsmeyer peppas model. Release mechanism was described by an equation.[19]

$$Mt/M\infty = kt^n$$

Followed by standard release mechanism

N value	Release mechanism
0.5	Fickian diffusion
0.5<n<1	Non-Fickian diffusion
1	Supercase II transport

The observed data of kinetic model shows the best fit model for prepared Aceclofenac liposomes was determined by regression coefficient (r^2) in all formulation. The highest r^2 value determine the best fit model, the observed data shows the First order release in F1, F2 and F3 formulation it shows the drug release is dependent on concentration and in F4 it shows zero-order release i.e., the drug release is independent of concentration. Formulation F1, F2 & F3 shows the non-Fickian diffusion and F4 shows the Fickian diffusion which means F1, F2 & F3 is anomalous drug release as it is erosion-controlled release rate and diffusion release rate. The best formulation is F4 formulation because the drug release is independent of concentration.

Entrapment Efficiency: - The drug entrapment efficiency of liposomes formulations is given in Table 12. The loading efficiency calculated for all liposome from batch F1 to F4 ranged from 79.15 to 95%. For this, it is clear that drug entrapment efficiency changed by changing the ratio of excipients. The highest loading efficiency was found for the F1 formulation is 95% and F3 is 89.45

Determination of PH: - The pH of prepared Aceclofenac liposomes was found to be 5 which is matched with the pH of skin so, the Aceclofenac liposomes was prepared successfully and have good therapeutic effect.

CONCLUSION

In this study, it has been concluded that the formulation of Aceclofenac liposomes provides the sustained action of drug. The Aceclofenac liposomes were successfully formulated by using cholesterol, Mannitol and chloroform for topical use. In this, the polymer used as a carrier for Aceclofenac drug release.[20] The Aceclofenac liposomes have a capability to penetrate the lipoidal structure easily and produce a prolonged action. When the Aceclofenac given orally, it will produce the gastrointestinal complication so, to overcome this, the topical preparation of Aceclofenac liposomes can be formulated; it is used in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.[21]

From the above experiments, it has been concluded that: -

i) Different pre-formulation studies were done on the sample of Aceclofenac.

ii) The liposome of Aceclofenac prepared by using different excipients such as methanol, chloroform, mannitol, lecithin, etc.

The liposome of Aceclofenac has been prepared by the thin-film hydration method and the rotary film evaporator equipment were used.[22]

The prepared Aceclofenac liposomes to be a novel drug approach for treating the arthritis through transdermal route in which drug can permeate through skin and also show a sustained action. The prepared formulation was found to have better bioavailability, analgesic activity and anti- inflammatory action as compared to existing formulations of the mentioned drug.[23]

According to the results obtained from this study, it was concluded that the Aceclofenac liposome were successfully prepared to obtain ointment. Aceclofenac ointment showed good pH value, Spreadability, good entrapment efficiency. The kinetic study was also performed for the prepared Aceclofenac liposomes and the observed data of kinetic model shows the best- fit model for prepared Aceclofenac liposome was determined by regression coefficient (r2) in all formulation. The best model of formulation F4 shows zero order release because the drug release is independent of concentration and it shows the Fickian diffusion.[24]

Therefore, it was concluded that the formulation could be very promising alternative for the topical or transdermal treatment.

Conflict Of Interest: The authors have no conflicts of interest regarding this investigation.

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