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Formulation and Evaluation of Liposome by Thin Film Hydration Method

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Article Info:

Abstract

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Liposomes are the most advance formulation for targeting and controlled drug delivery system. These liposomes are generally administered by intra-venous route. In this work the liposome was prepared by using thin film hydration method. The formulated liposome is evaluated or characterised by using zeta sizer, Encapsulation efficiency, Entrapment efficiency, In vitro drug release. Main things are drug which are used for formulation of liposome was Diclofenac sodium, it having anti-inflammatory and anti-pyretic effect. The Diclofenac sodium having several adverse effects, such as depression of renal function, Liver failure for repeated administration, Local mucosal irritation, gastritis. To avoid this adverse effect Diclofenac sodium are incorporate in liposomal formulation. By formulating liposomal formulation, the bioavailability of Diclofenac sodium increase. In conventional dosage form bioavailability of diclofenac sodium is 50%. But in liposomal formulation bioavailability of this drug increase. The final result includes that diclofenac liposome formulation shows more sustained and prolong anti-inflammatory activity.

Keywords: Diclofenac sodium, Liposome, Anti-inflammatory activity.

INTRODUCTION:

The liposome is tiny bubble and is made up of same material as cell membrane. The name of liposome is derived from two Greek words "Lipos" means Fat and "soma" means body. The membrane of phospholipid is having ability to self-assemble into tiny bilayer sphere. If single layer sphere is formed then called as micelles.[1] Liposome carries numerous molecules in pharmaceutical industry and cosmetic, these are used in cosmetic preparation also. The liposome is carrying both hydrophilic and hydrophobic drug also.[2] The liposomal preparation is also useful for oral local treatment.[3] These liposomes are having different types according to their structure such as Large Unilameller Vesicle (LUV), Multi-lamellar Vesicle (MLV) and Small Unilameller Vesicle (SUV). Liposomes are colloidal and small vesicular structure of lipidic bilayer. The main component of liposomal structure is cholesterol and non-toxic phospholipid. The liposome is having different property by changing lipid concentration, amount of cholesterol, method of preparation, charge present on surface and size of liposome. The unsaturated phosphatidylcholine species shows the less stable and more permeable bilayer structure of liposome. Whereas, saturated

phospholipid is form more rigid and less permeable structure of liposome. Generally, liposome is having structure spherical in nature and size start from 30nm to several micrometre size. The polar head of lipid are oriented toward the internal and external aqueous phase and non-polar tail of bilayer to each other. There are many factors which are affecting on liposomal formulation or preparation such as temperature, molecular shape, drug and their nature, type of phospholipid and their nature. Liposomes are vesicle in which nutrient and many pharmaceutical drugs incorporate. These liposomes are having several advantages but disadvantage also having such as it shows oxidation due to lipid as major component.[4]

There are several routes which are available for administration of liposome such as oral, Parenteral and Topical. The liposomal formulation is having targeted drug delivery action; due to that liposome are novel dosage form for administration many drugs in it. These liposomal structures are highly lipophilic in nature; these are made up of phospholipid. The topical absorption of liposome is taking place very easily due to lipidic nature. Many liposomal formulations are used for topical administration. The

biodegradation of liposome is easily taking place. These liposome formulations more advance formulation for administration of drug. The topical formulation of liposome which are act as local anaesthetics. The Diclofenac sodium is the NSAID drug, which is widely used for antipyretic, anti-inflammatory, and analgesic also for joint stiffness activity. Diclofenac sodium are having short half-life about 1-2 hr. In conventional dosage form diclofenac sodium are reaches in blood circulation as about only 50% but in liposomal formulation the bioavailability of diclofenac sodium increase. Due to that more adverse effect of diclofenac sodium such as depression of renal function, liver failure for repeated administration, local mucosal irritation and gastritis, hence drug is incorporated into liposome. Due to liposome incorporating the adverse effect reduces. The antiplatelets action is not appreciable due to sparing of COX-I. It is having 99% protein bound and excreted in both urine and bile. The particle size of liposome is in range of nanometre, these nanometric sizes of liposome and its lipidic nature increase the penetration property, due to that two things the bioavailability of this drug increase. When the liposomal formulation is administered by parenteral routes then first pass metabolism is also reducing.[5]

MATERIAL AND METHOD

Table-1: List of material used for the study

Sr.No	Ingridients	Supplier
1	Diclofenac Sodium	Alkha Pharmaceutical, Hyderabad
2	Soya lecithin	Sami Lab Lmt. Bangalore
3	Cholesterol	Laboratory
4	Rotary flash evaporator	KNF
5	Ultra-centrifugation	Beckman Coulter
6	Zeta sizer	Malvern
7	Magnetic stirrer	REMI
8	Weighing Balance	Jepson's

Drug Profile: -

Structure of Diclofenac Sodium

The Diclofenac sodium is the NSAID drug, which is widely used for antipyretic, anti-inflammatory, and analgesic also for joint stiffness activity. Diclofenac sodium are having short half-life about 1-2 hr. In conventional dosage form diclofenac sodium are reaches in blood circulation as about only 50%. The antiplatelets action is not appreciable due to sparing of COX-I. It is having 99% protein bound and excreted in both urine and bile. These are having water soluble in nature.[5]

Preperation of Liposome: -

By using Thin film hydration method were prepared multi lamellar liposome containing diclofenac sodium. Diclofenac sodium, soya lecithin and cholesterol were dissolved in chloroform and methanol mixture in ratio (9:1). These above solutions pour in round bottom flask of rotary flash evaporator. In rotary flash evaporator the organic solvent evaporates at 60^0c, for 15min. at 90rpm. After evaporating organic solvent thin layer which are form on inner surface of round bottom flask. These thin layers dried overnight by using vacuumed oven. Then this thin lipid layer suspension in phosphate buffer saline (PBS) having PH-7.4 by vertexing for 10min.and then it was allowed to hydrate for 1hr at 70^oc, 90 rpm. Then this liposomal suspension centrifuge by using ultra-centrifugation Machin at 3000rpm for 30 min. Then the settle liposome again centrifuge in PBS. Then this suspension of liposome sonicates for 15 min at 65^oc to get small unilamellar vesicle (SUV).[5] For this study the four batches were prepared and formula and their composition are show in below table:

Table 2: Composition of Diclofenac sodium liposome

Sr.No.	Batch Code	Amount of Drug	Soya Lecithin	Cholesterol
1.	F1	25 mg	100 mg	15 mg
2.	F2	50 mg	100 mg	15 mg
3.	F3	75 mg	100 mg	15 mg
4.	F4	100 mg	100 mg	15 mg

Characterization of Dliclofenac Sod. Liposome:

Particle size: -

The particle size of liposome is generally taken by zeta sizer instrument. This instrument containing Malvern PCS software. Before taking the result of sample solution the sample must be diluted with distilled water. The distilled water not interferes with result. Then after dilution the result were taken. The particle size must be required in nano range some time it goes to micron range if multilamellar vesicle are present. This software was taken the average particle size of liposome. The particle size of sample solution was determined by using light scattering technique and by transmission electron microscope. If the particle size of liposome increases then decrease the uptake and bioavailability of drug. The analysis of particle size was carried out for 60_s at 165^oc scattering angle of detection. The particle size is most important, the particle size of liposome in nano range are having more effective drug delivery as compare to micron range. The one advantage of large particle size liposome is having more area to fill more drug but it has very slow-release pattern. Various method is used

for administration of particle size of liposome such as SEM, TEM, XRD, AFM, Dynamic light scattering (DLS). [6,7,8,9,10]

PDI: -

PDI is also called "particle size distribution". If the sample having very broad size distribution then poly dispersed value goes to more than 0.7. The PDI of liposome is also obtained by photon Correlation spectroscopic analysis. During formulation of liposome the effort of manufacturer are must be to achieve lowest PDI value.[16]

Zeta Potential: -

The zeta potential means the charges which are present on the surface of liposome. The many time the charge is present on the surface of liposome. This charge is come due to the component or ingredient which was used during the manufacturing. Some charge is must be required on surface of all liposome present in formulation, due to some charge all liposome particle repeal to each other and coagulation of particle are avoided. The zeta potential of liposome was taken in zeta sizer instrument having Malvern software. The analysis of sample was carried out at 25°c with the angle of detection 90°. The ideal zeta potential value must be required in range between +30 to -30mV. These ranges prevent the aggregation of liposomal particle. [11,12,14]

Entrapment Efficiency:

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV spectrophotometer at 254 nm. A standard calibration curve of drug was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (W). Effectively, (W-w) will give the amount of drug entrapped in the liposome. [13,15,17,18,20]

$$\%Drug\ Entrapment = (W-w/W) \times 100$$

Loading Efficiency:

Drug content in the preparation was determined by extracting drug from the liposome with 0.1M hydrochloric acid. In this method liposome (50mg) were stirred in 50ml hydrochloric acid until dissolved. It was filtered by Millipore filter paper and drug content was determined, after suitable dilution. At 254nm by UV spectroscopy. The loading efficiency (L) of the liposome was calculated according to following formula.

$$L\ (\%) = (Qn\ /\ Wn) \times 100$$

Were, Qn is the amount of drug present in Liposome and Wn is weight of liposome.[19]

In vitro drug release: -

The Diclofenac sodium liposome was present in aqueous suspension they separated by using ultracentrifugation. Then 2mg of Diclofenac sodium liposome was taken and dispersed in 10ml 7.4-phosphate buffer. After this 10ml solution place in dialysis membrane bag. Then make 900ml 7.4 phosphate buffer and add it in dissolution apparatus beaker. Make the temperature 37°c. For the dissolution the USP paddle was used. At appropriate time intervals 1mL of the release medium was removed and 1mL fresh 7.4 phosphate buffer solution was added in to the system. The amount of Diclofenac sodium in the release medium was estimated by UV-Visible Spectrophotometer at 275 nm.[21]

RESULT AND DISCUSSION
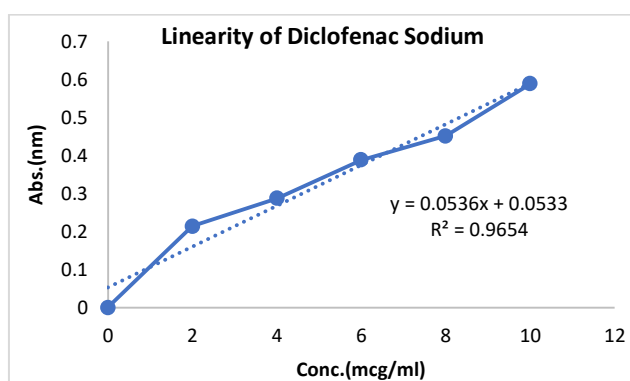
Graph 1: Linearity of Diclofenac sodium

Particle size: -

The particle size of liposome Increases by increasing the concentration of drug. In F1 batch the particle size occurs about 796.2 nm. Then final observation is as concentration of diclofenac increase then particle size is also increase. Very slightly particle size change occurs in all batches. The batch F3 shows the particle size about 836.4 nm.
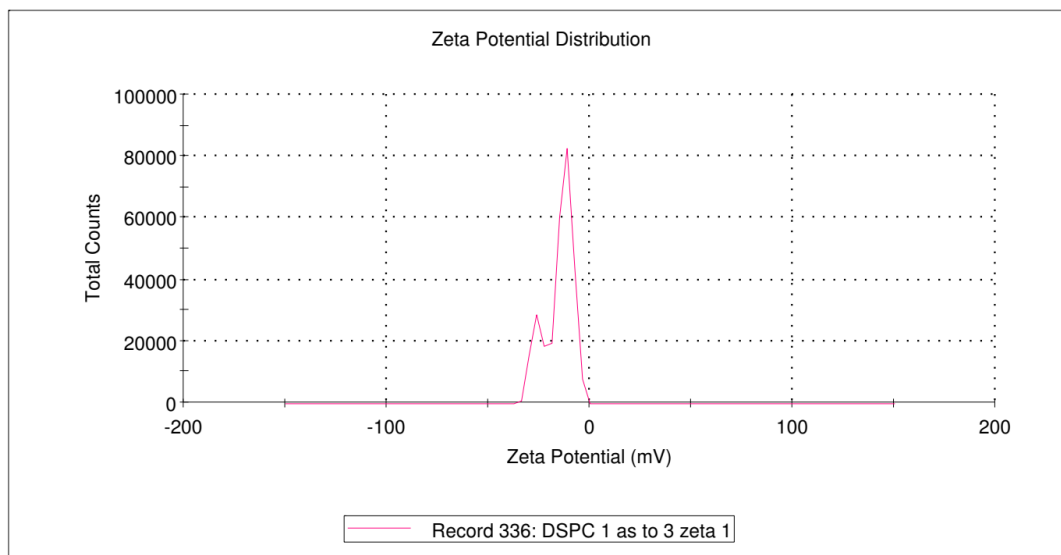
Results

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	836.4	Peak 1:	868.6	96.9	289.7
Pdl:	0.369	Peak 2:	5385	3.1	319.6
Intercept:	0.973	Peak 3:	0.000	0.0	0.000
Result quality :	Good				
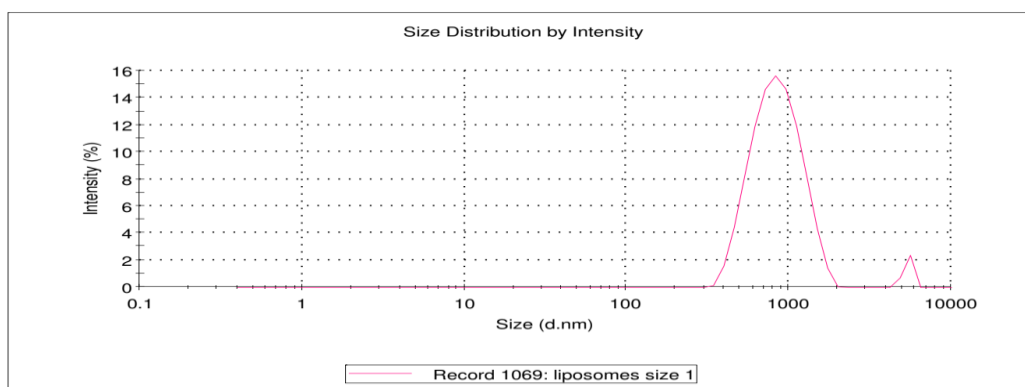
Zeta Potential: -

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -15.1		**Peak 1:**	-12.7	78.5	4.55
Zeta Deviation (mV): 6.94		**Peak 2:**	-26.1	21.5	2.88
Conductivity (mS/cm): 0.180		**Peak 3:**	0.00	0.0	0.00
Result quality : Good					

The zeta potential is most important Evaluation parameter. There are many drugs which are affecting the zeta potential value of liposome. Due to some drug means those drugs incorporated in liposomes which are causes the charge on the surface of liposome. Here in this work also observed that as amount of Diclofenac sodium increases then zeta potential also increases. If zeta potential value is not in range of -30mV to +30mV then the aggregation of liposome take place in formulation.

PDI: -

In this work only concentration of drug change as per batches. As the amount of drug increase in each batch the PDI also increase, but in batch F4 the PDI value decrease. PDI values are must be required below the 0.7 and this obtained all PDI value below 0.7.

Entrapment Efficiency:

The entrapment efficiency is also important evaluation parameter foe liposome. Entrapment efficiency of all batches occur different such as F1 is 93.64, F2is 94.86, F3 is 96.04 and F4 is 96.22%.

Loading Efficiency:

The loading efficiency also varies according to different drug concentration, such as in batch F1 is 4.2, F2 is 5.66, F3 is 7.41 and in F4 is 8.41%.
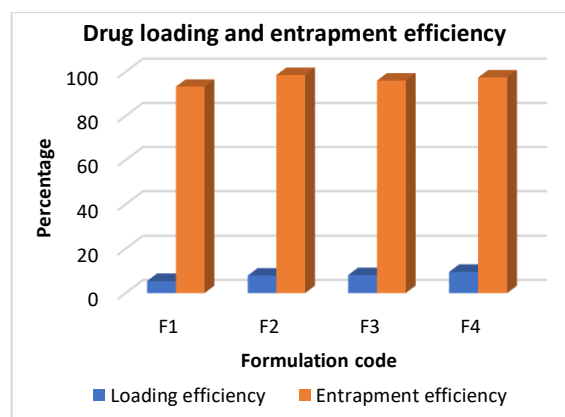
Graph 2: Effect of drug and polymer on Loading efficiency and Entrapment efficiency

Table-3: - Evaluation Parameter of Liposome

Sr.No.	Evaluation Test	F1	F2	F3	F4
1.	Entrapment Efficiency (%)	93.24	98.56	96.04	97.48
2.	Loading Efficiency (%)	5.44	7.88	8.14	9.56
3.	Particle Size (nm)	796.2	815.4	836.4	915
4.	Polydisperse Index (PDI)	0.245	0.346	0.369	0.298
5.	Zeta Potential (mV)	-15.4	-21.6	-22.3	-27.6

In vitro drug release: -
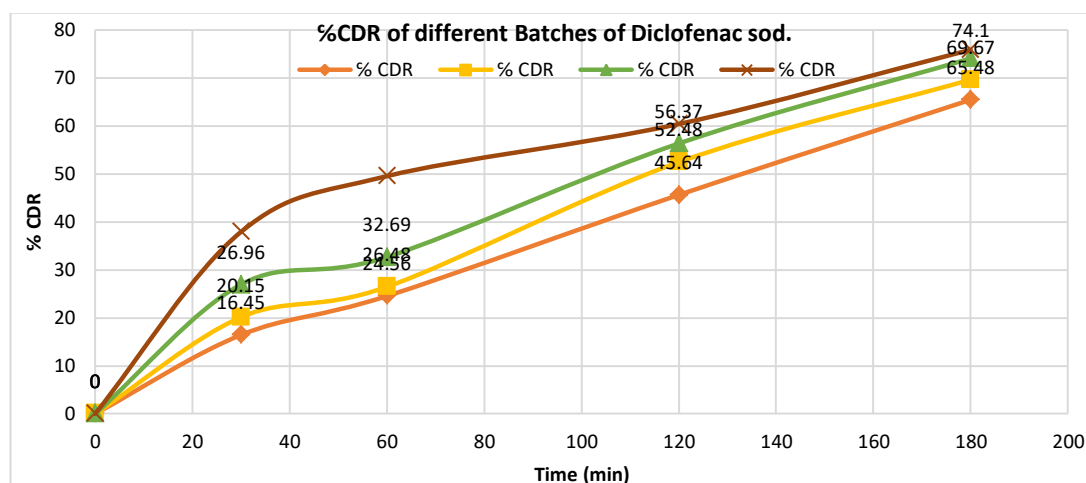
Graph-3: % CDR of Different batches of Diclofenac sodium.

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CONFLICT OF INTEREST

No conflict of interest.

CONCLUSION

Diclofenac sodium having several adverse effects. This side effect of diclofenac sodium reduces due to the liposomal formulation of diclofenac sodium. The bioavailability of diclofenac also increase's due to this novel formulation. As the concentration of drug increase's in formulation then entrapment efficiency, loading efficiency particle size of liposome and zeta potential also increase.

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