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Formulation And Evaluation Of Flucinolone Acetonide Loaded Liposome

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Abstract : Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphos phatidylcholine) form a rigid, rather impermeable bilayer structure.). The liposomes prepared by physical dispersion method showed better percentage drug entrapment when compared with ether injection method. The particle size was analyzed by Malven particle size analyzer. The results of the particle size showed, when the concentration of soya lecithin was increased the size of the particle was reduced. The *in vitro* release showed that as the concentration of soya lecithin was increased the release rate of drug was retarded. Among the two methods ether injection method showed prolonged action when compared to physical dispersion method.

Keyword: Liposome,Phospholipid,

Introduction: Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery. Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphos phatidylcholine) form a rigid, rather impermeable bilayer structure.

liposomes are definite as spherical vesicles with particle sizes ranging from 30 nm to several micrometers. They consist of one or more lipid bilayers surrounding aqueous units, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases. On the other hand, self-aggregation of polar lipids is not limited to conventional bilayer structures which rely on molecular shape, temperature, and environmental and preparation conditions but may self-assemble into various types of colloidal particles.

The liposome size can vary from very small (0.025 μm) to large (2.5 μm) vesicles. Moreover, liposomes may have one or bilayer membranes. The vesicle size is an acute parameter in determining the circulation half-life of liposomes, and both size and number of bilayers affect the amount of drug encapsulation in the liposomes. On the basis of their size and number of bilayers, liposomes can also be classified into one of two categories: (1) multilamellar vesicles (MLV) and (2) unilamellar vesicles. Unilamellar vesicles can also be classified into two categories: (1) large unilamellar vesicles (LUV) and (2) small unilamellar vesicles (SUV).In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. In multilamellar liposomes, vesicles have an onion structure. Classically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water.

Classification of Liposomes

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1.2. Method of Liposome Preparation

1.2.1. General methods of preparation

All the methods of preparing the liposomes involve four basic stages:

1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

1.2.2. Method of liposome preparation and drug loading

The following methods are used for the preparation of liposome:

1. Passive loading techniques
2. Active loading technique.

Passive loading techniques include three different methods:

1. Mechanical dispersion method.
2. Solvent dispersion method.
3. Detergent removal method (removal of non-encapsulated material)

1.2.3. Mechanical dispersion method

The following are types of mechanical dispersion methods:

1. Sonication.
2. French pressure cell:extrusion.
3. Freeze-thawed liposomes.
4. Lipid film hydration by hand shaking, non-hand. shaking or freeze drying.
5. Micro-emulsification.
6. Membrane extrusion.
7. Dried reconstituted vesicles

1.2.4. Solvent dispersion method

1.2.4.1. Ether injection (solvent vaporization)

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature.

1.2.4.2. Ethanol injection

A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high.

1.2.4.3. Reverse phase evaporation method

This method provided a progress in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid ratio and a capability to entrap a large percentage of the aqueous material presented. Reverse-phase evaporation is based on the creation of inverted micelles. These inverted micelles are shaped upon sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. The slow elimination of the organic solvent leads to the conversion of these inverted micelles into viscous state and gel form. At a critical point in this process, the gel state collapses, and some of the inverted micelles were disturbed. The excess of phospholipids in the environment donates to the formation of a complete bilayer around the residual micelles, which results in the creation of liposomes. Liposomes made by reverse phase evaporation method can be made from numerous lipid formulations and have aqueous volume-to-lipid ratios that are four times higher than hand-shaken liposomes or multilamellar liposomes.

1.3. Drug Loading In Liposomes

Drug loading can be attained either passively (i.e., the drug is encapsulated during liposome formation) or actively (i.e., after liposome formation). Hydrophobic drugs, for example amphotericin B taxol or annamycin, can be directly combined into liposomes during vesicle formation, and the amount of uptake and retention is governed by drug-lipid interactions. Trapping effectiveness of 100% is often achievable, but this is dependent on the solubility of the drug in the liposome membrane. Passive encapsulation of water-soluble drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation. Trapping effectiveness (generally <30%) is limited by the trapped volume delimited in the liposomes and drug solubility. On the other hand, water-soluble drugs that have protonizable amine functions can be actively entrapped by employing pH gradients, which can result in trapping effectiveness approaching 100%

Mechanism of transportation through liposome

The limitations and benefits of liposome drug carriers lie critically on the interaction of liposomes with cells and their destiny in vivo after administration. In vivo and in vitro studies of the contacts with cells have shown that the main interaction of liposomes with cells is either simple adsorption (by specific interactions with cell-surface components, electrostatic forces, or by non-specific weak hydrophobic) or following endocytosis (by phagocytic cells of the reticuloendothelial system, for example macrophages and neutrophils).

Fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal content into the cytoplasm, is much rare. The fourth possible interaction is the exchange of bilayer components, for instance cholesterol, lipids, and membrane-bound molecules with components of cell membranes. It is often difficult to determine what mechanism is functioning, and more than one may function at the same time.

Applications of liposomes in medicine and pharmacology

Applications of liposomes in medicine and pharmacology can be divided into diagnostic and therapeutic applications of liposomes containing various markers or drugs, and their use as a tool, a model, or reagent in the basic studies of cell interactions, recognition processes, and mode of action of certain substances.

Liposomes have been used in a broad range of pharmaceutical applications. Liposomes are showing particular promise as intracellular delivery systems for anti-sense molecules, ribosomes, proteins/peptides, and DNA. Liposomes with enhanced drug delivery to disease locations, by ability of long circulation residence times, are now achieving clinical acceptance. Also, liposomes promote targeting of particular diseased cells within the disease site. Finally, liposomal drugs exhibit reduced toxicities and retain enhanced efficacy compared with free complements. Only time will tell which of the above applications and speculations will prove to be successful. However, based on the pharmaceutical applications and available products, we can say that liposomes have definitely established their position in modern delivery systems.

Material and Methods:

Preformulation Studies

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rational development of dosage form. The objective of preformulation testing is to generate information useful to the formulation in developing stable and stable andbioavailable dosage forms.The use of preformulation parameters maximize the chancesin formulating an acceptable, safe, efficacious and stable product.

Solubility

Solubility of flucinolone acetonide in water, methanol, phosphate buffer pH 6.8 was determined at room temperature with the help of magnetic stirrer. Approximate solubility of drug was indicated from the following limits:

Very soluble: 1 part of the substance is soluble less than 1 part of the solvent.

Freely soluble: 1 part of the substance is soluble in 1 to 10 parts of the solvent.**Soluble:** 1 part of the substance is soluble in 10 to 30 parts of the solvent.

Sparingly soluble: 1 part of the substance is soluble in 30 to 100 parts of the solvent.

Slightly soluble: 1 part of the substance is soluble in 100 to 1,000 parts of the solvent.

Very slightly soluble: 1 part of the substance is soluble in 1000 to 10,000 parts of thesolvent.

Practically insoluble or insoluble: More than 10,000 parts of the solvent is requiredto dissolve 1 part of substance.

Melting Point

Melting point determination was done by using melting point apparatus. Small amount of pure drug of flucinolone acetonide was taken in a capillary tube and it was kept in the melting point apparatus and the melting point was noted.

Drug – excipients interaction studies:

FT-IR spectra were taken for the dried samples using FT-IR 8400S (Shimadzu,Japan) to determine the possible interactions between the drug and polymers. The plaindrug, individual lecithin and cholesterol, combination of drug with cholesterol and lecithin in three different ratio (1:1, 1:2 and 1:3) were taken and mixed with KBr.

The samples were compressed to form a pellet using a hydraulic press. The prepared pellets were transformed into disk. The disk was applied to the centre of the sample holding device and scanned from 4,500 to 400 cm-1 using FT-IRspectrophotometer.

Formulation of liposomes loaded with flucinolone acetonide:

The formulation of liposomes loaded with flucinolone acetonide was prepared by twodifferent techniques namely, physical dispersion method and ether injection method. Inboth the techniques ratio of cholesterol was kept as same and the lecithin concentrationwas increased as 1:1, 1:2 and 1:3.

Physical dispersion method:

Liposomes were prepared by physical dispersion method using different ratio of soya lecithin and cholesterol was kept as constant. In this method the soya lecithin and cholesterol were dissolved in chloroform. Then it was spread over flat bottom conical flask and allowed to evaporate at room temperature for overnight without disturbing the solution for a formation of lipid film. The drug was dissolved in phosphate buffer pH 6.8. It act as an aqueous medium. Then the aqueous medium wasadded to the lipid film for hydration. For this the flask was inclined to one side and aqueous medium was introduced down the side of flask and flask was slowly returned to upright orientation. Then the conical flask was kept on water bath and the temperature was maintained at 37± 2ºC for 2 hours for the completion of hydration. The conical flask was gently shaken until the lipid layer was removed from wall of conical flask and formation a liposomes suspension. Then the formed liposomessuspension was stored at 4ºC for one day for the maturation of liposomes. The preparedliposome suspension was centrifuged at 15,000 rpm for 20 mins. Then the precipitate was collected and diluted with distilled water for further studies[35]. Different batches ofliposomes were prepared as per the general method described above and composition for the

Determination of percentage drug preparation of liposomes is given in

Evaluation Of Liposomes:

Entrapment efficiency:

Drug entrapment efficiency was calculated by using centrifugation method. 10ml of liposome suspension was taken and centrifuged at 15,000 rpm for 20 mins. The supernatant liquid was collected and suitably diluted. Then the absorbance was taken at233 nm with the help of UV double beam spectrophotometer using pH 6.8 as a blank.

***In vitro* drug release study:**

Apparatus : USP TYPE II **(**Paddle type)

RPM: 50

Temperature: 37ºC ± 0.5ºC

Time: 30 min. interval Upto 8 hrs

The *in vitro* release for all the formulated flucinolone acetonide liposomes were carried out for 8 hours in phosphate buffer p H 6.8. The studies were carried in USP dissolution apparatus II (Paddle) at 37ºC ± 0.5ºC and 50 rpm speed. 900 ml of phosphate buffer p H 6.8 was used as a dissolution medium. Equivalent to 100 mg of flucinolone acetonide liposome was taken in a dissolution jar contains dissolution

medium and the paddle wasrotated at 50 rpm. 1 ml of samples were withdrawn at every 30 min. upto 480 mins and make upto 10 ml with pH 6.8 and analyzed for flucinolone acetonide content at 233 nm with pH 6.8 as blank using double beam UV double beam spectrophotometer.

Particle size determination:

The particle size determination is done by using Malven particle size analyzer.Groups of particles are dispersed in a liquid medium and measured as they are circulatedbetween the flow cell, which is placed in the measurement unit, and a dispersion bath in the sampler. The dispersion bath incorporates a stirrer and an ultrasonic sonicator. Apump delivers the dispersed suspension to the flow cell. The pump is specially designedto ensure both liquid medium and the particles are circulated. It can be controlled froma PC. Organic solvents can be used as dispersion media.

Stability studies:

The behavior of the liposome to retain the drug was studied by storing the liposome at two different temperature conditions, i.e., 4ºC (refrigerator RF), 25°C±2ºCfor a period of 1 month. The liposomal preparations were kept in sealed vials. At 30thday the samples were analyzed for the drug content following the same method described in % drug encapsulation efficiency and *in vitro* drug release. And also the liposomes were studied for their morphology.

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Result and Discussion:

Preformulation Studies

Solubility

The drug should be dissolve in solvents and also dissolution medium so the solubility analysis for the drug was important. The solubility of raw drug was determined by dissolving in distilled water, methanol and phosphate buffer pH 6.8.Thedrug was found to be freely soluble in water, soluble in methanol and phosphate bufferp H 6.8.

Melting point

The melting point was confirmed the Flucinolone acetonide present in raw material ofdrug. It was found to be 264ºC-265C within the specification range. So it confirmed flucinolone acetonide present in raw material of drug.

Drug – Excipients Interaction Studies:

The FT – IR studies were conduct to study the interaction between the drug and excipients. IR spectral analysis showed that the fundamental peaks and patterns of the spectra were similar both in pure drug and combination containing drug and highest proportion of excipients. This indicated that there was no chemical interaction between flucinolone acetonide and the other excipients used in the formulations. The spectral datas are presented in **Table No. 5 - 8** and spectral peaks were presented graphically in **FigureNo. 5 – 9**

Ft – Ir Spectrum Of Pure Flucinolone Acetonide: The major bands were observed at around 1600 cm-1 assigned to C=Cand around band 1728cm-1 was assigned to C=O group. The bands from 2995 to 2952 cm-1 were assigned to C-H Group.

Evaluaton Of Flucinolone Acetonide Liposomes

Percentage drug entrapment efficiency

The percentage drug entrapment efficiency of liposomes were prepared by physical dispersion method and ether injection method. The formulations was formulated by varying the cholesterol – soya lecithin ratio. It was found to be that percentage drug entrapment efficiency of formulations F 1, F 2 and F 3 were 86.60 %, 79.90 % and 73.10 % respectively and formulations F 4, F 5 and F 6 were 30.47%, 39.58% and 39.69% respectively. The results may adjudge physical dispersion method have better drug entrapment efficiency than ether injection method.

Particle size analysis

The particle size analysis was carried out by particle size analyzer for all the prepared liposome formulations. The particle size for all the formulated liposomes werefound be in the range of 30.617 µm to 0.031µm as shown in **Table No. 7.** The particle size data showed that when theconcentration of soya lecithin was increased the particle size was decreased for all the formulations of flucinolone acetonide liposomes in prepared by both methods. The particle size of flucinolone acetonide liposomes of F 3 and F 6 were found to be lower when comparedwith other formulations this may be due to higher concentration of soya lecithin.

The cumulative percentage drug release of formulations F 1, F 2 and F 3 were found to be 103.03±2.47, 91.92±2.72 and 82.12±2.51 respectively in 8 hours.

The formulation F 1 show faster release than formulations F 2 and F 3 due to the lower concentration of soya lecithin.

The cumulative percentage drug release of formulations F 4 was found to be 100.58 ± 1.58 at the end of 7 hours. And the cumulative percentage drug release of formulations F 5 and F 6 were found to 85.06±1.73 and 81.39±1.12 respectively in 8 hours. The formulation F 4 show faster release than formulations F 5 and F 6. While the concentration of soya lecithin was increase it decrease the release of drug.

The prepared liposomes F 1 to F 6 showed sustained release of drug. When increased ratio of soya lecithin also sustain the release of drug was increased in both method of preparations.

Stability Studies

Flucinolone acetonide liposomes for **F 1 – F 4** didn'tshow any characteristic changes after it was stored at 4ºC and 25ºC±2ºC for a period ofone month. F 5 and F 6 formulations were showed slightly reduced in the size after it was stored at 25ºC±2ºC for a period of one month but there was no changes for the same formulation when it was stored at 4ºC. Microscopic images of all the formulations(F 1 – F 6) of flucinolone acetonide liposomes were compared with before and after stabilitystability studies were shown in Figure

At storage condition 4°C showed better stability than another condition. This may due to their elevated temperature reduce the stability. But in both storage conditionhigher proportion of soya lecithin contains formulations like F3 and F6 showed betterstability than other their formulations.

Conclusion: This study concluded that flucinolone acetonide was successfully prepared as a liposomal drug delivery system by using two different techniques such as physical dispersion method and ether injection method. In this liposomes preparations, cholesterol ratio was constant and soya lecithin concentrations were gradually increased(like 1:1, 1:2 and 1:3). The liposomes prepared by physical dispersion method showedbetter percentage drug entrapment when compared with ether injection method. The particle size was analyzed by Malven particle size analyzer. The results of the particle size showed, when the concentration of soya lecithin was increased the size of the particle was reduced. The *in vitro* release showed that as the concentration of soya lecithin was increased the release rate of drug was retarded. Among the two methods ether injection method showed prolonged action when compared to physical dispersion method. The stability studies for all the formulations were performed by keeping the formulations at two different temperatures 4ºC±2ºC and 25ºC±2ºC for a period of 30 days. After the stability period the formulations were testedfor morphological analysis, percentage drug entrapment and *in vitro* drug release and compared with before stability study. There was no change in morphological charactersat 4ºC±2ºC, but there was a slight reduced in particles size at 25ºC±2ºC. The percentagedrug entrapment was reduced in all the formulations at both the conditions. The *in vitro*drug release was reduced for all the formulations. Liposomes prepared by physical dispersion method showed better stability compared with ether injection method.

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Table 1:Standard curve data of flucinolone acetonide using phosphate bufferpH 6.8

S. No.	Concentration (µg/ml)	Absorbance at 238 nm
1	2	0.189
2	4	0.370
3	6	0.524
4	8	0.699
5	10	0.858

Table No. 4 Formulation of Flucinolone acetonide liposomes

S. No.	Ingredients	Physical dispersion method			Ether injection method		
		F 1	F 2	F 3	F 4	F 5	F 6
1.	Cholesterol	100 mg	100 mg	100 mg	100 mg	100 Mg	100 mg
2.	Lecithin	100 mg	200 mg	300 mg	100 mg	200 Mg	300 mg
3.	Flucinolone acetonide	10 gm	10 gm	10 gm	10 gm	10 gm	10 gm
4.	Ether	-	-	-	7 ml	7 ml	7 ml
5.	Methanol	-	-	-	3 ml	3 ml	3 ml
6	Chloroform	5 ml	5 ml	5 ml	-	-	-
7.	Phosphate buffer pH 6.8	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Table No. 9 Stability study of percentage drug entrapment of liposomes flucinolone acetonide liposomes compared with percentage drug entrapment of immediately after preparation.

S. No.	Formulationscode	Immediately afterpreparation (%)	After one month	
			At 4°C	At 25°C±2°C
1.	F 1	86.61	85.93%	76.86%
2.	F 2	79.91 %	77.98%	70.97%
3.	F 3	73.11 %	72.07%	66.88%
4.	F 4	30.46%	29.36%	24.88%
5.	F 5	39.57%	38.43%	35.38%
6.	F 6	39.68%	38.35%	36.68%

Table No. 8 Cumulative percentage drug released of flucinolone acetonide from liposomes

S. No	Time (Mins)	F – 1	F - 2	F - 3	F – 4	F - 5	F – 6
1.	30	9.32±0.95	8.93±0.53	8.15±0.62	8.63±0.47	7.54±0.57	2.73±0.32
2.	60	15.75±0.58	12.18±0.62	11.23±0.81	16.64±0.68	13.74±0.36	5.33±0.93
3.	90	24.46±1.14	18.61±1.73	15.83±1.25	24.17±1.29	19.18±0.93	9.78±1.28
4.	120	32.71±2.53	25.23±1.48	20.77±2.42	31.47±1.87	26.47±0.72	15.74±0.58
5.	150	40.47±2.21	30.45±3.17	26.12±2.33	39.55±2.13	33.32±0.47	21.57±0.94
6.	180	45.48±1.84	36.57±3.53	30.61±2.45	47.32±2.31	38.17±0.44	26.57±0.43
7.	210	50.24±1.91	41.21±3.81	35.18±2.46	55.13±2.45	42.82±1.2	30.08±0.98
8.	240	57.44±1.73	47.64±3.86	38.48±2.62	62.45±2.33	47.43±2.57	33.32±1.52
9.	270	65.92±1.44	53.67±3.54	43.27±2.62	69.44±2.13	52.67±1.56	37.27±1.56
10.	300	74.01±3.12	58.44±3.01	46.97±2.39	76.55±1.48	57.28±1.37	42.41±2.0
11.	330	81.76±2.77	63.72±3.22	50.44±2.38	83.82±1.63	61.94±1.92	46.18±2.01
12.	360	88.03±2.82	69.83±3.55	55.84±2.36	91.05±1.56	66.52±1.35	51.42±1.87
13.	390	92.42±2.06	75.65±3.02	62.31±2.38	97.62±1.85	73.11±0.11	57.41±1.74
14.	420	95.84±2.12	80.71±2.62	69.24±2.52	100.57±1.57	79.12±1.62	64.05±1.54
15.	450	99.75±2.01	86.53±3.08	75.37±2.52	-	82.07±1.65	71.33±1.41
16.	480	103.02±2.46	91.93±2.71	82.13±2.52	-	85.05±1.7	79.04±1.04