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Formulation and Optimization of Ciprofloxacin Ocular Liposomal Hydrogel

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ABSTRACT:

Conventional ophthalmic dosage forms have lower bioavailability due to many factors like precorneal loss factor, tear dynamics, low residence time etc. Another reason for lower bioavailability is lower permeation of conventional dosage form. This research is based on formulation of liposomal suspension to increase the lipophilic cornea. This liposomal suspension was converted in to hydogel using carbopol 934 to increase residence time. Ciprofloxacin is broad – spectrum antibiotic effective against many Gram-positive and Gram-negative bacteria. Distearoylphosphatidylcholine (DSPC) is synthetic lipid which gives more stability to liposome as compare to natural lipid. The research work started with optimization of processing factors like CH: DSPC ratio, rotating speed, hydration time, drying time, hydration volume and drying temperature. Liposome characterization was done using scanning electron microscopy (SEM). Diffusion study was carried out for comparison between liposomal suspension and hydrogel.

Key words: Liposome, Hydrogel, Carbopol 934, DSPC

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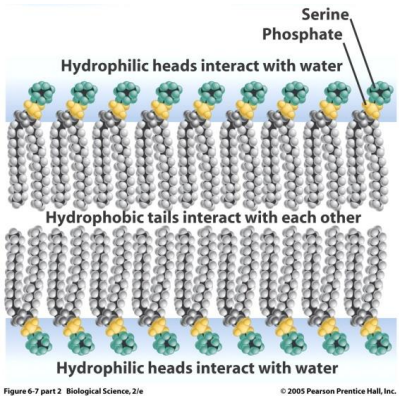
INTRODUCTION:

Eye is most interesting organ due to its disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy. Drugs administered by instillation must penetrate the eye and do so primarily the cornea followed by the non-corneal routes. These non-corneal routes involve drug diffusion across the conjunctiva and sclera and appear to be particularly important for drugs that are poorly absorbed across the cornea. Ophthalmic drugs are formulated to bring the active drugs in contact with the eye surface to allow for absorption. Extension of corneal contact time may result in increased drug penetration and higher intraocular drug delivery. In addition to the active drug, ophthalmic formulations should contain other ingredients to control various characteristics of the formulation, such as the buffering and pH, osmolality & tonicity, viscosity & antimicrobial preservatives. Although these ingredients are listed in active, they can affect permeability of drug across the ocular surface barriers & alter the therapeutic effectiveness of the drug.

Liposomes are spherical vesicles composed of lipid bilayers arranged around a central aqueous core. They can be composed of natural constitutes such as phospholipids and may mimic naturally occurring cell membrane. Liposome

has the ability to incorporate lipophilic amphiphilic drugs within their phospholipids membrane or them cam encapsulated hydrophilic compounds within the aqueous core. Liposome have several advantage such as ability to incorporate not only water soluble but also lipid soluble agents, specific targeting to required site in the body and versatility in term of fluidity, size, charge and number of lamellae. While the use of liposome as models for bio-membrane is confined to the research laboratory, their successful application in the entrapment and delivery of bioactive agent will depend not only on a demonstration of the superiority of the liposome carrier for the intended purpose, but also upon technical and economic feasibility of the formulation. Liposomal formulation has high entrapment efficiencies, narrow size distribution, long term stability and ideal release properties. Liposome can be formulated as a suspension, as aerosol, semisolid form such as a gel, cream or dry powder. In-vivo, they can be administered topically or perenterally. Liposome as a drug delivery system can improve the therapeutic activity and safety of drugs, mainly by delivering them to their site of action and by maintaining therapeutic drug levels for prolonged periods of time. Liposome also facilitates intracellular delivery via fusion with the plasma membrane, receptor-mediated endocytosis and phagocytosis.

The main components of liposome are phospholipids and cholesterol. Phospholipids are the major components of biological membrane such as the cell membrane. Two major type of phospholipids are phosphoglycerides and sphingholipids. The most common phospholipids used is phosphatidylcholine(PC). Phospholipids can be derived from natural and synthetic source, natural sources: Phosphotidylcholine, Phosphatidylethanolamine, phosphatidylserine. Synthetic sources: dioleoyl-phosphatidylcholine, Dioleoyl-phosphatidylethanolamine, Distearoyl-phosphatidylcholine, Distearoyl-phosphatidylethanolamine.



Incorporation of cholesterol in liposome bilayer brings about major changes in the preparation of membranes. Cholesterol by itself does not form a bilayer structure. However, cholesterol acts as a fluidity buffer, i.e. below the phase transition temperature, it makes the membrane less ordered and slightly more permeable; while above the phase transition temperature it makes the membrane more ordered and stable. cholesterol can be incorporated into phospholipids membranes in very high concentration up to 1:1 even 2:1 molar ratios of cholesterol to PC. Cholesterol inserts into the membrane with its hydroxyl groups oriented towards the aqueous surface and aliphatic chain aligned parallel to the acyl chains in the center of the bilayer. Cholesterol incorporation increases the separation between the choline head groups and eliminates the normal electrostatic and hydrogen-bonding interactions thus pushing the phospholipids apart making the layer less ordered at lower temperatures.

There are mainly two type of liposome preparation. Passive loading involves loading of the entrapped agents before or during the manufacturing procedure. Active loading involves introduction of certain types of compounds with ionizable group and those with both water and lipid solubility after formulation of intact vesicles.

Application of liposomes in pharmaceutical industry

Liposome utility	**Current Application**	**Disease State Treated**
Solubilization	Amphotericin B, Minoxidil	Fungal infections
Site Avoidance	Amphotericin B – reduced Nephrotoxicity, Doxorubicin – Decreased cardiotoxicity	Fungal infections, Cancer
Sustained release	Systemic antineoplastic drugs, Hormones, Corticosteroids, Drug depot in the lung	Cancer, Biotherapeutics
Drug protection	Cytosine arabinoside, interleukins	Cancer
Specific targeting	Cell bearing specific antigens	Wide therapeutic applicability
Liposome utility	Current application	Disease state Treated
Extravasation	Leaky vasculture of tumours,	Cancer, Bacterial infectioin

	Inflammation, Infections	
Accumulation	Prostaglandins	Cardiovascular diseases
Enhanced penetration	Topical vehicles	Dermatology
Drug depot	Lungs, Subcutaneous, Intramuscular	Wide therapeutic applicability
RES targeting	Immunomodulators, Vaccines, Antimalarial macrophages-located disease	Cancer, MAI, tropical parasites

Hydrogels are water swollen polymer matrices, with a tendency to imbibe water when placed in aqueous environment. This ability to swell, under biological conditions, makes it an ideal material for use in drug delivery and immobilization of proteins, peptides, and other biological compounds. Due to their high water content, these gels resemble natural living tissue more than any other type of synthetic biomateria1. These networks, have a three dimensional structure, crosslinked together either physically (entanglements, crystallites), or chemically (tie-points, junctions). This insoluble crosslinked structure allows immobilization of active agents, biomolecules effectively, and allows for its release in well-defined specific manner. Thus the kdrogels" biocompatibility and crosslinked structure are responsible for its varied applications.

Materials and methods

Material

Ciprofloxacin was procured on gratis from Sakar Healthcare (Ahmedabad, India). Cholesterol, Methyl paraben, Carbopol 934 was obtained from Astron Chemicals (Ahmedabad, India). Distearoylphosphatidylcholine was purchased from Lipoid, Germany.

Methods

Preparation of Liposomes:

Liposomes of Ciprofloxacin were prepared by thin lipid film hydration technique using rotary flask evaporator as described by method of Bangham, reported by Juliano and Daoud.

The two steps of liposomal suspension preparation by thin film hydration technique. In the first step, CH: DPSC in the proper ratio was dissolved in Chloroform. This solution was taken in round bottom flask. The flask was attached to rotary flask evaporator and rotated in it at appropriate speed for 30 minutes at 40°c temperature under vacuum. The organic solvent was slowly removed by this process such that a very thin, smooth and dry film of lipid was formed on the inner surface of the flask. The film was allowed to dry for one hour. In the second step, the dry lipid film was then slowly hydrated with aqueous phase containing Ciprofloxacin. The flask was once again rotated at the same speed as before for 1.5 hour at room temperature. Liposomal dispersion was left to mature overnight at 4°c to ensure full lipid hydration.

Preparation of liposomal hydogel

Conventional ophthalmic formulation are having lower bioavailability due to lo residence time. For conversion of liposomal suspension into hydrogel, gelling agent was used. Carbopol is best gelling agent which forms good gel in lower concentration. Carbopol 934 is widely used as gelling agent and which is permeated for ophthalmic delivery system. Appropriate amount of carbopol was dissolved in water. It was kept to allow hydration of carbopol. This solution was added in liposomal suspension and adjusts pH.

Experimental design

The ratio of cholesterol and DSPC was increased and the effect on encapsulated efficiency was studied using 3^2 full factorial designs. It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of hours and raw materials. Traditionally pharmaceutical formulations are developed by changing one variable at time and keeping other constant. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variable are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulation by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a variable and their interactions.

Particle size and particle size distribution

Most precise method to determine size of the liposome is by electron microscopy, since it allows to view each individual liposome and to obtain exact information about the profile of liposome population over the whole range of sizes. Unfortunately it is very time consuming and requires equipments that may not always be immediately available to hand. In contrast, laser light scattering (quasi-elastic laser light scattering) method (Malvern Zetasizer) is very simple and rapid to perform but having disadvantage of measuring an average property of the bulk of the liposome. All the methods require very costly equipments. If only approximate idea of size range is required then gel exclusion chromatographic techniques are recommended, since only expense incurred is that of buffers and gel materials.

Surface charge

A method using free flow electrophoresis is used to determine the surface charge of MLVs. A technique has been developed that separates extruded vesicles on the basis of their surface charge by electrophoresis on a cellulose acetate plate in a sodium borate buffer pH 8.8. The lipid samples (5 nmoles) are applied to the plate and electrophoresis is carried out at 4°c on a flat bed apparatus for 30 min at 18 V/cm. The plate is dried and the phospholipids are visualized by the molybdenum blue reagent. Liposome up to 0.2 µm in diameter can migrate on this support and with this technique as little as 2 mole % of charged lipids can be detected in a liposome bilayer. This sensitive assay should prove valuable for examining the charge heterogeneity in liposome preparation for following fusion between two populations of vesicles with different charge and for determining.

Percent Capture (entrapment)

It is essential to measure the quantity of material entrapped inside liposomes before the study of behavior of this entrapped material in physical and biological system, since the effects observed experimentally will usually be dose relates. After removal of unincorporated material by the separation techniques, one may assume that the quantity of material remaining is 100% entrapped, but the preparation may change upon storage. For long term stability test and for developing new liposome formulation or method of preparation, a technique is needed for separating free from entrapped material. In general two methods used are Mini column centrifugation and Protamine aggregation.

In Mini column centrifugation method, the hydrated gel (sephadex G-50) is filled in a barrel of 1ml syringe without plunger which is plugged with a whatman GF/B filter pad. This barrel is rested in a centrifuge tube. This tube is spun at 2000rpm for 3 min to remove excess saline solution from the gel. After centrifugation the gel column should be dried and have come away from the side of the barrel. Then, eluted saline is removed from the collection tube. Liposome suspension (0.2mL undiluted) is applied dropwise to the top of the gel bed, and the column is spun at 2000 rpm for 3 min. to expel the void volume containing the liposomes into the centrifuge tube. The elute is then removed and set aside for assay.

Lamellarity

The average number of bilayers present in a liposome can be found by freeze electron microscopy and by 31P-NMR. In the latter technique, the signals are recorded before and after the addition of broadening agent such as manganese ions which interact with the outer leaflet of the outermost bilayers. Thus, a 50% reduction in NMR signal means that the liposome preparation is unilamellar and a 25% reduction in the intensity of the original NMR signal means that there are 2 bilayers in the liposome. Nowadays, freeze fracturing electron microscopy has become a very popular method to study structural details of aqueous lipid dispersions.

Phase behaviour of Liposome

An important feature of lipid membrane is the existence of a temperature dependant, reversible phase transition, where the hydrocarbon chains of the phospholipids undergo a transformation from an ordered (gel) state to a more disordered fluid (liquid crystalline) state. These changes have been documented by freeze fracture electron microscopy, but most easily demonstrated by differential scanning calorimeter. The physical state of the bilayers profoundly affects the permeability, leakage rates and overall stability of the liposomes. The phase transition temperature (Tc) is a function of phospholipids content of the bilayers. The Tc can give good clues regarding liposomal stability, permeability and whether drug is entrapped in the bilayers or the aqueous compartment.

Drug release

The mechanism of drug release from the liposomes can be assessed by the use of a well calibrated *in-vitro* diffusion cell. The liposome based formulations can be assisted by employing *in-vitro* assays to predict pharmacokinetics and bioavailability of the drug before employing costly and time-consuming *in-vivo* studies. The dilution-induced drug release in buffer and plasma was employed as predictor for pharmacokinetic performance of liposomal formulations and another assay which determined intracellular drug release induced by liposomes degradation in the presence of mouse-liver lysosomes lysate was used to assess the bioavailability of the drug.

3^2 FULL FACTORIAL DESIGN LAYOUT

Batch code	X_1	X_2
F1	-1	-1
F2	-1	0
F3	-1	1
F4	0	-1
F5	0	0
F6	0	1
F7	1	-1
F8	1	0
F9	1	1

TRANSLATION OF CODED VALUE IN ACTUAL UNITS

Coded value	CH:DSPC (molar ratio) X_1	Rotating speed (rpm) X_2
-1	1:2	80
0	1:3	100
1	1:4	120

Result and discussion

Diffusion study

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	11.96	13.97	13.99	9.11	13.97	16.16	10.91	12.96	15.10
2	20.07	24.11	24.93	21.09	25.10	32.04	19.12	23.04	26.06
3	25.02	31.22	30.67	28.05	31.95	38.09	25.03	29.06	30.12
4	32.03	36.93	39.41	36.00	40.18	43.09	29.99	37.07	35.89
5	36.01	42.10	45.04	44.08	45.88	49.09	38.05	44.01	43.11
6	41.10	51.01	50.19	49.09	51.01	55.92	43.09	48.76	48.07

Comparison of diffusion for hydrogel and suspension:-

Hydrogel

Diffusion of batch F6 through cornea

Time (hr)	Absorbance	Concentration	Dilution factor	Concentration mg/ml	% Permeation
0	0	0	0	0	0
1	0.2124	2.0350	20	0.4070	27.13± 2.14
2	0.2981	2.9185	20	0.5837	38.91± 1.62
3	0.3497	3.4505	20	0.6901	46.01± 0.65
4	0.3934	3.9010	20	0.7802	52.01± 0.6
5	0.4368	4.3484	20	0.8697	57.98± 0.13
6	0.4879	4.8752	20	0.9751	65.00± 0.85

Suspension

Difussion of batch F6 through cornea

Time (hr)	Absorbance	Concentration	Dilution factor	Concentration mg/ml	% Permeation
0	0	0	0	0	0
1	0.2324	2.2415	10	0.241	14.94± 0.34
2	0.1964	1.8701	20	0.3740	24.93± 0.51
3	0.2481	2.4031	20	0.4806	32.04± 0.68
4	0.2842	2.7753	20	0.5551	37.00± 1.42
5	0.3214	3.1588	20	0.6318	42.12± 0.86
6	0.3648	3.6062	20	0.7212	48.08± 0.71

This may explained by that an electrostatic attraction force occur between liposome and negatively charged corneal membrane. We speculate then that liposomes, adsorbed to the corneal surface, transfer their membrane associated

drug delivery to the corneal epithelial cell membrane, there facilitating drug transport across the cornea. Other mechanism such as endocytosis of liposomes of fusion of liposome membrane with the membrane is also involved in enhanced transport. While liposomal hydrogel show higher permeation than aqueous solution, this may explained by the fact that mucoadhesive properties of hydrogel base ensure an intimate contact between liposomes and corneal membrane which prolong the retension of the formulation at the site of administration, which is beneficial for enhancing permeation.

Release kinetic study:-

Kinetic model	Regression	Slope	Intercept
Higuchi	0.999	25.34	0.744
Zero	0.991	7	21.2
Krosmeyer peppas	0.997	0.4907	-0.584
Hixon	-0.991	-2.409	26.226
First order	0.9673	0.073	1.393

The release kinetic was checked for the data obtained from diffusion study of liposomal hydrogel through cornea.

Value of slope in Krosmeyer peppas kinetic is 0.4907 which is less tha 0.5, so we can say that drug release is diffusion controlled. The value of regression co-efficient (r^2) in Highuchi model is near to 1. The diffusion from hydrogel is following higuchi model. The release from liposome must follow reservoir type release (Zero order), but with hydrogel there is effect of concentration gradient in initial period.

Parameter of optimized batch

% Entrapment efficiency	**71.67%**
% Permeation	65%
Release model	Higuchi model
Particle shape	Sphere
Particle size	2360nm
Viscosity	3160cp
pH	7.2±0.2

Stability study:-

Storage condition	Absorbance	Conc(µg/ml)	Dilution factor	Conc(µg/ml)	%EF
Refrigeration temperature	0.8391	8.6495	80	691.96	69.20± 1.25
Room temperature	0.523	5.3918	80	431.34	43.13± 0.84

Parameter after stability study

Parameter	Refrigeration temperature	Room temperature
Color	Not changed	Changed
Sedimentation	Not changed	Observed
Creaming	Not changed	Observed
Vesicle shape	Spherical	Non-uniform
Extent of leakage	1.64%	28.54%

It is observed from stability study that there is no significant change in sample which was stored at refrigeration temperature. But there was significant change in characteristic of sample which was stored at room temperature. It was concluded that the batches which was stored refrigeration temperature is stable while sample stored at room temperature is not stable.

Conclusion

Liposomal hydrogel containing Ciprofloxacin can be formed using Cholesterol and DSPC. Batches F1-F9 was prepared by applying 3^2 full factorial design. There was increased in entrapment efficiency with increase in rotating speed. The maximum entrapment efficiency was observed in batch F6 Which was prepared with 1:3 CH:DSPC ration and 120 rotating speed. Liposomal suspension was converted in to hydrogel using Carbopol 934 (0.7%w/w). Compariso of diffusion study was carried out between Liposomal suspension and 'liposomal hydrogel. Liposomal hydrogel was giving better permeation due to mucoadhesion with cornea. Release kinetic of liposomal hydrogel(Batch F6) was following Highuchi model.

References

1. Chaudhry NA, Flynn HW, Murray TG and Miller MO. Emerging ciprofloxacin resistant Pseudomonas aeruginosa, Am J Ophthalmic 1999; 128: 509-510.

2. Wagh VD, Inamdar B and Samanta MK. Polymer used in ocular dosage form & drug delivery system, Asian J Pharma 2008; 2: 12-17.
3. Sasaki H, Yamarapura K and Nakamurat J. Delivery of drug to the eye by topical application, Prog Retin Eye Res 1996; 15: 553-620.
4. Brandl M. Liposomes as drug carriers: a technological approach, Biotechnol Annu Rev 2001; 7: 59-65.
5. Gregoriadis G and Florence AT. Liposomes in drug delivery, Clinical, diagnostic and ophthalmic potential drugs, Trends Biotechnol 1995; 45: 15-28.
6. Sreekamth N, Rao CB and Mukkani K. Method Development and validation of Ropinirole hydrochloride in bulk and pharmaceutical dosage form, Int J of Pharmacy and Pharm Sci 2009; 1: 186-192.
7. Szoka F and Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse phase evaporation, Proc Natl Acad Sci 1978; 75: 4194-4198.
8. Szoka F and Papahadjopoulos D. Comparative properties and methods of preparation of lipid vesicles (liposomes), Annu Rev Biophys Bioeng 1980; 9: 467-508.
9. Taylor K, Taylor G, Kellaway I and Stevens J. Drug entrapment and release from multilamellar and reverse-phase evaporation liposomes, Inr J Pharm, 1990; 58: 4945.
10. Vadiei K, Lopez-Berestein G, Peres-Soler R and Luke D. Tissue Distribution and in vivo immunosuppressive activity of liposomal cyclosporine, Drug Metab Dispos 1991; 19: 1147-1151.
11. Papahadjopoulos, D, Gabizon A, Mayhew E, Matthay K and Martin FJ. Sterically stabilized liposomes: Improvement in pharmacokinetics and anti-tumor therapeutic efficacy, Proc Nutl Acad Sci 1991; 88: 11460-11464.
12. Gruner SM, Lenk RP, Janoff AS and Ostro M. Novel multilayered lipid vesicles: Comparison of physical characteristics of multilamellar liposomes and stable plurilamellar vesicles, Biochemistry 1985; 24: 2833-2842.
13. De Gier J, Mandersloot JG and Van Deenem L. Lipid composition and permeability of liposomes, Biochim Biophys Actu 1968; 150: 666-675.