



Novel Approach for Design and Characterization Of Muco Adhesive Buccal Ganciclovir *In Situ* Gel

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ABSTRACT

The present study was design to prepare periodontal gel of Ganciclovir for the treatment of inflammation condition. In this study, it was found that as the phospholipid concentration was increased, it resulted in corresponding increase in the entrapment efficiency of reconstituted liposomes. In conclusion, a sustained delivery of Ganciclovir can be achieved by proliposomal drug delivery system. Phospholipids, being the major component of liposomal system, can easily get integrated with the skin lipids and maintain the desired hydration conditions to improve drug permeation. Fusion of lipid vesicles with skin contributed to the permeation enhancement effect. In –vitro studies concluded that enhance skin permeation and retention of Ganciclovir was observed and was due to liposolubulized state of drugs with in proliposomes which helped to produce the depot effect. The prepared proliposomes are in incorporated in to gel. The data show that liposomal systems can make the drug molecule more accessible with in skin layers. This conclusion compares favourably with early study showed that drug associated with liposomes bi-layer bounds and better routed into skin. Other studies have shown that liposomal ambiaence may help modify the permeability characteristics of the stratum corneum, and the system keep the drug molecules with in skin layers so that sustain release of drug can be achieved. The results advocate the extension of this work on the preliminary clinical trails and commercialization of proliposomal gel formulation of anti viral drugs for effective topical pharmacotherapy in treatment of virus.

Keywords: Ganciclovir, Gellan gum , Mucoadhesive in situ gel

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INTRODUCTION

The unique environment of the oral cavity offers its potential as a site for drug delivery. Because of the rich blood supply and direct access to systemic circulation, the oral mucosal route is situated for drug, which are susceptible to acid hydrolysis in the stomach or which are extensively metabolized in the liver (first pass effect). The total area of the oral cavity is about 100 cm³. Out of this about one third is the buccal surface, which is lined with an epithelium of above 0.5 mm thickness. The oral mucosal surface is continuously washed by the saliva (daily turn out is about 0.5 to 2 liters).

Delivery through Buccal Mucosa:

Administration of a drug via the buccal mucosa (the lining of the cheek) to the systemic circulation is defined as buccal delivery. Despite, the buccal mucosa is significantly less permeable than the sublingual mucosa and usually not able to provide rapid drug absorption or good bio availability, it is relatively more permeable than sublingual floor makes it more desirable site for sustained drug delivery. Apart from avoiding enzymatic degradation and first pass metabolism, the non acidic conditions and lipophilic nature of the buccal tissue provide potential and promises for successful delivery of peptide and proteins.

The various strategies Employed for Buccal Delivery

*Bio adhesive buccal Gels

Bioadhesive buccal Gels:

Viscous adhesive gels have been designed for local therapy using polyacrylic acid and polymethacrylate as gel forming polymers. Gels are reported to prolong residence time on the oral mucosa to a significant level. This not only improves absorption but also allows for sustained release of the active principle.

Include solutions, suspensions, gels, micro particles, suppositories, creams, foams and tablets. And all have relatively short contact time. Robinson et al reported on a system of treatment using a gel containing the mucoadhesive epolycarbophil that remained on vaginal tissue for 3-4 days and hence served as a platform for delivery of drug such as progesterone.

MUCOADHESIVE DRUG DELIVERY

The potential route of buccal mucosal route of drug administration was first recognized by Walton and others reported in detail on the kinetics of buccal mucosal absorption.

Mucoadhesion, or the attachment of a natural or synthetic polymer to a biological substrate, is a practical method of drug immobilization or localization and an important new aspect of

controlled drug delivery. The unique environment of the oral (buccal) cavity offers its potential as a site for drug delivery. Because of the rich blood supply and direct access to systemic circulation. The Buccal route is suitable for drugs, which are susceptible to acid hydrolysis.

LIPOSOMES

- Liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. The water soluble compounds/drugs are present in aqueous compartments while lipid soluble compounds/drugs and amphiphilic compounds or drugs insert themselves in phospholipid bilayers.
- The liposomes containing drugs can be administered by many routes (intravenous, oral inhalation, local application, ocular) and these can be used for the treatment of various diseases.
- For drug delivery applications liposomes are usually unilamellar and range in diameter from about 50 - 150 nm.
- Depending on the lipid composition, methods of preparations and the nature of the encapsulated agents, many types of liposomal products can be formulated.

Advantages of liposomes

- Provides selective targeting to tumour tissues.
- Increased efficiency and therapeutic index.

Disadvantages of liposomes

- Liposomes are bio compatible.
- Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions, yet do not cause undesirable side reactions.

Preparation methods:

The formulation of an appropriate liposomal system as a carrier for a given drug is dependent on the type of the lipid used and the method of preparation.

Ethanol Injection Method:

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol.

Ether Infusion Method:

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature.

Reverse Phase Evaporation Method:

First water in oil emulsion is formed by brief sonication of a two phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure.

Lipid Hydration Method:

This is the most widely used method for the preparation of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time.

Freeze-Thaw Method:

SUVs are rapidly frozen and followed by slow thawing. The brief sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is due to the fusion of SUV during the processes of freezing and or thawing.

Solvent Spherule Method:

The process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath

Applications of liposomes

- (1) Liposomes act as carriers for drug both in vitro and in vivo.
- (2) In targeted drug delivery system, liposomes are used as carriers of anticancer drug such as methotrexate, actinomycin- D etc.
- (3) Liposomes encapsulation has been used as a step in enzyme purification.

DRUG PROFILE

Drug name : Ganciclovir

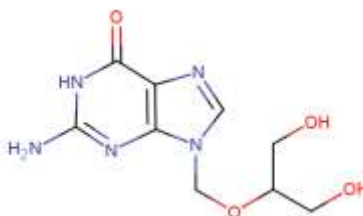
IUPAC name : 2-amino-9-[[[1,3-dihydroxypropan-2-yl)oxy]methyl]-6,9-dihydro-- purin - 6- one.

Synonyms : Ganciclovir , GA2, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine,

Solubility : Soluble in 0.1M HCl(10mg/ml), DMSO (5mg/ml), water(2 mg/ml), hot methanol , and ethanol (<1mg/ml).

Melting point : 250°C

Structure :



Molecular formula : C₉H₁₃N₅O₄

Molecular weight :Average: 255.2306 Monoisotopic: 255.096753929 g/mol.

Bioavailability : 5%(oral)

Half-life : 2.5 to 3.6 hours

Protein binding : 1 to 2%

Dosage forms : injection, gel, powder, capsule.

Dose : 250, 500mg. 500mg/10ml.

Category :Antiviral Agents

MATERIALS AND METHOD

The following materials are used for project work

S.No.	Materials	Source
1	Ganciclovir	A gift sample from Dr. Reddy's laboratories, Hyderabad. AP.
2	Mannitol	SD Fine-Chem. Pvt., Mumbai, India.
3	Cholesterol	SD Fine-Chem. Pvt., Mumbai, India.
4	Phosphotidyl choline	Dr. Reddy's laboratories, Hyderabad, AP.
5	Chloroform	Merk laboratories, Mumbai
6	Methanol	Merk laboratories, Mumbai
7	Gellan gum	Yaro chemicals, Mumbai
8	Triethanolamine	Fisher scientific, Mumbai

List of chemicals used with supplier

Instruments Used:

S.No.	Instrument name	Manufacturer	Model
1	UV spectrophotometer	Lab India	32000
2	Rota evaporator	Helidopath	Sonics-569-00050-00-0
4	Optical microscope	Metzer	5000DTM
5	Viscometer	Brook field	LVDV II pro+
6	Magnetic stirrer	Remi	1ML

7	Zeta sizer	Malvern	
8	Hot air oven	Navtug	D5247
9	Weighing balance	Shimadzu	Apx224
10	Water purifier	Labindia	SGLABOSTAR3TWF
11	Scanning electron microscope		
12	P ^H meter	Lab India	SAB 5000
13	FTIR	Brucker	Alpha-T
14	KBR Pellet Press	Horizon	WC-56
15	Ultra Sonicator	Citizen	CD4520
16	Cooling centrifuge	REMI	TR-01
17	Homogenizer	REMI	RQT-124A

List of Equipments used

METHODOLOGY:

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 forms.

Objective

The overall objective of preformulation testing is to generate information useful to the formulation in developing stable and bioavailable dosage forms.

Scope

The use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product.

Determination of absorption maxima:

Absorption maxima is the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.

Procedure:

For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed drug in 100ml of methanol (1mg/ml). Further 1ml of the stock solution was pipette out into a 100 ml volumetric flask and volume was made up with phosphate buffer (7.4 PH). From this stock solution pipette out 1ml and dilute to 10 ml with phosphate buffer and subject for UV scanning in the range of 200-400 nm using double beam UV spectrophotometer. The absorption maxima was obtained at 270 nm with a characteristic peak.

Drug-excipients interaction studies by FTIR :

The compatibility between pure drug and mannitol, phosphatidyl choline, cholesterol were detected by FTIR (Bruker Alpha- T) spectra. The potassium bromide pellets were prepared on

KBR press (Horizon WC-56). To prepare the pellets the solid powder sample were ground together in a mortar with 100 times quantity of KBR. The finely grounded powder introduced into a stainless steel die. The powder was pressed in the die between polished steel anvils at a pressure of about 10t/in². The spectra's were recorded over the wave number of 4000 to 600 cm⁻¹

Preparation of Ganciclovir loaded proliposomes:

Proliposomes could be prepared by many methods including

- Film deposition on carrier method
- Crystal film method
- Fluidized bed method
- Powder bed grinding method
- Freeze drying method
- Spray drying method

Based on the laboratory conditions film-deposition on the carrier method was chosen to prepare Ganciclovir proliposomes.

Variables for optimization of proliposomes:

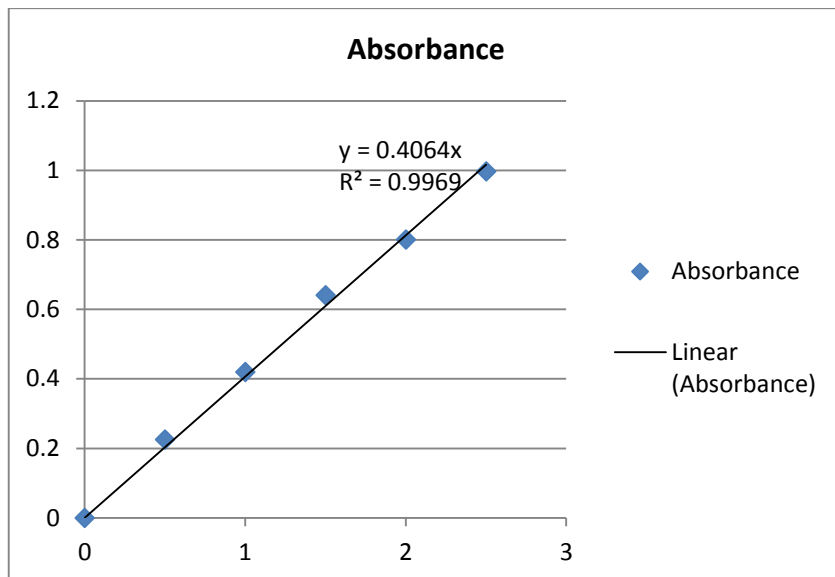
- Mannitol
- Phosphatidyl choline
- Cholesterol

RESULTS AND DISCUSSION

Preformulation studies:

Melting point determination: Melting point of Ganciclovir was determine by capillary method. Melting point of Ganciclovir was found to be in the range of 152-155⁰C which compiles the standards thus indicating that purity of the drug sample.

Construction of calibration curve using phosphate buffer (P^H 7.4)

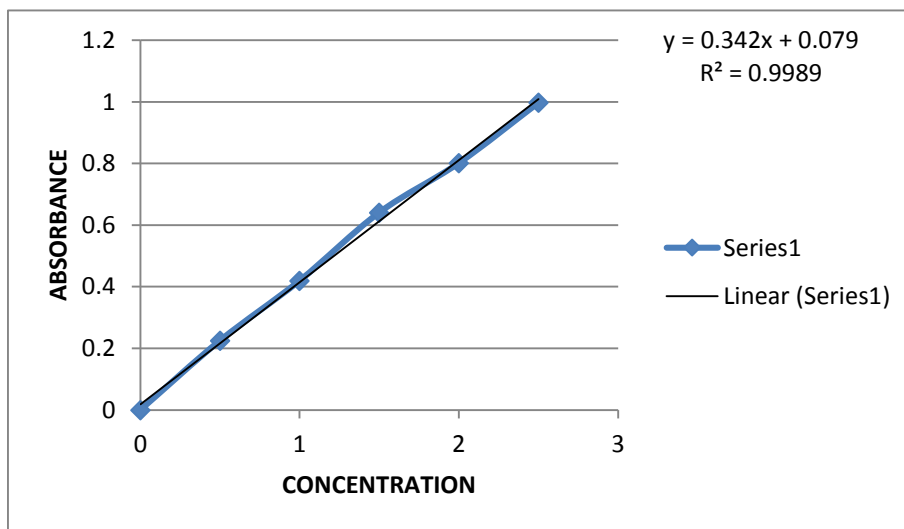


λ max of drug in phosphate buffer (PH 7.4)

Standard calibration curve of drug in phosphate

S.No	Concentration	Absorbance
1	0.5	0.2251
2	1	0.4195
3	1.5	0.6407
4	2	0.8013
5	2.5	0.9971

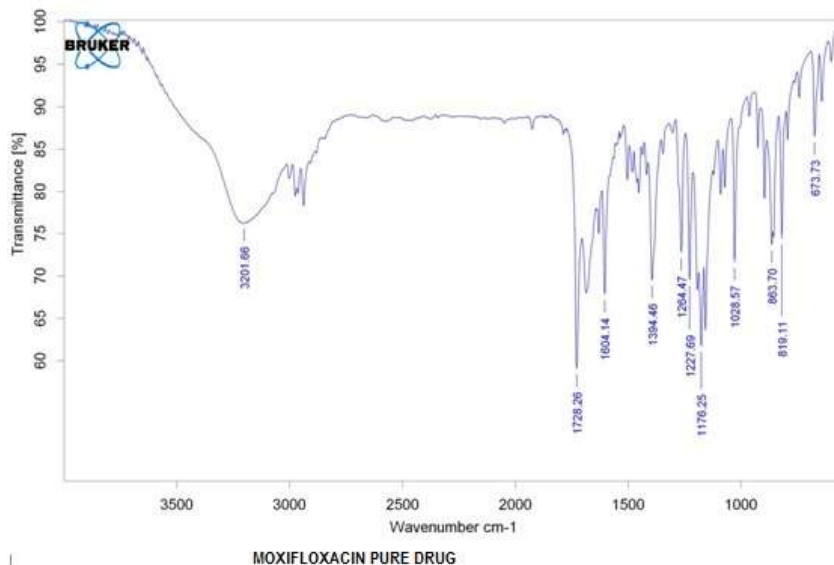
Buffer (PH 7.4)



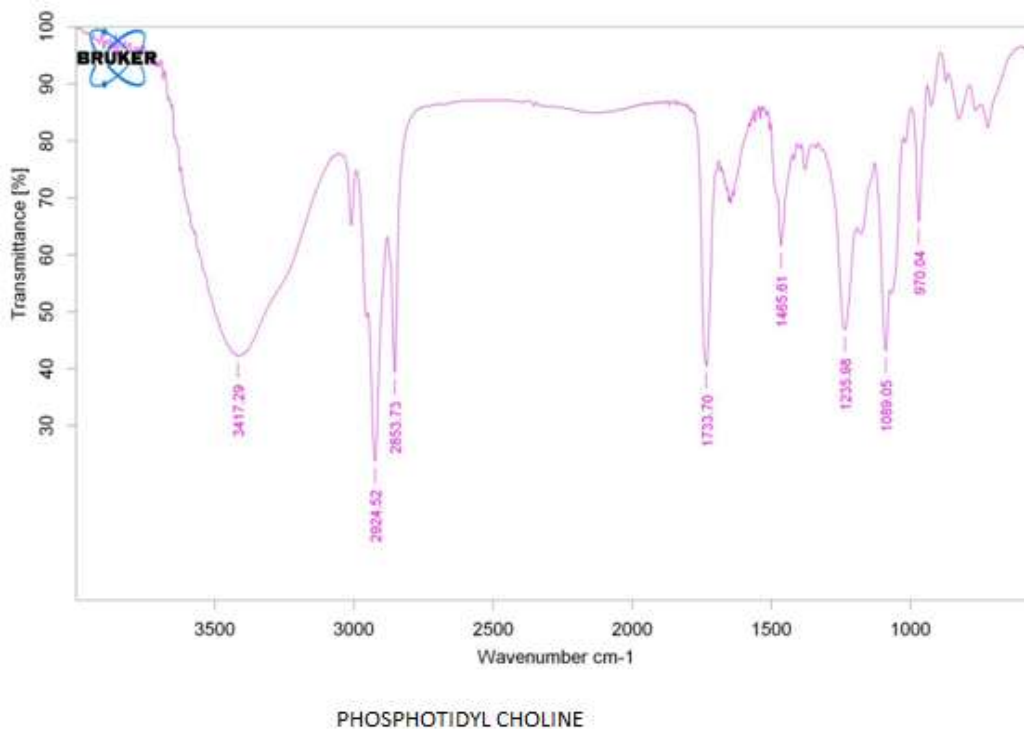
Standard curve of Ganciclovir in phosphate buffer (PH 7.4)

Drug- excipient compatibility studies using FTIR:

IR spectra's for pure drug as well as excipients used mannitol, phosphotidyl choline and cholesterol and also proliposomal formulation were recorded and compared to identify possible interaction.



FTIR spectra of Ganciclovir pure drug



FTIR spectra of Phosphotidyl choline

S.NO	Wave number in formulation (cm ⁻¹)		Characteristic Wave number range (cm ⁻¹)	Bond nature and bond attributed
	Pure drug	Optimized formulation		
1	3201	3404	3300-2500	O-H stretching Carboxylic acids
2	1720	1753	1760-1690	C=O stretching Carboxylic acids
3	1600	1422	1600-1400	C-C stretch in ring aromatics

4	1264	1282	1320-1000	C-O stretch Esters
5	883	880	900-675	C-H oop aromatics
6	673	680	1000-650	=C-H bend Alkenes

FTIR data interpretation

On comparison of IR spectra of proliposomes, pure Ganciclovir drug, mannitol, cholesterol and phospholipid it was clear that, there was no significant interaction of encapsulated drug with the phospholipid and water soluble solid support (mannitol) with formulations

Drug content of proliposomal formulations

S.No	Formulation	%Drug content \pm SD
1	F1	95.03 \pm 0.543
2	F2	86.4 \pm 0.734
3	F3	93.7 \pm 0.664
4	F4	96.8 \pm 0.249
5	F5	94.7 \pm 0.984
6	F6	94.8 \pm 0.860
7	F7	92.4 \pm 1.70
8	F8	90.6 \pm 0.748
9	F9	87.5 \pm 0.953

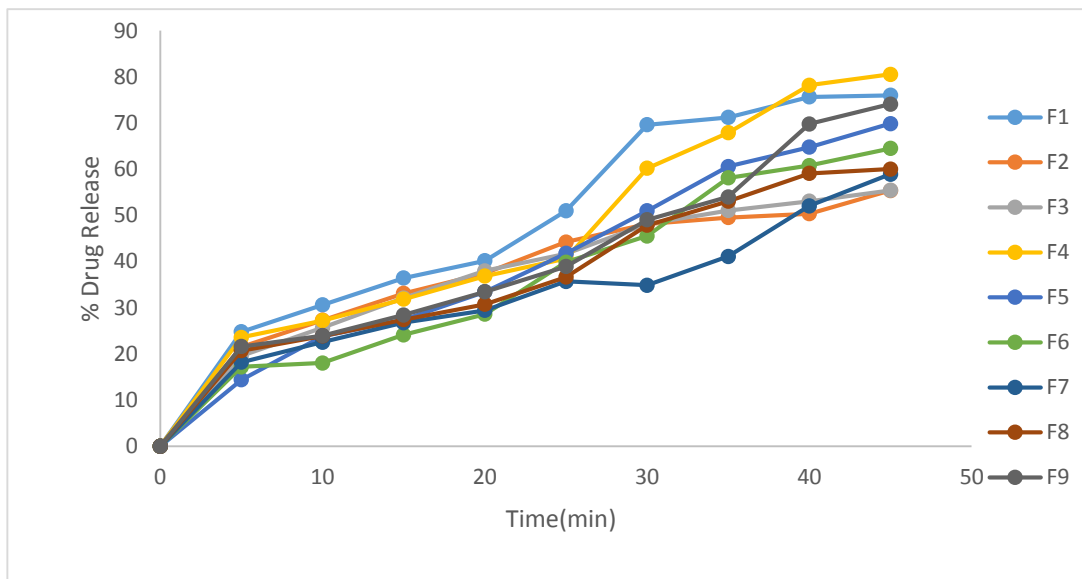
The Ganciclovir content in the proliposomes were observed in the range of 86.4% to 96.8% at various drug to phospholipid ratios. From the above results it is concluded that F4, F1, F5 and F6 formulations showed maximum drug content when compare to other formulations

In -vitro studies:

The cumulative amount of drug release of various proliposomal formulations and conventional (Conventional) Ganciclovir gel after 16hrs were shown in table

Cumulative percentage drug release of proliposomal formulations

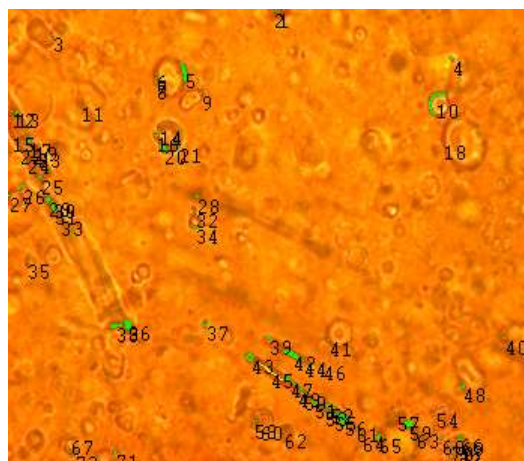
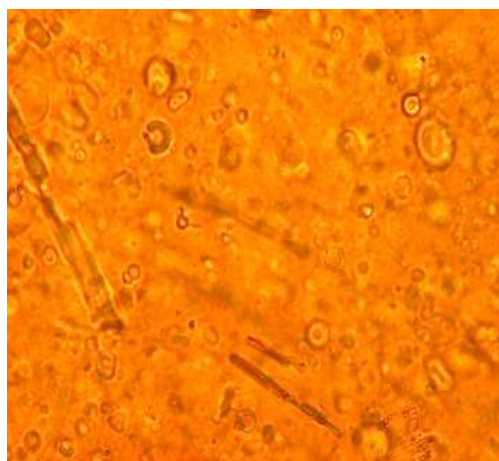
Time(MIN)	% Drug Release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	24.8	21.5	19.6	23.6	14.4	17.22	18.22	20.7	21.6
10	30.61	27.31	25.66	27.22	23.8	18.04	22.56	23.92	23.98
15	36.43	33.12	32.12	31.87	27.45	24.15	26.78	27.45	28.45
20	40.2	37.48	38.02	36.87	33.45	28.6	29.45	30.78	33.45
25	50.98	44.23	41.67	40.67	41.77	39.87	35.71	36.67	38.95
30	69.65	48.13	48.15	60.22	50.98	45.56	34.87	47.89	49.07
35	71.22	49.55	51.06	67.89	60.61	58.15	41.12	53.08	54.02
40	75.66	50.38	53.07	78.2	64.77	60.79	52.06	59.1	69.81
45	76.01	55.4	55.44	80.55	69.88	64.5	58.94	60.05	74.1



Diffusion data of various proliposomal formulations and conventional

From the above results f4 formulation has selected as optimized one which is used for further study.

Particle size count and distribution:



Particle size distribution of optimized formulation

Determination of zeta potential

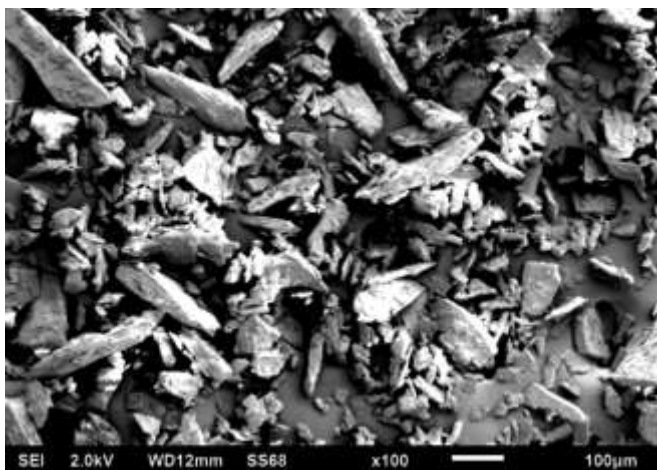
Zeta potential was measured by using zeta sizer (Melvern, INDIA) and was found to be -16mv which is sufficient to avoid aggregation of vesicles. From the reports of zeta sizer vesicle size was found to be 990nm.

Zeta size distribution by intensity

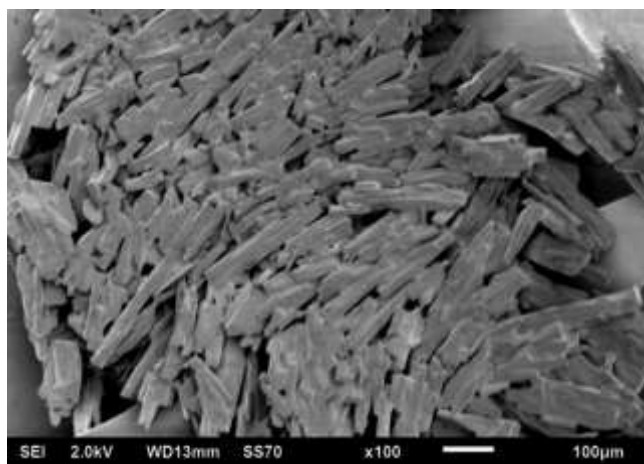
Results	Peaks	Diameter(nm)	% Intensity	Width (nm)
Z-average(d. nm) -990	Peak 1	91.50	93.3	13.07
PDI-0.82	Peak 2	13.91	6.7	1.404
Intercept-0.432	Peak 3	0.000	0.0	0.00

Surface morphology:

The surface morphology of proliposome granules, pure drug and mannitol granules was examined by scanning electron microscopy (SEM) and the images were photographed at 100 resolution.



SEM image of Ganciclovir



SEM image of Ganciclovir proliposomes

The surface morphology of proliposome granules and plain mannitol granules were examined by scanning electron microscopy. The surface morphology of proliposome powder was different as compare to plain mannitol powder as shown in SEM. From SEM photographs it is clear that, the surface of mannitol crystals becomes illegible due to deposition of phospholipid on mannitol surface.

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itraconazole were prepared different concentrations of carbopol934 and in combination with different viscosity-enhancing agents such as hydroxypropyl methylcellulose (HPMC), xyloglucan and hyaluronic acid to formulate a pH-triggered system that undergoes sol-to-gel transition on change in pH to the physiological pH . The formulations were evaluated for physical appearance , pH , gelation capacity , rheological properties , spreadability ,muco adhesive force , and in vitro release of drug

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