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R E S E A R C H A R T I C L E

Formulation and In-Vitro Evaluation of Ganciclovir Liposome

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A B S T R A C T

Ganciclovir, is an antiviral medication used to treat cytomegalovirus (CMV) infections. In the present work an attempt was being made to formulate and evaluate ganciclovir liposomes. Phosphatidylcholine, Cholesterol, Methanol and Chloroform were used as excipients in the preparation of liposomes. The drug and excipient compatibility was studied by using FTIR. Prepared liposomes of ganciclovir were incorporated into a gel using Carbopol 934. The developed formulation was evaluated for drug entrapment efficiency, pH measurement, in vitro drug release, drug content. F8 formulation shown maximum drug release which is considered as the optimized formulation.

Keywords: Ganciclovir, Liposomes, Phosphatidylcholine, Cholesterol.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Liposomes are one of the promising drug delivery systems, which are widely investigated for targeted drug delivery .They are lipid bilayer vesicles, which are composed mainly of phospholipids and cholesterol. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation . It also has affinity to keratin of horny layer of skin and can penetrate deeper into skin and hence give better absorption. In the formulation of topical dosage forms, attempts are being made to utilize drug carriers that ensure adequate localization or penetration of drug within or through the skin in order to enhance the local and minimize the systemic effects or to ensure adequate percutaneous absorption. Applied on the skin, liposomes may act as a solubilizing matrix for poorly soluble drugs, penetration enhancer as well as local depot at the same time

diminishing the side effects of these drugs. Topical liposome formulations could be more effective and less 6 toxic than conventional formulations.

2. Materials and Methods

Ganciclovir, Cholesterol, Phosphatidylcholine, Chloroform, Methanol, Carbopol 934, Triethanolamine all the chemicals were laboratory grade.

Preparation of Ganciclovir liposome:

Based on the laboratory conditions film-deposition on the carrier method was chosen to prepare Ganciclovir liposome. The Ganciclovir liposome was prepared by film deposition on carrier method using vacuum rotary evaporator (Helidopath - Sonics-569-00050-00-0). There are various process variables which could affect the preparation and properties of the liposomal. The optimization of Ganciclovir liposome was done by preparing the different formulations by varying the concentration of cholesterol was placed in 100ml round bottom flask which was held at 60-70^{0}C temperature and the flask rotated at 80-90 rpm for 30 min under vacuum. After complete drying the temperature of water bath was lowered to 20-30^{0}C. Ganciclovir (10 mg), cholesterol and Phosphatidylcholine were dissolved in mixture of organic solvents (chloroform: methanol, 6:4,v/v) and 5ml of aliquot of organic solution was slowly introduced into the flask via the solvent inlet tube. After complete drying second aliquot (5ml) was introduced. After complete dying, the vacuum was released and liposome were placed in a desiccator over night and then sieved with 100mesh. The collected powder was transferred into a glass bottle and stored at the freeze temperature.

Preparation of carbopol gel base:

1gm of carbopol 934 was weighed and dispersed in distilled water. Then, propylene glycol was added and the mixture was neutralized by drop wise addition of 1% triethanolamine Mixing was continued until the transparent gel was obtained and allowed to swell for 24 hours.

Preparation of liposomal gels: Prepared liposomes were incorporated into 2% carbopol gel.

Characterization of liposomal gel

Liposomal gel were characterized by following parameters like Surface morphology, Drug content, Entrapment efficiency, Yield of liposomal. Gel base was evaluated for following parameters for both plain gel and gel loaded with liposomal Physical appearance, pH of formulation, Rheological properties, Homogeneity, Drug Content, In-vitro studies, Franz diffusion cell was used for the in vitro drug release studies.

3. Results and Discussion

Table 2: Standard calibration curve of drug in phosphate Buffer (pH 6.8)

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

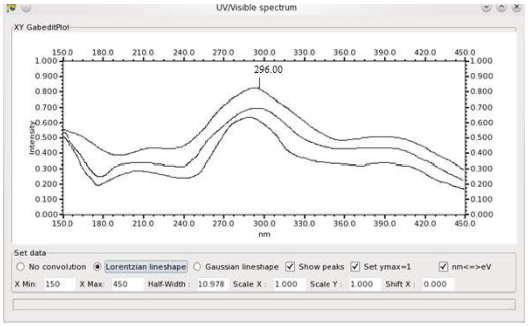

Fig 1: UV Absorption maxima

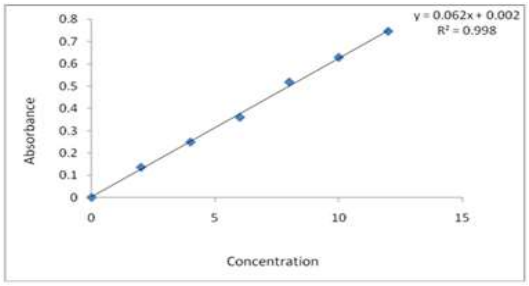

Fig 2: Standard curve of Ganciclovir in phosphate buffer (pH 6.8)

Characterization of liposomes and liposomal gels:

Determination of entrapment efficiency: Determination of entrapment efficiency is an important parameter in case of liposomes as it majorly effects the drug release and skin deposition. Entrapment efficiency is expressed as the fraction of drug incorporated into liposomes relative to total amount of drug used. A positive correlation was observed for both variables Phosphatidylcholine and cholesterol. Results show that with increase in the concentration of Phosphatidylcholine and cholesterol entrapment efficiency found to be increased. In the present study, the observed entrapment efficiency for all batches of Ganciclovir liposome formulation in the range of 72 to 90%. Among all Ganciclovir liposomal formulations F1-F9 had maximum vesicle size and entrapment efficiency which were selected for the further study.

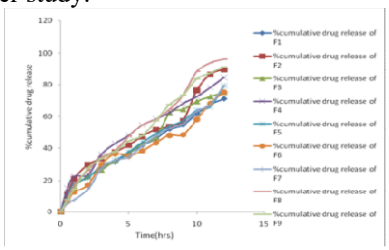

Fig 3: Diffusion data of various liposomal formulations

From the above results F8 formulation has selected as optimized one which is used for further study.

Surface morphology:

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

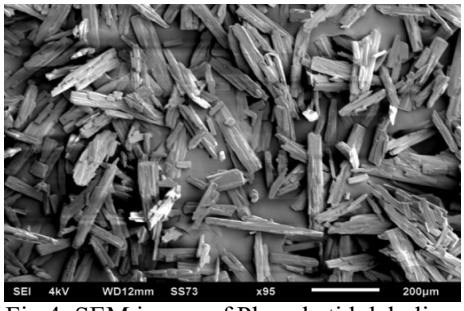

Fig 4: SEM image of Phosphatidylcholine

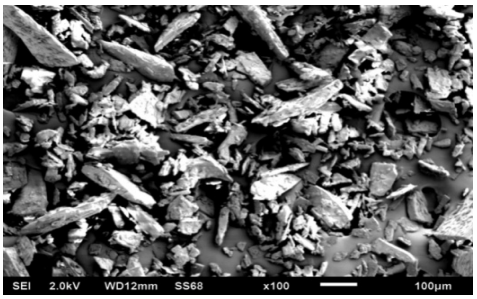

Fig 5: SEM image of Ganciclovir

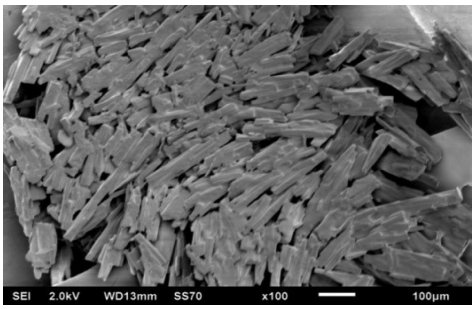

Fig 6: SEM image of Ganciclovir liposomes

The surface morphology of liposome granules and plain Phosphatidyl cholinegranules were examined by scanning electron microscopy. The surface morphology of liposome powder was different as compare to plain Phosphatidylcholine s powder as shown in SEM. From SEM photographs it is clear that, the surface of Phosphatidylcholine s were clear.

Viscosity measurement: Viscosity of the gel was measured by Brookfield viscometer (LVDV II pro+). Viscosity of liposomal gel showed 1156cps at 100rpm.

P^H measurement:

The P^H of the developed formulation was in accordance with human skin P^H rendering them more acceptable. Therefore formulated liposomal gel was suitable for topical application. The P^H values of prepared liposomal gels were within the limits of 5.5 to 5.8.

Release kinetics:

Various mathematical models were selected to evaluate the kinetics and mechanism of drug release from liposomal gel formulation. Best model was selected for release data which showed high correlation coefficient (r) value. In-vitro drug release over semi permeable membrane and skin was performed and release kinetics was calculated.

Release kinetic graphs of optimized formulation:

The mechanism of release for the optimized liposomal formulation based on regression coefficient (R^2) value. For most of the liposomal formulation the R^2 value nearer to1. Hence it can be concluded that the drug release follow peppasmodel. The n value of peppas model of the liposomal formulations are in the range of 0.1 to 0.5 which confirms that release of liposomal formulation was fickian diffusion.

4. Conclusion

Breast cancer is the most common cancer in women worldwide. In 2011, an estimated 230,000 women were diagnosed with breast cancer in the U.S. alone, with an estimated 40,000 deaths, making it the second most common cause of cancer related death in women. Most women with breast cancer will have some type of surgery to remove the tumor. Initial investigations for breast cancer begin with a physical examination, mammography and ultrasound scan. In some cases, breast magnetic resonance imaging (MRI) will also be performed. If a tumour is found, a biopsy will be taken to assess the cancer before any treatment is planned. Depending on the type of breast cancer and how advanced it is, you might need other types of treatment as well, either before or after surgery, or sometimes both. The mainstays of breast cancer treatment are surgery, radiation, chemotherapy, hormone therapy, and targeted therapy. But scientists continue to study novel treatments and drugs, along with new combinations of existing treatments.

Table 3: Entrapment efficiency of liposome formulations

S.No	Formulation	Entrapment efficiency
1	F1	94.7
2	F2	85.11
3	F3	91.03
4	F4	96.3
5	F5	92.7
6	F6	94.1
7	F7	88.1
8	F8	89.2
9	F9	86.02

Table 4: Drug content estimation

S.No	Formulation	%drug content
1	F1	95.4
2	F2	86.7
3	F3	93.8
4	F4	96.9
5	F5	94.2
6	F6	94.5
7	F7	92.2
8	F8	90.5
9	F9	87.3

The Ganciclovir content in the liposomes were observed in the range of 86.4% to 96.8% at various drug to Phosphatidylcholine ratios.

Table 5: Percentage yield of liposomal formulations

S.No	Formulation	Percentage yield
1	F1	93.8
2	F2	90.6
3	F3	89.3
4	F4	95.2
5	F5	94.5
6	F6	94.4
7	F7	88.8
8	F8	89.4

Percentage yield for F1 – F9 formulations was found to be with increase in the Phosphatidylcholine concentration. The % yield of formulations was found to be increase with increase in Phosphatidylcholine concentration. The results of % yield of various formulations were found to be in the range of 86.2 -95.2 % as the drug to Phosphatidyl choline ratio in liposomes was changed.

Table 6: Cumulative percentage drug release of liposomal formulations

Time (Hrs.)	F1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9
0	0	0	0	0	0	0	0	0	0
0.5	7.67	11.16	7.22	15.23	9.31	6.32	5.31	7.41	7.61
1	17.41	21.15	16.35	22.56	18.25	12.52	7.55	17.38	14.55
2	22.24	29.76	22.57	23.57	21.75	17.31	14.31	26.31	27.65
3	28.24	33.45	26.54	36.21	29.24	29.87	27.44	34.31	33.44
4	31.41	37.54	32.34	42.84	32.44	36.47	32.57	38.55	37.84
5	36.87	41.87	36.89	47.81	37.57	35.21	34.44	47.31	44.34
6	41.77	47.22	42.68	54.77	43.54	38.24	41.45	54.87	47.61
7	45.89	51.67	46.78	58.54	48.76	43.25	47.41	58.44	57.44
8	51.87	53.84	62.32	62.57	53.87	47.88	52.41	64.67	67.64
9	54.84	57.54	64.58	68.34	57.42	48.15	55.37	73.54	73.44
10	61.87	76.42	69.47	72.51	63.89	58.34	63.34	88.44	83.64
11	67.76	86.41	72.54	78.31	66.34	68.24	67.31	94.21	87.34
12	71.24	89.61	75.24	84.56	79.24	75.32	79.44	96.35	91.54

Table 7: Correlation coefficients (R2) values of different kinetic models

Cumulative (%) RELEASE Q	TIME (T)	ROOT (T)	LOG (%) Release	LOG (T)	LOG (%) Remain
0	0	0			2.000
7.41	0.5	0.458	0.870	1.987	1.967
17.38	1	1.000	1.240	0.000	1.917
26.31	2	1.414	1.420	0.301	1.867
34.31	3	1.732	1.535	0.477	1.817
38.55	4	2.000	1.586	0.602	1.789
47.31	5	2.236	1.675	0.699	1.722
54.87	6	2.449	1.739	0.778	1.654

58.44	7	2.646	1.767	0.845	1.619
64.67	8	2.828	1.811	0.903	1.548
73.54	9	3.000	1.867	0.954	1.423
88.44	10	3.162	1.947	1.000	1.063
94.21	11	3.317	1.974	1.041	0.763
96.35	12	3.464	1.984	1.079	0.562

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