Formulation and *In-vitro* characterization of Verapamil hydrochloride Ufasomes

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ABSTRACT

Verapamil Hydrochloride is a Cardio-Vascular calcium channel blocking agent preferably used to treat high blood pressure and Cardiac Arrhythmia. On its oral administration 90% is absorbed and only 20-30% of the drug is available systemically. The present aim of the work is to prepare the suitable dosage vehicle for the transdermal delivery of Verapamil Hydrochloride. The ufasomes are proved to be good vesicles for the delivery of the drugs through transdermal route. They are effective in transporting the drug through stratum corneum which is superficial barrier in the transdermal drug delivery. Oleic acid is indulged for the formation of drug vesicles by thin film hydration technique. Other, excipients used are cholesterol and glyceryl mono stearate. The prepared Ufasomes are evaluated for different parameters like Entrapment efficiency, Zeta potential, Poly dispersity index, TEM studies, *In-vitro* diffusion and Drug release kinetics. The drug release studies of all formulations were conducted for 12 hours. Among the 9 formulations F5 have the better drug entrapment efficiency of 74.3% and mean particle size of 321.6 nm of drug release of 79.40% at end hour. By this we conclude that formulation F5 was chosen as the best formulation. Kinetic release study of release data in best curve-fitting method of drug Verapamil hydrochloride shows Zero order kinetics stating that the release of the drug from dosage form doesn't depends on its concentration.

Keywords: Verapamil Hydrochloride, Cholesterol, Glyceryl mono stearate, Oleic acid, thin film hydration technique, Ufasomes and %CDR.

1. INTRODUCTION

Topical and transdermal application of active pharmaceutical constituents to the skin is most up took manoeuvre by formulation developers to treat diseased states diminishing the oral drug delivery. Though the drug delivery across the skin is the beneficial, most desired and reputed one it's too the hurdle some path for the drug delivery, as there is a barrier for the drug delivery through the external veil which is the stratum corneum[1].In the field of the dermal or transdermal drug delivery the skin represents the application site and sometimes also the target, but it is the main

obstacle for efficient drug and/or carrier penetration. The main barrier is the so-called horny layer, or stratum corneum[2]. Stratum corneum is the top most layer of the skin which is exposed to external environment. This layer is the barrier in the permeation of the drugs which has less lipophilicity as complies with its lipophilic nature ultimately results in less permeation of drug through the stratum corneum of the skin[3]. Notably, the pathway of the drugs (dermal or transdermal) involves a couple of steps, the drug has to get deposited at sufficient concentrations near the stratum corneum, followed by diffusion into the living epidermis[4] further the drug has to pass through the dermis, and finally reach the systemic circulation.

Lipid mediated vesicular systems are carriers for the travel of drugs passing the stratum corneum dermal drug delivery and thereby reaching into the bloodstream for systemic action transdermal drug delivery. These systems are multi rated from conventional dosage forms[5]. These differ with the conventional forms as they are not strengthened to possess certain traits such as transportation through various barriers and drug holding capacity. On designing the lipid vesicular drug systems grew a newer perception of disease treatment overcoming the several path obstacles thereby shift in the drug effectiveness. The lipid vesicles are epitomes of biological membranes that have rose up successfully as carriers for many forms of drug delivery. Ufasomes are nano colloidal suspensions of lipid bilayers that comprises of fatty acids and their ionized species. These ufasomes contains two amphiphiles one of them is the nonionized neutral form and the negatively ionized form. The important factor for vesicle stability is the ratio of nonionized neutral form and the ionized form amphiphiles. These are actually fatty acid and soap mixture vesicles in simple terms we can term them as fatty acid vesicles. The theory of ufasomal formation was first reported by Gebicki and Hicks[6] in 1973 which were named as "ufasomes or unsaturated fatty acid liposomes". Further studies have suggested that not only these fatty vesicles[7] form with unsaturated fatty acids like oleic and linoleic acid but also saturated fatty acids like octanoic acid and decanoic acid.

Calcium channel blockers like verapamil hydrochloride inhibit the entry of calcium by blocking the L-type of calcium channels and bind to both open and inactivated state of Ca^{+} channels thus resulting in less opening of the channel as this there will be less influx of the calcium ions. Due to these the cardiac and vascular smooth muscles relaxes and lowers the blood pressure. Hypertension could become the root cause to many other diseases like myocardial infraction, arrythmias, angina pectoris and several other diseases associated with cardiovascular system and central nervous system. The exact reasons for the genesis of hypertension[8] is cannot be envisaged there could be many reasons like poor lifestyle, smoking tobacco, alcohol consumption, obesity and even the hypertension is carried genetically. The present study was commenced as it was known that 80% of the orally administered drug is subjected to biotransformation to bypass this drug is incorporated into ufasomes and administered through transdermal route.

2. MATERIALS AND METHODS

Materials

Verapamil hydrochloride was bought from Appcure laboratories and remaining excipients were procured from the below sources respectively. Glyceryl mono stearate from sigma Aldrich, Cholesterol from divya associates, Oleic acid from SDFCL, Methanol from SD Fine and Chloroform from SDFCL.

Methods

Compatibility studies by FTIR[9,10]

FTIR is the spectrophotometric technique used to find any incompatibilities between drug and excipient. In this technique the drug and other excipients used in the formulation is blended thoroughly with diluting substance like potassium bromide in the ratio of 1:100 and made into a fine pellet by using pressed pellet technique. The prepared pellets are subjected for the FTIR investigation. Thus, the obtained graphs show the functional groups present in the drug and excipients which are interpretated for the incompatibilities.

Compatibility studies by DSC[11]

Thermal analysis of the formulation is an important evaluation technique to find any possible interaction between drug and other used additives. The thermal behavior of the drug along with oleic acid, cholesterol and GMS was studied using DSC instrument. Calibration of the heat flow scale was done before adding the samples for analysis. A small sample of 15mg was weighed in aluminum pans followed by crimping. The thermogram was recorded at 20 mL/min nitrogen gas flow rate and a heating rate of $5^{O}C$ per min over a temperature range of $20^{O}C$ to $350^{O}C$.

Formulation of Verapamil Hydrochloride Ufasomes by Thin film hydration technique[12]

The formulation table was built with 9 different formulations and each formulation contains different proportions of drug, oleic acid, cholesterol and glyceryl mono stearate. To the formulations which contain cholesterol methanol is used as the solvent and to those formulations which contain GMS acetone is used as solvent. According to the formulation the formulation contents are mixed are transferred into the round bottom flask and the flask is connected to the rotary evaporator apparatus. The apparatus is functioned at $40^{O}C$ at 35 rpm until the solvent present in the formulation is evaporated and a thin layer is formed. Now the formed film is hydrated with 10ml of phosphate buffer pH 8.0 and the suspension was sonicated and collected in a glass tube.

Table 1: Composition of various verapamil HCl ufasomes formulations

S.No.	Ingredients (mg)	V1	V2	V3	V4	V5	V6	V7	V8	V9
1	Verapamil Hydrochloride	25	25	25	25	25	25	25	25	25
2	Oleic acid	100	100	100	100	100	100	100	100	100
3	Glyceryl mono stearate	10	15	20	25	-	-	-	-	25
4	Cholesterol	-	-	-	-	25	50	75	100	25
5	Methanol	5ml	5ml	5ml	5ml	10ml	10ml	10ml	10ml	5ml
6	Chloroform	5ml	5ml	5ml	5ml	-	-	-	-	5ml

Evaluation studies of formulated ufasomes

Entrapment efficiency[13]: Entrapment efficiency was the procedure used to determine the percentage concentration of drug entrapped in the ufasome of the dispersion medium. The prepared samples were placed in centrifuge wells and centrifuged at 7000 rpm for 30 mins. The precipitated solution was diluted with deionized water and sonicated for 15 mins and measured by using UV visible spectrophotometer at 278nm.

Zeta potential: Dynamic light scattering was used to measure the average particle size, zeta potential and poly dispersity index of the prepared ufasomes. The samples were diluted ten times with distilled water. The zeta potential of the formulation was measured under an electric field of 40V/cm. The measurements were conducted at a scattering angle of 90 and temperature of 25 ± 2^{O}C. Helium–neon gas laser was the light source at intensity of 4mW and wavelength of 633 nm. All measurements were performed in triplicates[14].

Surface morphology by TEM[15]: Morphological characteristics of the ufasomes including sphericity and aggregation were examined using TEM. A drop of sample is placed on a piece of para film, the carbon coated copper grid and wait for 5-10 mins and drain the excess with help of filter paper, wash with distilled water and stained with 2% uranyl acetate and dried now observed under transmission electron microscopy at various magnificent views.

In-vitro Diffusion studies[16]: The in-vitro diffusion studies were carried out in modified diffusion cell one side of the cell is dipped in the 6.8 pH phosphate buffer medium by the means of the diffusion layer through which the drug diffuses from the dosage form into the diffusion medium. The setup is maintained at 37±0.5^{O}c with magnetic stirring. At regular time intervals of 1 to 12 hours sample of 4 ml was withdrawn and 4 ml was replaced with 6.8 phosphate buffer into the beaker to maintain the sink conditions. The drew samples are absorbed spectrophotometrically by using UV spectrophotometer at lambda max of 278nm and calculate the cumulative drug release of the drug.

Drug release kinetics model fitting of the release data[17,18]: The results obtained from the diffusion studies of the dosage form are fitted into the several kinetic models like zero order, first order, Higuchi and Korsmeyer-Peppas model and the results are as mentioned below.

3. RESULTS & DISCUSSION

Compatibility studies by FTIR: The FTIR spectra of the pure drug verapamil hydrochloride shows the NH stretching at 3424.68 cm^{-1} indicates the presence of aliphatic primary amine, CH stretching at 2934.25 cm^{-1} signifies the presence of alkane, CH bending at 1384.98 cm^{-1} witness the presence of aldehyde and CH bending at 812.84 cm^{-1} shows the presence of 1,2,4 trisubstituted benzene derivative. All the significant peaks related to drug

which are appeared in the spectra of the drug are appeared in the other FTIR spectra of drug and excipients mixture stating that there are no incompatibilities between drug and other excipients.

Differential calorimetry studies: The DSC studies of drug + oleic acid + cholesterol and was done. From these thermograms it was concluded that there was a slight shift in the melting point peaks of the formulations this shows that there were no existing incompatibilities between drug and other lipids.

Entrapment efficiency: Entrapment efficiency of formulations F1 to F4 which contains glyceryl mono stearate as the lipid shows the entrapment efficiency as follows 55.25%, 64.32%, 56.36%, 63.2%.And the entrapment efficiency of formulations F5 to F8 which contains cholesterol as the lipid shows the entrapment efficiency as follows 74.3%, 69.8%, 71.2% and 75.23%. And the formulation F9 which contains cholesterol and glyceryl mono stearate as lipids shows entrapment efficiency 55.52%.

Mean particle size: The particle size of the formulation F5 was analyzed by using the HORIBA Malvern zeta sizer and the average particle size of the particles dispersed in the suspension was 321.6 nm and poly dispersity index was 1.342.

Zeta Potentiometric studies: Zeta potential analysis of the formulation was preceded by using HORIBA zeta potential instrument. And the mean zeta potential was found to be -21.1 mV and electrophoretic mobility mean was found to be -0.000163.

In-vitro drug diffusion studies: The %CDR of the formulations F1-F4 are 55.16%, 54.33%, 60.07% and 58.73% respectively at 12^{th} hour which contains glyceryl mono phosphate as lipid. And the % CDR of formulations F5-F8 are 79.40%, 68.23%, 68.52% and 66.82% respectively at 12^{th} hour which contains cholesterol as lipid. And the %CDR of the formulation F9 at 12^{th} hour was found to be 62.94%.

To analyze the drug release mechanism in-vitro release data were employed into zero order, first order, Higuchi, Korsmeyer-Peppas model. Drug dissolution has been described by kinetic models in which the dissolved amount of drug is a function of test time.

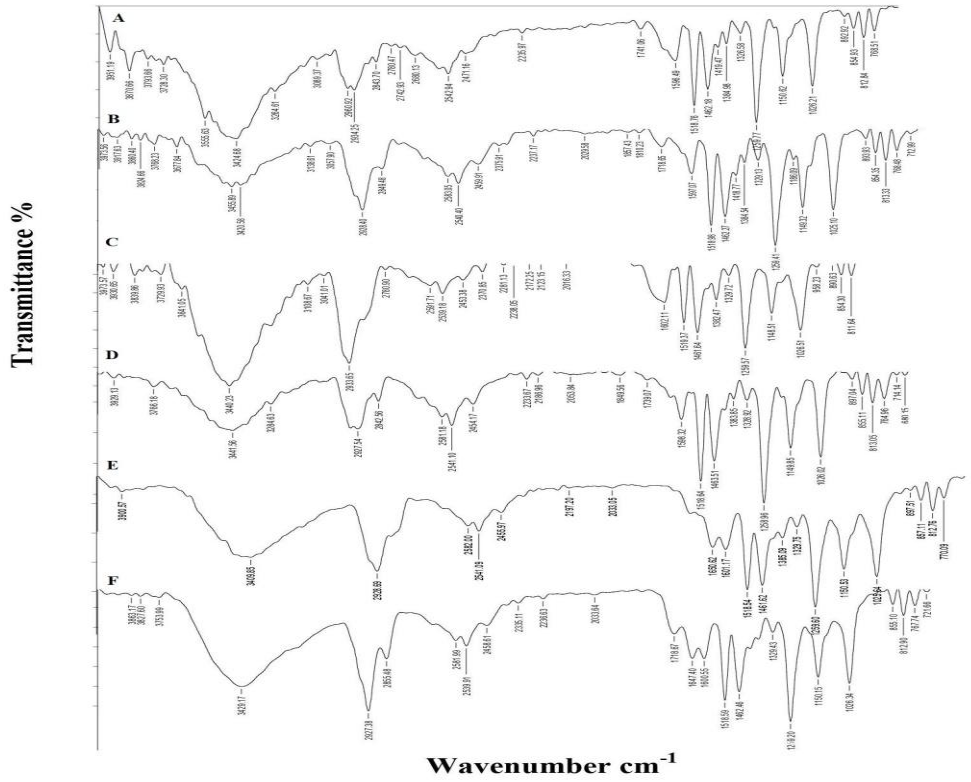


Fig 1: FTIR spectra of A) drug B) Drug + oleic acid C) drug and cholesterol D) drug and glyceryl mono stearate E) oleic acid and cholesterol F) drug, oleic acid and glyceryl mono stearate

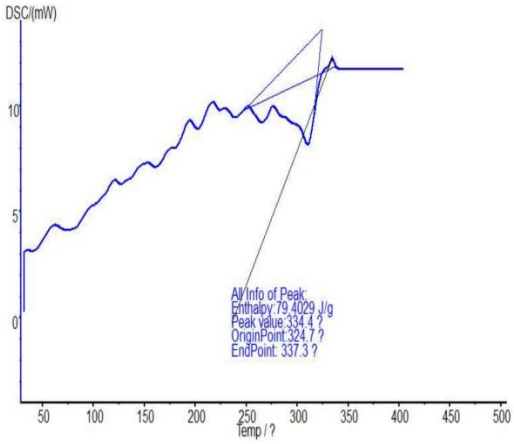
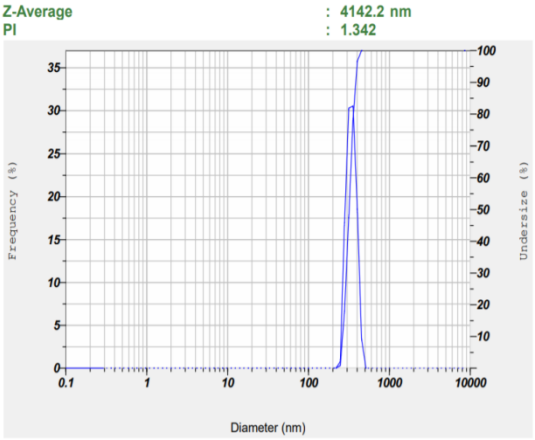


Fig 2: DSC thermogram of drug and other excipients drug and other excipients used



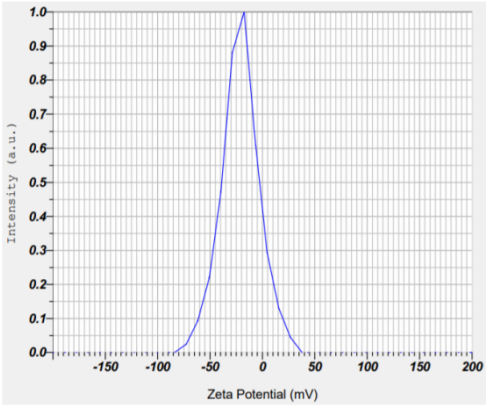


Fig 3: Mean particle size of V5

Fig 4: Zeta potential of V5

Table 2: Entrapment efficiency results

S.No.	Formulation code	Entrapment Efficiency
1	F1	55.25
2	F2	64.32
3	F3	56.36
4	F4	63.2
5	F5	74.3
6	F6	69.8
7	F7	71.2
8	F8	75.23
9	F9	55.52

Table 3: *In-vitro* drug diffusion studies

S.No.	Time (hours)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	0	0	0	0	0	0	0	0	0	0
2	1	5.16	3.89	5.80	3.57	9.64	4.60	4.88	7.36	6.31
3	2	12.62	10.84	13.45	7.71	24.31	12.68	11.90	10.77	7.65
4	3	18.55	13.71	19.45	13.71	40.73	19.69	20.26	14.32	9.18
5	4	23.16	19.13	28.82	20.79	46.63	24.51	26.14	20.19	9.94
6	5	31.76	24.55	32.65	23.27	54.04	29.12	34.43	22.66	13.13
7	6	36.73	26.21	37.56	25.57	63.61	34.65	40.46	27.35	14.66
8	7	44.06	29.20	38.39	26.27	70.55	35.43	44.78	36.91	20.98
9	8	50.76	30.29	43.98	29.27	76.92	48.54	49.03	49.39	26.78
10	9	51.14	32.65	44.96	32.52	77.32	51.37	53.71	54.06	33.29
11	10	51.72	33.86	49.93	37.11	78.52	56.76	58.74	57.53	37.69
12	11	52.61	43.94	55.16	48.21	78.84	62.71	64.48	62.07	45.91
13	12	55.16	54.33	60.07	58.73	79.40	68.23	68.52	66.82	62.94

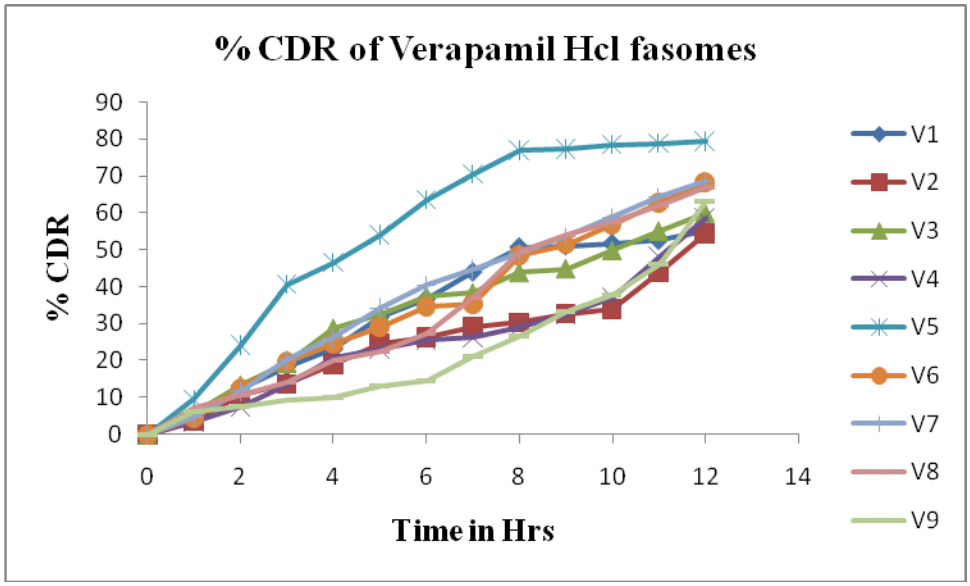


Table 5: %CDR of Verapamil HCl Ufasomes

Table 4: Slope and Regression coefficients from all the Kinetic models

Formulation	Zero order		First order		Higuchi		Korsmeyer-Peppas		Release mechanism
	Slope	R^2	Slope	R^2	Slope	R^2	N	R^2	
V5	9.104	0.992	0.4325	0.6925	24.689	0.980	1.0484x + 1	0.7613	Super case-II transport

Discussion

FTIR investigations and the conception of the obtained graphs showed the characteristic peaks related to the various functional groups present in the drug and the super imposable functional peaks are obtained in the drug and other excipients mixture stating that there are no incompatibilities between drug and other excipients. The drug and excipients compatibility studies were examined by differential scanning calorimetry technique reveals that there are no incompatibilities between drug and other excipients used. The extent of drug entrapment in the dosage form was evaluated. Among all formulations F5 and F8 showed the entrapment efficiency 74.3% and 75.23% respectively. Particle size characterization was done by Malvern zeta sizer and formulation F5 shows less particle size of 321.6nm and poly dispersity index 1.342. Zeta potential and mean electrophoretic mobility of formulation F5 was found to be -21.1 mV and -0.000163 correspondingly. In-Vitro diffusion studies of the drug from the dosage form were studied for all the formulations and %CDR of the formulation F5 at 12^{th} hour was found as 79.40%.

4. CONCLUSION

From the study and results obtained it was concluded that the Verapamil hydrochloride Ufasomes were prepared by thin film hydration technique and these were evaluated. The yielded results predict that the ufasomes are the effective drug carrier which increases the bioavailability of the drug. And considering all other evaluation parameters of all formulations F5 formulation can be selected as the best formulation which has particle size of minute range of 321.6 nm, drug entrapment efficiency of 74.3% and drug release at 12^{th} hour was 79.40%. The release exponents reveal the value of 'n' was 1.0484, which says that the dosage follows Zero order kinetics and Super case-II transport mechanism. So, the excerpt of the study is the ufasomes loaded with verapamil hydrochloride are much potential lipoidal drug carriers and the ufasomes loaded with the drug verapamil hydrochloride increases the systemic bioavailability of the drug.

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6. CONFLICT OF INTEREST:

The authors declare no conflict of interest

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