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PREPARATION AND EVALUATION OF LIPID MICROSPHERE OF VERAPAMIL HYDRO-CHLORIDE

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Keywords:

Microspheres, Release kinetics, Melt solidification, Verapamil HCL

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ABSTRACT: Microspheres drug delivery system have improved patient compliance, decreased toxicity, and increased efficacy. This work aimed to design a controlled-release drug-delivery system for lipid microspheres of Verapamil hydrochloride that would retain the drug in the stomach and continuously release the drug in a controlled manner up to a predetermined time. Lipid microspheres were prepared by using a melt solidification method. Microspheres with spherical shapes and narrow size distribution were formed. In the present investigation, two lipids were used in various concentrations; Ceresine wax & Bees Wax. *In-vitro* performance was evaluated, such as particle size analysis, drug entrapment efficiency, compatibility study, Preformulation study, flow properties, *in-vitro* drug release studies, stability studies, *etc*. The drug in microspheres was found to be stable and compatible with waxes, as confirmed by DSC and FTIR methods. The mixing ratio of lipids with drug affected the size, size distribution, Yield, drug content, and drug release of microspheres. Results showed that the solid, discrete, reproducible free-flowing microspheres were obtained. In most cases, good in vitro microsphere behavior was observed, and a broad variety of drug release patterns could be achieved by variation of the drug-lipid ratio. The release kinetics followed different transport mechanisms. The surface morphology of microspheres was characterized by scanning electron microscopy (SEM). The release of the drug was controlled for more than 12h.

INTRODUCTION: The need to design new drug delivery systems will reduce or eliminate the variable plasma concentration. Controlled-release drug delivery systems are developed to address many of the difficulties associated with traditional methods of administration [1]. The real issue in developing oral controlled release dosage forms is to prolong the residence time of the dosage form in the stomach or upper gastrointestinal tract until the drug is completely released [2].

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The transit of a drug or formulation through the gastrointestinal tract will determine how long a compound will be in contact with its preferred absorptive site [3]. Prolonged gastric retention improves bio-availability, reduces drug waste, and improves solubility for less soluble drugs in a high pH environment. It has also applicable for local drug delivery to the stomach and proximal small intestine [4].

Controlled-release drug delivery employs devices such as polymer-based disks, rods, pellets, or microspheres - that encapsulates the drug and release it at controlled rates. Microspheres can encapsulate many drugs, including small molecules, proteins, and nucleic acids, and are easily administered through a syringe needle. Microspheres are small spherical particles; with diameters in the micrometer range (typically 1μm

to 1000μm) [5]. They are generally biocompatible, can provide high bioavailability, and are capable of controlled release for long periods. The microsphere formulation method is a governing factor in the encapsulation and release of therapeutics. In addition, difficult factors including the type of lipids, the lipids molecular weight, the nature of any excipients added to the microsphere formulation and the microsphere size, can strongly impact the delivery rates [6].

Microsphere drug delivery systems have been formulated by various techniques, including phase separation or precipitation, emulsion/solvent evaporation, and spraying, melt congeal /dispersion technique [7]. The principle of lipid microsphere preparation offers a simple and practical approach to achieve increased gastric residence time for the dosage form and controlled drug release [8].

Verapamil hydrochloride belongs to the calcium channel antagonists used in treating several cardiovascular disorders, particularly angina pectoris, supraventricular tachycardia, and hypertension [9]. In medical practice, it is mostly used in a conventional tablet, forming a minimal dose of 40 mg and a maximal dose of 180 mg [10]. The remaining part of the Verapamil hydrochloride dose undergoes a first-pass effect, mainly in the liver [11]. However, due to its extensive first-pass effect, it has much low bioavailability (10-20%). It has a shorter half-life (4 h); hence dosing frequency is high [12]. Verapamil's physicochemical properties and shorter half-life make it a suitable molecule for the preparation of lipid microspheres. The present study aims to develop suitable gastro retentive lipid microspheres of Verapamil HCL and to study release kinetics of the drug to reduce the dose frequency and achieve a controlled drug release with improved bioavailability.

MATERIALS AND METHODS:

Materials: Verapamil hydrochloride was kindly supplied by Dr. Reddy's Lab, Hyderabad (India). All other reagents and chemicals used were of analytical grade.

Preformulation Studies: The calibration curves of Verapamil Hydrochloride were prepared in distilled water/phosphate buffer pH 7.4 / acidic buffer pH 1.2. Then absorbance of the solutions was measured spectrophotometrically at 278nm for Verapamil Hydrochloride. Shake flask method Distilled water did a solubility study, Simulated Gastric Fluid or Hydrochloric Acid Buffer (SGF; pH 1.2) Simulated Intestinal Fluid SIF; pH 6.8) Phosphate Buffer (PB; pH 7.4). Drug-Drug and Drug lipids Compatibility study was done by FTIR Spectroscopy.

Preparation of Wax Microspheres: The weighed amount of ceresin wax was melted separately in a china dish using water baths. The drug previously passed through sieve no.100 was dispersed in the melted wax mass and stirred to obtain a homogeneous melt.

These individual mixtures were poured into 200 ml of a mixture of dispersant medium containing 100ml of pH 7.4 Phosphate buffer solution (to minimize the solubility of the drug) and 100ml of PVA (1%), which was previously heated to a temperature higher than the melting point of wax (>+ 5°). Tween 80 (2% w/w) was added to the mixture containing waxes; the whole mixture was mechanically stirred at 900 rpm using a stirrer (RQ-127D).

Spherical particles are produced due to the dispersion of molten wax in the aqueous medium. The mixture was stirred continuously at 900 rpm at a higher temperature (>+ 5°) of the melting point of waxes/fat for 3 min. The mixture's temperature in the beakers was cooled rapidly to 4° C by adding cold water. The resultant solid spheres collected by filtration were extensively washed with water to remove any drug and surfactant residues.

TABLE 1: COMPOSITION FOR VERAPAMIL HCL MICROSPHERES

Formulation Code	Quantity of Lipids		Drug(mg)
	Ceresin Wax (mg)	Bees Wax (mg)	
F1	120	-	40
F2	160	-	40
F3	200	-	40
F4	-	120	40
F5	-	160	40
F6	-	200	40

Air drying was carried out at room temperature for 48 hr produced discrete, free-flowing solid microspheres. Similarly, the above process was carried out with Bees wax and melted in a china dish at a temperature of 75°C. Total 6 formulations were prepared by varying concentrations of both lipids, as shown in **Table 1.**

Characterization of Lipid Microspheres [13-18]:

Measurement of Micromeritic Properties: The flow properties of prepared lipid microspheres were investigated by measuring the bulk density, tapped density, Carr's index, Housner's Ratio, and angle of repose. The bulk and tapped densities were measured in a 10 ml graduated cylinder.

The sample in the measuring cylinder was tapped mechanically by a constant velocity rotating cam. The initial bulk and final tapped volumes were noted, from which their respective densities were calculated. Results are shown in **Table 2.**

TABLE 2: MICROMERITIC PROPERTIES OF THE DRUG IN LIPID MICROSPHERES

Formulation	% Yield (%w/w)± S.D	Mean particle size (microns)	Angle of repose ± S.D	Bulk Density± S.D	Tap Density± S.D	Compressibility Index± SD	Housner's Ratio ± SD
F1	80.72±0.18	31.60±2.51µm	28.437±0.57	0.443 ±0.0115	0.503 ±0.012	11.925±0.277	1.135±0.004
F2	83.82±0.016	34.13±5.93µm	27.426±0.699	0.54 ±0.01	0.62 ±0.01	12.905±0.208	1.148±0.003
F3	89.35±0.016	39.22±0.91µm	23.1±0.725	0.663 ±0.006	0.776±0.012	14.587±0.522	1.171±0.007
F4	86.03±0.028	38.22±1.67µm	28.823±0.99	0.467 ±0.006	0.527 ±0.006	11.393±0.126	1.128±0.002
F5	91.88±0.016	41.50±4.53µm	27.693±0.903	0.567 ±0.005	0.636 ±0.005	12.566±0.114	1.144±0.001
F6	96.92±0.029	49.25±3.91µm	26.97±0.86	0.653 ±0.005	0.766 ±0.011	14.777±0.526	1.173±0.007

% Compressibility index = (TD-BD/TD) x 100

Housner's Ratio = TD/BD

Where,

TD = Tapped Density and BD = Bulk Density.

Particle size Analysis: The particle size was determined using an optical microscope under regular polarized light, and mean particle size was calculated by measuring 200-300 particles with the help of a calibrated coulometer. Results are shown in **Table 2.**

Yield of Microspheres: The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components used to prepare the microspheres. Results are shown in **Table 2.**

% Yield = (Actual weight of product / Total weight of excipient and drug) x 100

Scanning Electron Microscopic Studies and Sphericity Determination: SEM photographs were taken using a scanning electron microscope JEOL 5400, Tokyo, Japan, at suitable magnification at room temperature. The photographs were observed for morphological characteristics and to confirm the spherical nature of the microspheres. To determine the sphericity, the tracings of lipids microspheres (magnification 45 X) were taken on a black paper using Camera Lucida, (Model-Prism type, Rolex, India), and circulatory factor was calculated [14]. The sphericity of microspheres was calculated using the equation,

$$S = p^2 / (12.56 \times A)$$

Where A is area (cm^2) and p is perimeter (cm^1).

Differential Scanning Calorimetry (DSC): DSC studies were carried out on a Netzsch thermal analyzer with 200F DSC module. Calorimetric measurements were made with the help of an empty cell as the reference. The instrument was calibrated using high-purity indium metal as standard. The dynamic scans were taken in a nitrogen atmosphere at a heating rate of 10°c/min. The sample wash thermetically sealed in an aluminum crucible. Nitrogen gas was purged at the rate of 10 ml/min. for maintaining the inert atmosphere.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectra of pure drug, empty microspheres, and drug-loaded microspheres were obtained using KBr pellet method (applying 600 kg/cm^2). The drug excipient interactions were measured by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Model 8033,USA)in the wave number region 400 -4000CM^{-1} .

X-ray Diffractometry (XRD): The crystalline nature of the drug is performed by XRD analysis technique. The X-Ray Diffraction pattern of Pure drugs Verapamil HCL and drug in lipid microsphere were recorded using (Phillips Xpert pro, Germany) X-ray diffractometer with a Cu-tube (k(alpha) 1.541, 45 kV, 40 mA), at a scanning speed of 0.30°C /min and the peaks were indexed in Bruker (Germany).

Loose Surface Crystal Study: This study was conducted to estimate the amount of drug present on the surface of the pellets. 100 mg of pellets were suspended in 100 ml phosphate buffer (pH 7.4). The samples were shaken vigorously for 15 min in a mechanical shaker. The amount of drug leached out from the surface was analyzed spectrophotometrically at 278 nm. The percentage of drug released concerning the entrapped drug in the sample was recorded.

Estimation of Drug Loading: Drug-incorporated lipid microspheres of each batch were selected and powdered in a mortar. 100 mg of drug-loaded lipid microspheres was accurately weighed and added into 100 ml volumetric flask. To this, 100 ml dichloromethane was added. This solution was stirred for 60 min, till the entire drug leached out.

The solution was filtered, and 1 ml was withdrawn from this solution and added to 10 ml volumetric flask, and the volume was made to 10 ml (10 µg/mL) with phosphate buffer pH 6.8. Drug content was estimated UV spectrophotometrically at 278 nm.

***In-vitro* Studies:** USP XX1 dissolution apparatus type II was employed to study the percentage of drug release from various formulations prepared. Accurately weighed quantities of drug (Verapamil HCL 40 mg) lipid microspheres of each batch were taken in 900 ml dissolution medium (2 hr in pH 1.2 hydrochloric acid buffer and 3hr upto 24hr in pH 7.4 phosphate buffer) and stirred at 100 rpm by maintaining at a temperature of 37 °C ± 0.5°. At prefixed time intervals, 1ml of the sample was withdrawn and filtered through a 0.4 µm membrane filter. Then the withdrawn is diluted to 10ml. The volume of the dissolution medium was adjusted to 900ml at every sampling time by replacing the same 1ml of dissolution medium to maintain the sink condition. Then the samples were analyzed Spectrophotometrically at 278 nm, and the cumulative percentage drug release was obtained from the following formulae.

Amount of drug present = Concentration × Dilution factor × Conversion factor × Amount of stock solution

Cumulative % drug release: Amount of drug present / Amount of drug to be present

Kinetic Analysis of Drug Release Data:

Zero Order Kinetics: Drug dissolution from pharmaceutical dosage Forms that do not disaggregate and release the drug slowly can be represented by the following equation:

$$f_t = f_o t$$

Where, f_t represents the fraction of drug dissolved in time t and k_o the apparent dissolution constant or Zero order release constant.

First Order Kinetics: the pharmaceutical dosage Forms following this dissolution profile, such as Those containing water-soluble drugs in porous Matrices, release the drug remaining in its Interior in such a way that the amounts of drug Released by a unit of time diminish.

$$\log Q_t = \log Q_0 + k_1 t / 2.303$$

Where, Q_t= amount of drug released in time t

Q_0= amount of drug initially.

K1= first order rate constant here, the graphical Representation of the log cumulative of % drug Remaining vs. Time will be linear.

Higuchi Model: Higuchi was the first to derive an Equation by describing the release of a drug from an Insoluble matrix as the square root of a time-Dependent process based on fickian diffusion.

$$Q_t = K_h (t)^{0.5}$$

Where, Q_t is the amount of drug released in time t, and K_h is the release rate constant for the Higuchi model.

Korsmeyer - Peppas Model: In 1983, Korsmeyer *et al.,* developed a simple, semi-empiric model when diffusion is the main drug release

Mechanism, relating the drug release To the elapsed time t exponentially.

$$Q_t = k.t^n$$

Where, Q_t is the percent drug release at time 't', k Is a constant incorporating structural and geometric Characteristics of the drug dosage form, n is the Release exponent, indicative of the drug release Mechanism and the function t is the fractional release of the drug.

The value of n (release exponent) in the Korsmeyer Peppas equation is used to indicate different release Mechanisms. A value of n = 0.5 indicates fickian (case i) release ;> 0.5 but < 0.89 for non-fickian (anomalous) release; n = 1 indicates case-ii Transport (zero-order release) and >1 indicates Super case ii type of release. Case ii generally Refers to the erosion of the polymeric chain, and Anomalous transport (non-fickian) refers to a combination of diffusion and erosion Controlled drug release. Only the linear portion of the graph was used to calculate the time exponent 'n' value. The plot made: log cumulative % drug release *vs.* log time (Korsmeyer-Peppas model).

Stability Studies: The prepared lipid microspheres that showed an appropriate balance between the in vitro and the percentage release were selected for stability studies. A stability study of an optimized batch of lipid Microsphere was performed under accelerated stability conditions (40 ± 2 °C / 75 ± 5% RH) for 3 months according to ICH guidelines for stability testing of new products. The samples were withdrawn at different intervals (0, 1 and 3 months) and evaluated in vitro and the percentage release.

RESULTS AND DISCUSSION: In the present study, a modified novel congeable dispersion emulsified cooling-induced solidification method was employed using inert lipids and non-toxic solvents to entrap the drug. In this study, various parameters were studied, such as drug and wax ratio, stirring speed and time, the concentration of emulsifier added, the volume of the aqueous phase used, the effect of pH on drug entrapment, the temperature of the aqueous phase and rapid cooling studies. Therefore the influence of the above parameters was highlighted. When the pH value of the external aqueous phase was highly alkaline, the solubility of the drug was reduced, and the encapsulated amount of the drug increased. The maximum drug load was obtained at pH 7.4. When pH value changes from 7.4 to 5.0, the percent of drug loading reduced from 11.31 to 16.66%, 12.15 to 16.66% for ceresine wax and beeswax formulations, respectively.

The present study found that 200 ml of aqueous phase or dispersant medium was suitable for producing the spherical microspheres. The resultant microspheres did not have any surface irregularities and were non-aggregated. As the volume of the external phase increased, the yield was reduced and the resultant microspheres were irregularly shaped. When the volume of the aqueous phase was less than 150 ml, the resultant microspheres were highly aggregated and highly impossible to distinguish as individual microspheres. To avoid the formation of irregularly shaped larger particles, in the present method, 200 ml of aqueous phase containing 100ml of pH 7.4 Phosphate buffer solution (to minimize the solubility of drug) and 100ml of PVA (1%) was used.

Incorporating Verapamil HCL into ceresin wax, beeswax microspheres required the addition of tween 80 as a surfactant or emulsifier at an optimum concentration to reduce the interfacial tension between the hydrophobic material and external aqueous phase. An attempt was made to incorporate the drug in the wax microspheres without adding a surfactant. But the process failed, as it resulted in an aggregate cake-like mass during the solidification of wax. This may be due to repulsion from high interfacial tension between the hydrophobic waxy material and the external aqueous phase. It was found that tween 80, having an HLB value of 15 was suitable to substantially increase the waxy material's dispersion in the external aqueous phase and promote drug incorporation in the wax microspheres. Various concentrations ranging from 1.0 to 2.0% (w/w) of the total formulation were tested to obtain an optimal surfactant concentration. Discrete microspheres with good flow properties using an optimum concentration of surfactant 2 % w/w (tween 80) were used. Concentrations of tween 80 ranging from 1.0 to 1.9 % w/w failed to produce reproducible microspheres. The resultant lipids microspheres were composed of irregular masses,

which could not be distinguished as individual microspheres. A similar emulsifier concentration was reported for wax microspheres prepared by melt dispersion method [17]. The temperature of the aqueous phase was maintained at 5 °C higher than the melting point of the lipids in the corresponding formulations. SEM studies showed that the resultant microspheres were free from surface irregularities, except for some wrinkles. It was also observed that when the temperature of the aqueous phase was less than 5 °C than the melting point, big waxes flakes were produced.

In the present study, an optimum drug to lipids phase ratio of 1:5 w/w was used to produce the spherical discrete microspheres. A higher amount of drug to the wax ratio (2:5) was found to produce aggregate masses during the cooling process. It may be due to the reduced melting point of the lipids materials. SEM photographs also indicated the crystals' presence on the microspheres' surface. The resultant microspheres were unsuitable for pharmaceutical uses. Hence, an optimum 1:5 ratio was used to prepare microspheres. Sieve analysis data obtained for prepared lipid microspheres were in size range of 106 to 500 µm, and 55.43 to 74.18 % were of size fraction 250 µm. It was observed that the average size of the microspheres ranged between 310 to 320 µm. The optimum stirring speed and stirring time is the important factor that influences the size distribution of microspheres.

A stirring speed of 900 rpm and a stirring time of 3 min was used to obtain reproducible microspheres. It was observed that with the increase in the stirring speed from 900 to 1200 rpm there was a decrease in the average size of the spheres and recovery yield of the microspheres due to small-sized microspheres, which were lost during successive washings. When the stirring speed was lower than 900 rpm, larger pellets were formed. It was also found that an increase in stirring time, from 4 to 8 min (at a stirring speed of 900 rpm), decreased the recovery yield of microspheres. When the stirring time was lower than 3 min, melted waxes/fat materials adhered to the sides of the beaker during the cooling process, resulting in lower yield recovery. The Particle Size for drug-ceresine wax formulations F1 to F3 was found in the range of 31.60±2.51µm to 39.22±0.91µm, and drug-bees Wax formulations F4 to F6 was found in the range of 38.22±1.67µm to 49.25±3.91µm.The Percentage Yield for drug-ceresine wax formulations was found in the range of 80.72% to 89.35%, and for drug-bees wax, formulations was found in the range of 86.03% to 99.92%. The Entrapment Efficiency increased from 39.722±0.481% to 67.944±0.722% for ceresine wax and 46.667±0.833%to 72.946±0.722% for beeswax. Micromeritics analysis of the microspheres plays an important role in the various pharmaceutical processing such as mixing, filling, and packaging of the pharmaceutical dosage form.

Different micromeritics parameters (such as angle of repose, bulk density, Carr's index, and Hausner's ratio) of all batches of lipid microspheres have been shown in **Table 2.** The percent compressibility of the microspheres was less than 14.58%, Hausner's ratio was found to be within 1.17, and Angle of repose within 28.82, which is an appreciable limit for microspheres to show good flow properties while formulating in the dosage form. The density of all the batches was found to be less than $1g/cm^{3,}$ which is essential for diffusion properties in the intestinal fluid. The dissolution studies were carried out, and mean values were plotted as a percentage of cumulative drug release against time in **Fig. F5**.

The drug release in drug-ceresine wax microspheres formulations F1 to F3 showed biphasic behavior consisting of initial burst release followed by a slow release phase. But ceresine wax optimized batch F3 gave 91.40% release in 12 hours. Drug-bees wax microspheres formulations gave the best release results F4 to F6 showed 87.83% release in 12 hours. The formulations F3 and F6 showed a longer duration of drug release for 24 hrs in simulated intestinal fluid, in addition to completing retarding the drug release in gastric medium. This is due to the polymer Bees wax. The drug release from waxy microspheres was considerably retarded from the waxes. So F6 was taken as the best formulation to achieve prolonged maintenance of effective drug concentrations. The release kinetics study was done for batches F1 to F6 of all the formulation factors. Highest r^2 value was obtained in the first-order kinetics model for all the batches, so it is concluded that release kinetics followed the first-order model (R^2= 0.984), and most of the drug was released in 12 hours. The

release of the drug from the microspheres was due to the diffusion of the drug from the lipid surface. The Korsmeyer- Peppas modelling showed n > 0.45. Hence, it can be interpreted that the drug release from the formulation followed non-Fickian diffusion, and the drug is uniformly distributed within the lipid as in the matrix system. Also r^2 is higher for the first order; hence release was diffusion controlled.

TABLE 3: DRUG LOADING PROPERTIES OF LIPIDS MICROSPHERES

Formulation	Drug Content (Mg)	Theoretical Drug Loading (%)	Actual Drug Loading ±S.D	Drug Entrapment Efficiency ± Sd
F1	11.64	25	9.931±0.12	39.722±0.481
F2	12.13	20	10.556±0.121	52.778±0.601
F3	11.73	16.66	11.319±0.121	67.944±0.722
F4	14.43	25	11.667±0.208	46.667±0.833
F5	15.83	20	11.18±0.1	55.902±0.601
F6	15.83	16.66	12.153±0.12	72.946±0.722

TABLE 4: *IN-VITRO* RELEASE KINETICS OF VERAPAMIL HCL LIPIDS MICROSPHERES

Formulation code	Mathematical models (release kinetics)				
	Zero order kinetics	First order kinetics	Higuchi's	Peppa's	
	r^2	r^2	r^2	r^2	n
F1	0.797261	0.984683	0.922912	0.961232	0.820325
F2	0.801704	0.975229	0.921378	0.96002	0.826744
F3	0.795612	0.938432	0.914782	0.95866	0.835165
F4	0.805052	0.98394	0.927185	0.961849	0.824783
F5	0.791261	0.9433	0.914391	0.960514	0.83586
F6	0.792254	0.927385	0.91682	0.960683	0.831819

The surface morphology, shape of batch F6 was studied by using scanning electron microscopy shown in **Fig. 1** The images showed spherical structure of microspheres. The magnified view of microsphere surface revealed that surface of the microspheres was covered with the free crystal drug, which in turns responsible for initial burst release of drug from the surface of the microspheres in the acidic buffer of pH 1.2. The pure drug Verapamil Hydrochloride's DSC curve indicates its crystalline state. The DSC thermogram is characterized by an endothermic melting peak at 149°C as shown in **Fig. 2**. In the formulation, the characteristic peak of Verapamil Hydrochloride is not retained but a single endothermic peak at 57 °C and a glass transition at 38 °C shown in **Fig. 2**. It can be concluded that the drug changes from crystalline to amorphous form in the formulation, which is also responsible for the increase in the solubility of the drug.

TABLE 5: STABILITY DATA FOR OPTIMIZED FORMULATION

Optimized Batch F6	Months	% Drug Release at 40 ± 2^0C at RH 75±5%
	15 Days	88.55
	1	87.57
	2	89.99
	3	87.54

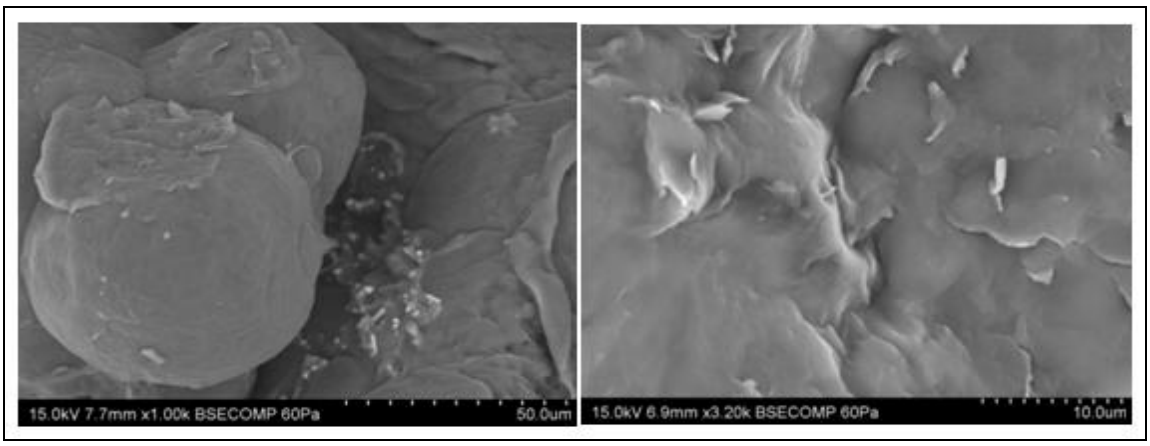

FIG. 1: SEM IMAGE FOR VERAPAMIL HCL LIPID MICROSPHERES FOR OPTIMIZED FORMULATION F6

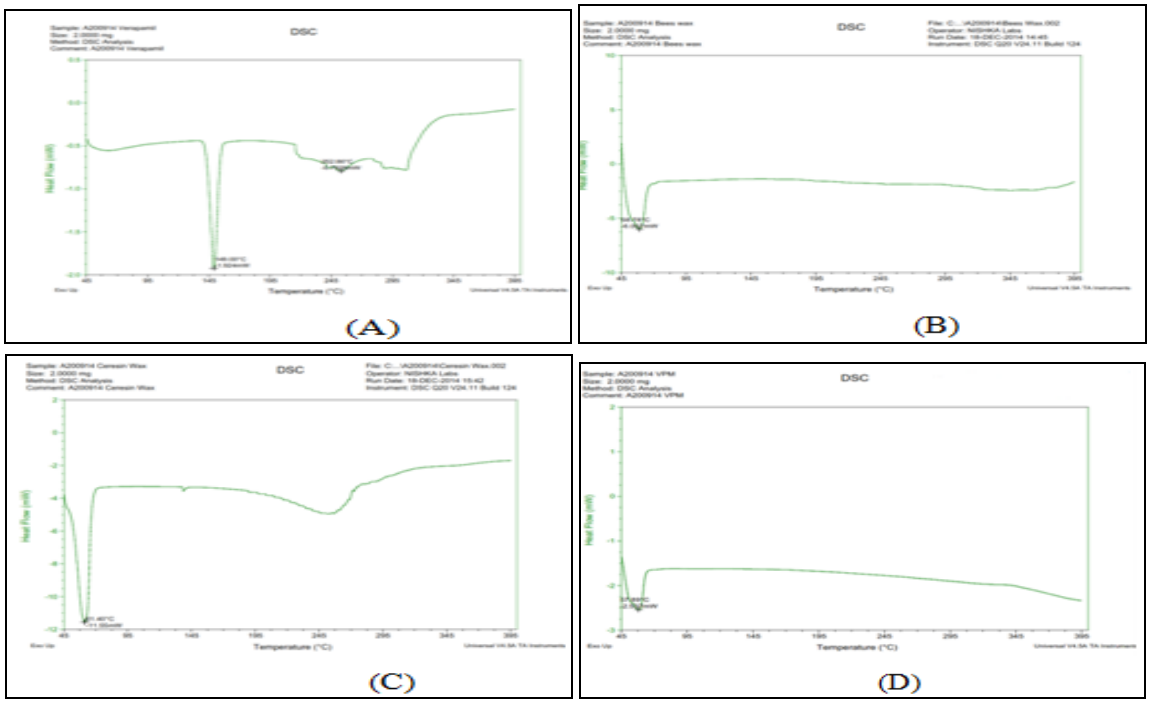

FIG 2: DSC CURVE OF VERAPAMIL HCL (A), BEES WAX (B), CERESINE WAX (C), FORMULATION F6 (D)

In X-RD studies the prominent peaks from pure verapamil HCl at 2θ of 10.59°, 14.45°, 17.07°, 18.1°, 18.84°, 20.29°, 21.32°, 23.06°, 23.75°, and 26.29°, *etc*. The some changes in peak position of verapamil HCl were observed in hollow microsphere (Batch F6).

The prominent peaks from pure verapamil HCl at 2θ of 10.59°, 14.45°, 17.07°, 18.1°, 18.84°, 20.29°, 21.32°, 23.06°, 23.75°, and 26.29°, *etc*. were clearly seen at the same position in the bees wax microsphere (Batch F6) but the peak intensities were decreased to some extent **Fig. 3.**

From the stated observations, we can conclude that the crystalline nature of the drug was still maintained, but the small reduction of diffraction intensity of verapamil HCl in lipid microsphere suggests that the quality of the crystals was reduced and/or presence of high-concentration lipids.

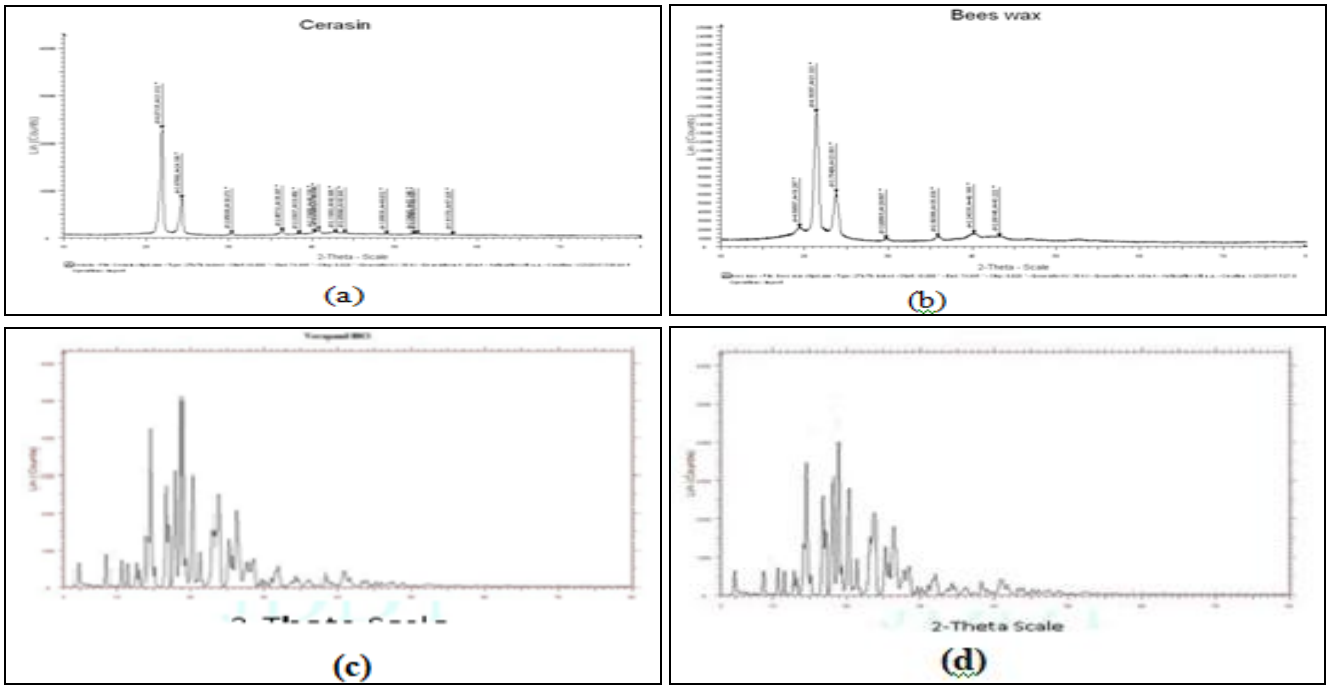

FIG. 3: XRD SPECTRUM OF CERESINE WAX (A) BEES WAX (B) VERAPAMIL HCL(C) FORMULATION F6(D)

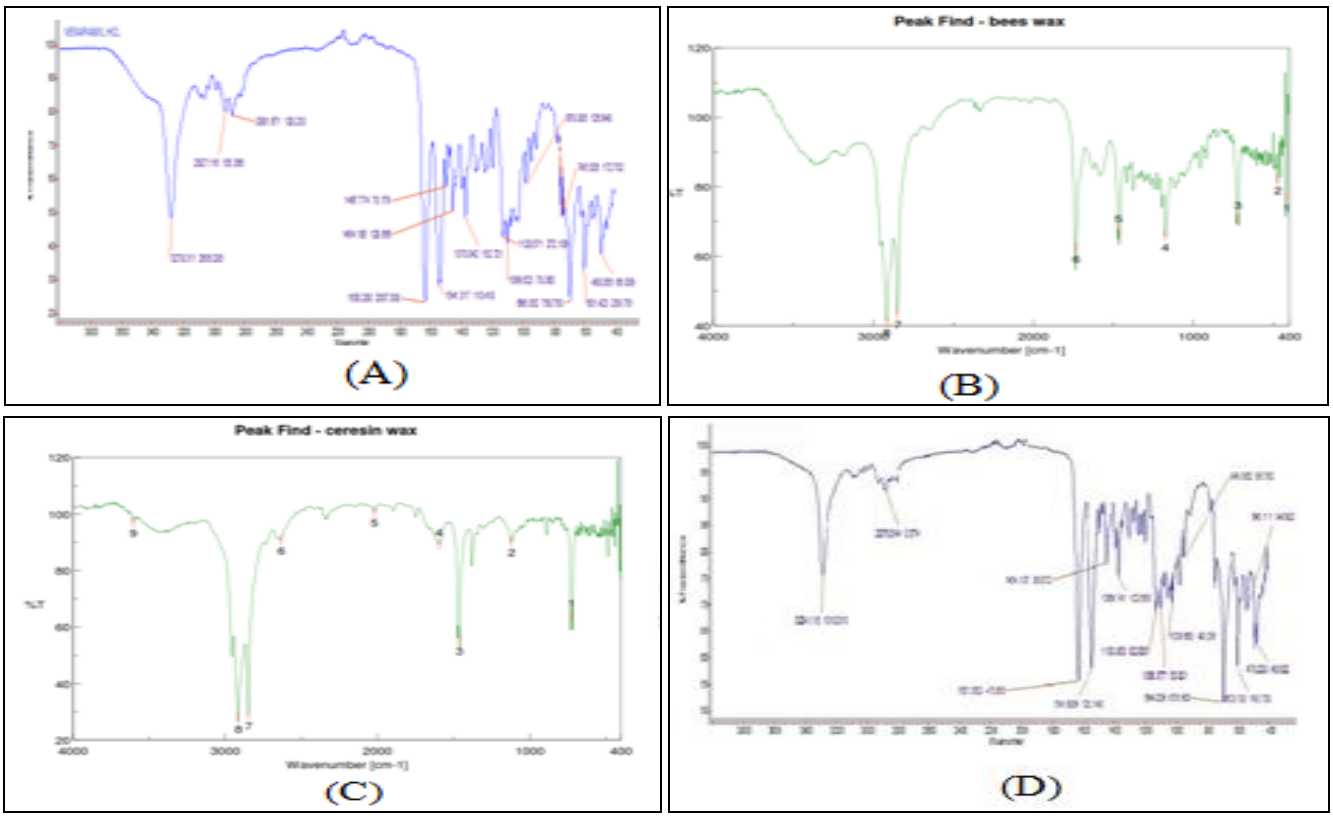

FIG. 4: FTIR OF VERAPAMIL HCL (A), BEESWAX (B), CERESINE WAX (C), FORMULATION F6 (D)

From the FTIR studies, the characteristic bands for important functional group of pure drug Verapamil HCL empty microspheres and drug-loaded lipid microspheres were identified. It was observed that 1695-1649 cm-1 (C=O stretch), 1250-1000(C-O stretch), 1000-650 (Aromatic C=CH stretch), are presented in **Fig 4**. FTIR spectra showed that the characteristic bands of Verapamil HCL were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and lipid used. Hence the FTIR spectrum shows the same characteristic peaks when it is formulated into Microspheres.

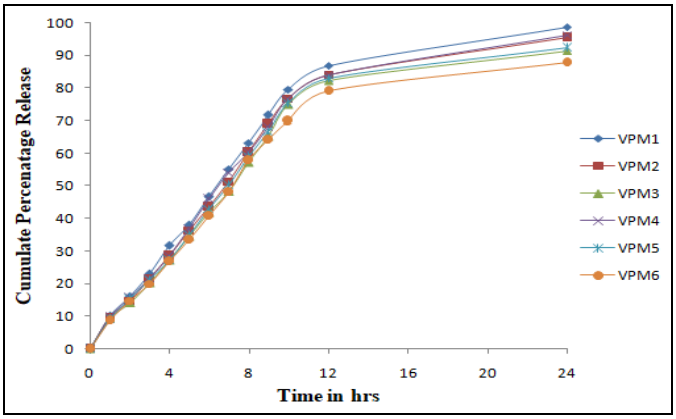

FIG. 5: *IN-VITRO* DRUG RELEASE DATA FOR FORMULATION F1 TO F6 MICROSPHERES

The stability was evaluated on the basis of percentage entrapment efficiency and cumulative percentage release in 15days intervals up to 45 days. No significant change in percentage entrapment efficiency and cumulative percentage release was observed for all the storage time, indicating that batch F6 was stable, as shown in **Table 5.**

CONCLUSION: From the study, it was concluded that there is the possibility of formulating Verapamil hydrochloride lipid microspheres of ceresine wax and beeswax by congeable melt dispersion method. Formulation factors like a drug: Lipids proved to be important for forming Verapamil hydrochloride lipid microspheres. Verapamil lipid microspheres were stable, white colored, spherical, free flowing in nature, and showed controlled release up to 12 hours.

The drug release from the lipid microspheres followed the first-order kinetic model, and Korsmeyer- Peppas modelling showed n>0.45, indicating diffusion-controlled non-Fickian drug release. Optimized formulation batch F6 showed a percentage yield of 87.83, particle size of 49.25±3.91µm, and percentage drug entrapment

efficiency of 72.946±0.722%. Moreover, in vivo pharmacokinetic, bio-distribution, and preclinical studies are required to be done. As a part of future work, the work will continue at a lab scale with *in-vivo* pharmacokinetic and bio-distribution studies.

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