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Development and In-Vitro Evaluation of Capecitabine Microspheres by Emulsification Solvent Evaporation Method

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ABSTRACT

This manuscript deals with the formulation of floating microspheres of Capecitabine by the o/w emulsification and solvent evaporation method in the presence of tween 80 as an emulsifying agent. The influence of formulation factor Drug: Polymer ratio on particle size, encapsulation efficiency and in-vitro release characteristics of the microspheres were investigated. The microspheres have been analyzed for their size, drug loading capacity and drug release study. Spherical and smooth surfaced microspheres with desired encapsulation efficiencies were obtained. Slow drug release from microspheres observed up to 12 h. for formulation F4, F5. Optimized formulation F4 was evaluated for FTIR, DSC, SEM. DSC and FTIR studies showed that the nature of pure drug Capecitabine remains unaffected till the completion of process of microspheres formation. SEM photographs showed that the Floating microspheres were spherical in nature with smooth surface and uniform distribution of the drug within the microsphere.

INTRODUCTION

Microspheres are one of the multiparticulate drug delivery systems and are prepared to obtain prolonged (or) controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000µm, containing dispersed drug in either solution (or) microcrystalline form[1,2].

Ethyl cellulose is non-biodegradable, biocompatible, non-toxic natural polymer and widely used in oral and topical formulation. [3] The microspheres can be produced by several methods utilizing emulsion system (o/w, w/o, o/w/o and w/o/w). The common emulsion system used oil-in-water (o/w), with microspheres being produced by the emulsion solvent evaporation method [4]. The main objective of this study was to formulate the floating microspheres of the Capecitabine for the sustained release of the drug and low dose dumping and increased therapeutic efficiency. Microsphere can be defined as "solid, approximately spherical particles ranging in size from 1 to 1000 micrometer and are made of polymeric materials". Substances can be incorporated within microspheres in the liquid or solid state during manufacture or subsequently by absorption. (5) In floating types, the bulk density is less than the gastric fluids and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, if the system is floating on gastric content, increases gastric residence and fluctuation in plasma concentration. It also reduces chances of striking and dose dumping and produces prolonged therapeutic effect (6, 7).

Capecitabine is a prodrug that is selectively tumour-activated to its cytotoxic moiety, fluorouracil, by thymidine phosphorylase, an enzyme found in higher concentrations in many tumors compared to normal tissues or plasma. Fluorouracil is further metabolized to two active metabolites, 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP), within normal and tumour cells. These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor, N5-10-methylenetetrahydrofolate, bind to thymidylate synthase (TS) to form a covalently bound ternary complex. This binding inhibits the formation of thymidylate from 2'-deaxyuridylate. Thymidylate is the necessary precursor of thymidine triphosphate, which is essential for the synthesis of DNA, therefore a deficiency of this compound can inhibit cell division. Secondly, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of

uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis through the production of fraudulent RNA. (8, 9)

MATERIALS AND METHODS

MATERIALS

Capecitabine was obtained as kind gift sample from Cipla Pvt. Ltd. Mumbai., Ethyl Cellulose was purchased from RANKEM Pharma Ltd Mumbai, India. All other materials used of analytical/pharmaceutical grades.

METHODS

Drug & Excipients Compatibility Study

Differential Scanning Calorimetry (DSC) study

Assessment of possible incompatibilities between an active drug substance and different excipients forms an important part of the preformulation stage during the development of solid dosage form. Differential Scanning Calorimeter (DSC PerkinElmer 4000) allows the fast evaluation of possible incompatibilities because it shows changes in the appearance, shift of melting endotherms and exotherms, and/or variations in the corresponding enthalpies of reaction. The DSC thermograms of pure drug, other excipients and optimized film were recorded. The thermal analysis was performed in a nitrogen atmosphere at a heating rate of 10°C/min over a temperature range of 40°Cto 300°C. DSC study was performed for Capecitabine and physical mixture of all ingredients of Microspheres. (10)

FT-IR Study

The FTIR of pure drug and physical mixture of formulation ingredients of optimized batch was measured using Fourier transform infrared spectrophotometer (Model FTIR- Agilent carry 630, United States). The amount of each formulation ingredient in the physical mixture was same as that in the optimized batch. The pure drug and physical mixture were then separately mixed with IR grade KBr. This mixture was then scanned over a wave number range of 4000 to 400cm-1. (11-13).

Preparation of Floating Microspheres of Capecitabine by Emulsification Solvent Evaporation Method

Capecitabine microspheres were prepared based on o/w emulsion solvent evaporation technique by using ethyl cellulose as a polymer. Different formulations were prepared by dissolving the polymer and the drug in ethyl acetate (oil phase) and acetone. This solution was poured slowly in the 500 ml of distilled water (aqueous phase) containing tween 80 as the emulsifying agent with continuous stirring on propeller stirrer. The resultant mixture was emulsified at speed of 1000 RPM for 4 h. The dispersed drug and polymer solution was immediately transformed into fine droplets, which subsequently solidified into rigid microspheres due to the solvent evaporation. The particles were collected by filtration, washed to remove excess oil by distilled water and dried in hot air oven at 60˚C.

Table 1: Formulation of Floating Microsphere

Sr. No.	Ingredients	F1	F2	F3	F4	F5
1.	Capecitabine (g)	1	1	1	1	1
2.	Ethyl Cellulose (g)	1	1.5	2	2.5	3
3.	Ethyl Acetate (ml)	20	20	20	20	20
4.	Acetone (ml)	10	10	10	10	10
5.	Tween 80 (ml)	0.4	0.4	0.4	0.4	0.4
6.	Distilled Water (ml)	250	250	250	250	250

Evaluation of Microspheres

Particle size analysis

Particle size of the microspheres was determined by optical microscopy. The eyepiece micrometer was calibrated with the help of a stage micrometer. The particle diameters of more than 50 microspheres were measured randomly. The average particle size was determined by using Edmondson's equation. (14)

$$D = \Sigma nd / \Sigma n$$

Where, n = Number of microspheres checked; D = Mean of the size range.

Percentage Yield

The dried microspheres were weighed and percentage yield of the prepared microspheres was calculated by using the following formula, (15)

Percentage yield = {the weight of microspheres / (The weight of polymer + drug)}*100

Drug entrapment efficiency

Microspheres were crushed using a glass mortar by pestle and equivalent to 5 mg of Capecitabine weighed. These microspheres were suspended in 25 ml of phosphate buffer pH 6.8. After 24 h, the solution was filtered; 1 ml of the filtrate was pipette out and diluted to 10 ml and analyzed for the drug content using UV Visible spectrophotometer at 265 nm.

The drug entrapment efficiency was calculated using following formula:

% Drug entrapment efficiency = (Practical Drug content / Theoretical Drug content) X100

Percent drug content

The microsphere was powdered, accurately weighed a quantity of the powder equivalent to 250 mg of Capecitabine, transfer to a 500ml volumetric flask using 300 ml of methanol, the resulting suspension was heated to 60 and shaking for 15 minutes. Cool, dilute to 500.0 ml with methanol dilute a suitable volume of the filtrate with sufficient methanol to produce a solution containing 0.01% w/v of Capecitabine. Measure the absorbance of the resulting solution at the maximum at about 304nm. (16).

Floating ability of microspheres

Floating microsphere (50 mg) were placed in 0.1 N HCl (100 ml) containing 0.02% tween 20. The mixture was stirred at 100 rpm in a magnetic stirrer. The layer of buoyant microsphere was pipetted and separated by filtration at 1, 2, 4 and 8 hours. The collected microsphere was dried in a desiccator overnight. The percentages of microspheres were calculated (17).

***In-vitro* release studies**

In-vitro dissolution test was carried out using USP type I apparatus at 37 ± 0.5°C in 900 ml of phosphate buffer solution pH 6.8. Microspheres equivalent to 250 mg Capecitabine was tied at the bottom of the paddle using muslin cloth and rotated at 100 rpm. A sample of 5 ml was

withdrawn at various time intervals like 30, 60, 120, 180, 240, 300, 360 and 420 min and filtered. Analyze the filtered sample by UV Spectrophotometer at 304 nm and find the amount of drug release at each interval to calculate cumulative % of drug release after each time interval.

Surface morphology study

From the formulated batches of floating microspheres, formulation which slowed an appropriate balance between the buoyancy and the percentage release was examined for shape using scanning electron microscope. (18, 19).

Accelerated stability studies

From the prepared floating microsphere, formulation which showed an appropriate balance between the buoyancy and the percentage release was selected for stability studies. The floating microspheres (F) were placed in borosilicate screw capped glass containers and stored at temperature (40°C ± 2°C) with relative humidity (75% ± 5% RH) For a period of 60 days. The samples were assayed for drug content at regular intervals of 15 days. (20).

RESULT AND DISCUSSION

Drug-Excipient Interaction Study

DSC Analysis

The preformulation study was performed by DSC and found that there was no any interaction between Capecitabine and excipients. By DSC conclude that Capecitabine gives peak at 141.6^{0}C which has its Melting point peak which is correlated with formulation Melting point peak. So, there was no interaction between Drug and Polymers.

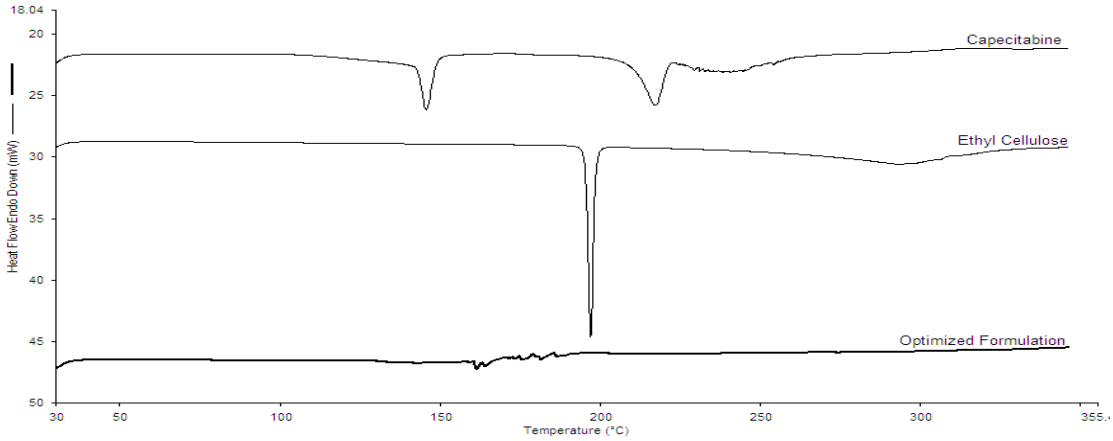

Fig. No. 1: Overlay of all DSC graphs.

Fourier Transforms Infrared Spectroscopy (FT-IR) Analysis:-

The FT-IR spectra of the pure drug and physical mixture of drug-polymers were recorded to check interaction between drug and polymers. The characteristic pick of Capecitabine appeared in the spectra of physical mixture without any makeable change in the position. This indicates that there was no chemical interaction between Capecitabine and polymers.

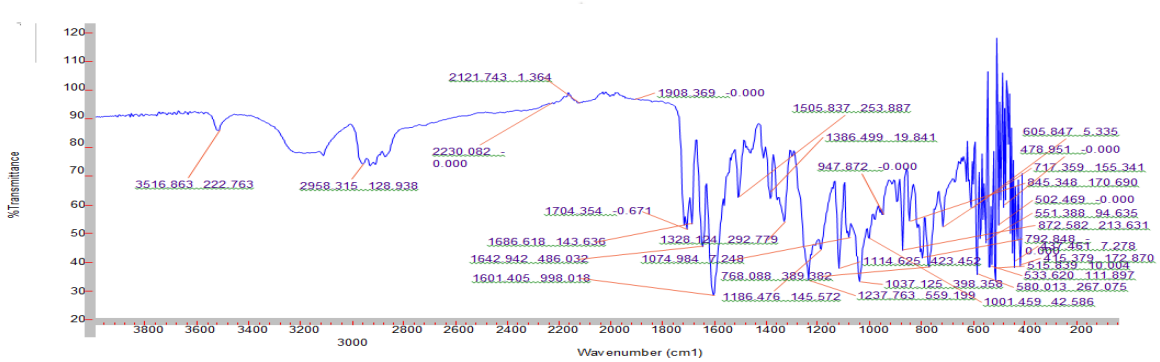

Fig No. 2: Capecitabine IR Spectra.

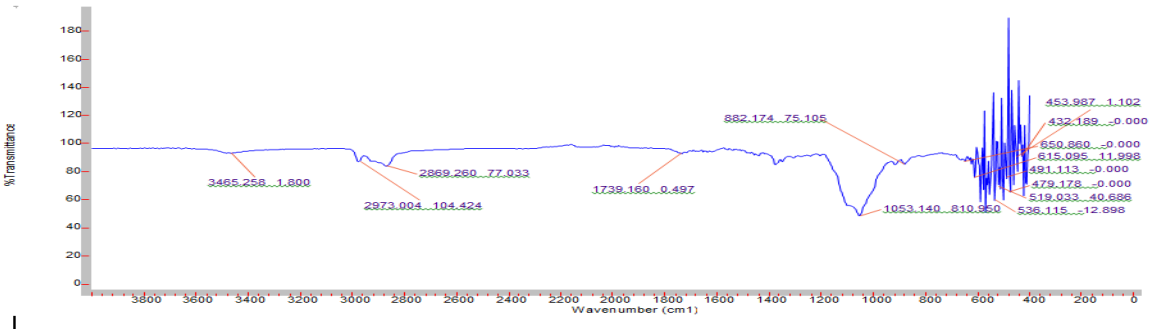

Fig. No. 3: IR Spectra of Drug + Ethyl Cellulose + Ethyl Acetate + Tween 80.

Evaluation of Floating Microspheres:

Particle size analysis:

The particle size of floating microspheres varied somewhat among the formulation due to variation in the composition of formulations. The mean particle size of floating microspheres formulation which showed relatively higher percentage of entrapment was in the range of 102 – 118 microns. Formulation showed relatively small size floating microspheres. Smaller the microspheres, floating ability will be less and faster will be the release rate of drug from microspheres, While larger the size, floating ability will be more and sustained will be the release of drug.

Table No. 2: Particle size of Microspheres

Batches	particle size (µm)	Angle of Repose	Bulk density	Tapped density	Carrs index
F1	102 ± 0.38	17°.91± 0.12	0.361± 0.02	0.428± 0.02	15.65± 0.02
F2	106 ± 0.69	19°.66± 0.11	0.385± 0.09	0.464± 0.12	17.02± 0.03
F3	112 ± 0.52	20°.26± 0.01	0.389± 0.07	0.474± 0.19	17.93± 0.07
F4	113 ± 0.31	22°.64± 0.03	0.450± 0.01	0.522± 0.03	13.79± 0.01
F5	118 ± 0.62	20°.52± 0.05	0.480± 0.03	0.567± 0.01	15.34± 0.05

n=6

Angle repose of floating microspheres was observed in range of 17°.91 ± 0.12'- 22°.64'± 0.03 i.e. less than 30 as shown in Table 2. All formulation showed good free flowing nature. The bulk density value of different microspheres ranged 0.361 ± 0.02 to 0.480± 0.03gm/cm^3.The tapped density value of microspheres ranged from 0.428 ± 0.02 -0.567± 0.01gm/cm^3. The density of microsphere was less than the density of gasric fluid (1.004g/cm^3) thereby, it will have good buoyancy property in stomach. The percentage compressibility index values ranged between 15.65 ± 0.02%-17.93± 0.07%. The percentage compressibility value less than 20 for all formulation suggested excellent flow property of floating microspheres.

Percentage yield:

The percentage yield of different batches was determined by weighing the floating microspheres after drying. The percentage yield of different formulation was found to be in range of 68.58 ± 0.55% to 78.39 ± 0.58% as shown in Table 3. The percentage yield of floating microspheres appeared unchanged by changing polymer ratio.

Drug entrapment efficiency:

The entrapment efficiency of different batches of floating microspheres was found in the range of 76.39 ± 0.84% to 82.39 ± 0.39% w/w as shown in Table 3. Drug entrapment efficiency was decreased with the increased drug concentration and increased with increasing polymer concentration in floating microspheres. This may be due to solubility of Capecitabine in water and water can penetrate in polymer which facilitates the diffusion of a part of entrapped drug to surrounding medium preparation of floating microspheres.

Table No. 3: % Entrapment Efficiency of Microspheres

Sr. No.	Batch	Entrapment Efficiency (%)	Percentage Yield. (SD)	% Drug Content
1.	F1	82.39 ± 0.39	78.39 ± 0.58	99.80 ± 0.01
2.	F2	82.56 ± 0.42	72.35 ± 0.39	99.78 ± 0.02
3.	F3	81.39 ± 0.31	70.56 ± 0.63	99.81 ± 0.03
4.	F4	78.58 ± 0.62	68.38 ± 0.34	99.72 ± 0.05
5.	F5	76.39 ± 0.84	68.58 ± 0.55	99.56 ± 0.09

n=6

Percent drug content:

The drug content of different batches of floating microspheres was found in the range of 99.78± 0.02 - 99.56 ± 0.09%.

Floating ability of Microspheres:

The floating test was carried out to investigate the floating ability of the prepared microspheres. Floating Microsphere was dispersed in 0.1 N HCl containing Tween 20 (0.02%w/v). Tween 20 was added to counteract the downward pulling at the liquid surface by lowering surface tension.

Floating ability of different formulation was found to be differed according to polymer ratio. F1-F5 formulations showed best floating ability (99.02 – 88.58 %) in 12 hours. F4 formulation showed best floating ability (99.23 - 89.71 %) in 12 h as showed in table.

Table No. 4:- Evaluation of floating efficiency of microspheres.

Sr. No.	Batch	1 hr.	2 hrs.	4 hrs.	8 hrs.	12hrs.
1.	F1	99.38 ± 0.03	98.32 ± 0.12	97.02 ± 0.02	97.63 ± 0.05	94.33 ± 0.19
2.	F2	99.28 ± 0.09	97.22 ± 0.01	96.39 ± 0.01	95.53 ± 0.06	91.47 ± 0.17
3.	F3	99.24 ± 0.07	98.69 ± 0.04	95.52 ± 0.09	94.68 ± 0.09	90.38 ± 0.12
4.	F4	99.23 ± 0.01	97.69 ± 0.13	94.87 ± 0.01	93.57 ± 0.03	89.71 ± 0.01
5.	F5	99.02 ± 0.02	96.89 ± 0.14	93.01 ± 0.06	92.35 ± 0.16	88.58 ± 0.03

n=6

***In-vitro* drug release studies:**

Release of Capecitabine from floating microsphere was evaluated in 0.1 N HCl (PH 1.2). Polymer ethylcellulose is of low permeability were insoluble in water. Floating microsphere showed sustained release of the drug acidic condition (pH 1.2) and the drug release was found to be approximately linear. Approximately 20% of the drug was released initially. Furthermore, drug release from the floating microspheres matrix was controlled by the polymer.

Ethyl cellulose is not a water soluble polymer and it does not show pH dependency. As the polymer content was increased and the drug loading was decreased, the release of drug was decreased significantly. In order to increase the release rate of drug, the ratio of drug and polymer is decrease and increase respectively. Formulation F4 showed best appropriate balance between buoyancy and drug release rate.

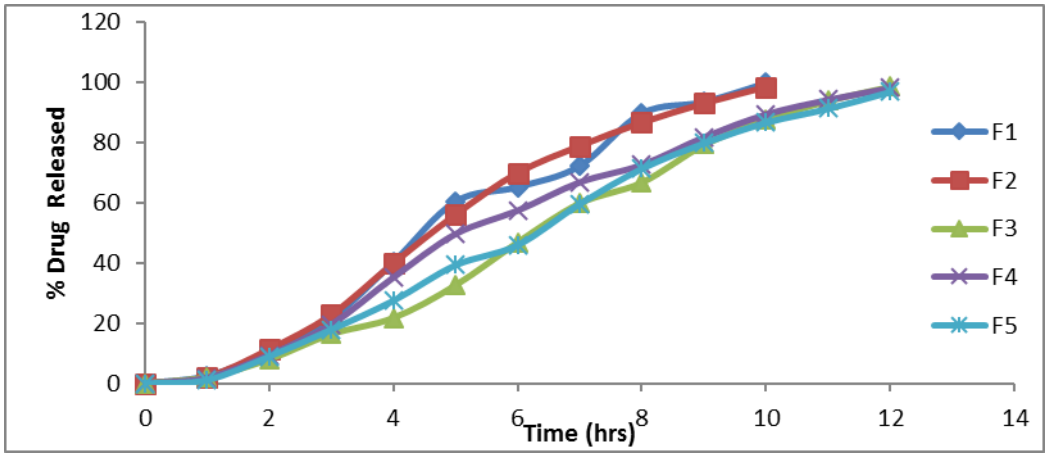

Fig. No. 4: Graphical % Drug release of microspheres

Surface morphology study:

The floating microspheres were examined by surface morphology study as shown in Fig. 5. These figure illustrating the microscopes were spherical with no visible major surface irregularity. Few wrinkles and inward dents appeared at the surface and some crystal shape particles appeared. It may due to collapse of floating microspheres during the in-situ drying process.

The surface morphology of both formulations was examined at higher magnification (1800X) which illustrates the smooth surface of floating microsphere. Some small pores and cavities were present on the surface of floating microspheres, Some small pores and cavities were present on the surface of floating microspheres, probably arising as a trace of solvent evaporation during the process.

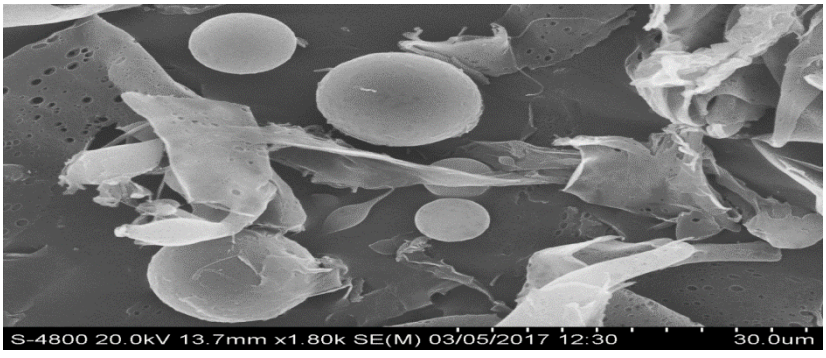

Fig. No. 5: SEM of Formulation F4 at 1800X

Kinetic modeling

The *in-vitro* release data was applied to various models to predict the drug release kinetic mechanisms. The release constant was calculated from the slope of appropriate plots and the regression coefficient (r) was determined. It was found that the in-vitro drug release of floating microspheres was best explained by first order kinetics as the plots shows highest linearity.

Table No. 5: Kinetic Modeling

Batches	Zero Order		First Order		Matrix		Korsmeyer Peppas		
	(R)	(K)	(R)	(K)	(R)	(K)	(R)	(K)	(n)
F1	0.932	8.484	0.998	-0.162	0.199	24.729	0.985	16.458	0.711
F2	0.924	9.077	0.995	-0.193	0.993	26.503	0.994	19.781	0.650
F3	0.927	8.902	0.997	-0.182	0.992	25.978	0.6991	18.698	0.669
F4	0.919	9.006	0.998	-0.186	0.992	26.318	0.994	19.793	0.647
F5	0.932	8.730	0.998	-0.173	0.991	25.450	0.993	18.001	0.678

n=6

Accelerated stability studies of formulation F4:

Accelerated stability study was carried out for optimized batch (F4) by exposing it to temperature 40°C ± 2°C and 75 ± 5% RH for 60 days. The sample was analyzed for drug content at the regular intervals for 15 days. It was found that no remarkable change in the drug content of F4 formulation.

Table No. 6:- Accelerated Stability Study of formulation F4:-

Sr. No.	Days	Colour	% Drug Content	% Drug Release
1.	0	Off White.	99.72 ± 0.05	98.10 ± 0.01
2.	15	No Change.	99.70 ± 0.02	98.07 ± 0.06
3.	30	No Change.	99.15± 0.06	97.61 ± 0.07
4.	45	No Change.	98.89± 0.01	97.42 ± 0.09
5.	60	No Change.	97.67 ± 0.07	96.87 ± 0.10

n=6

The IR spectrum did not show presence of any additional peaks for new functional groups indicating no chemical interaction between drug &excipients used in formulations.

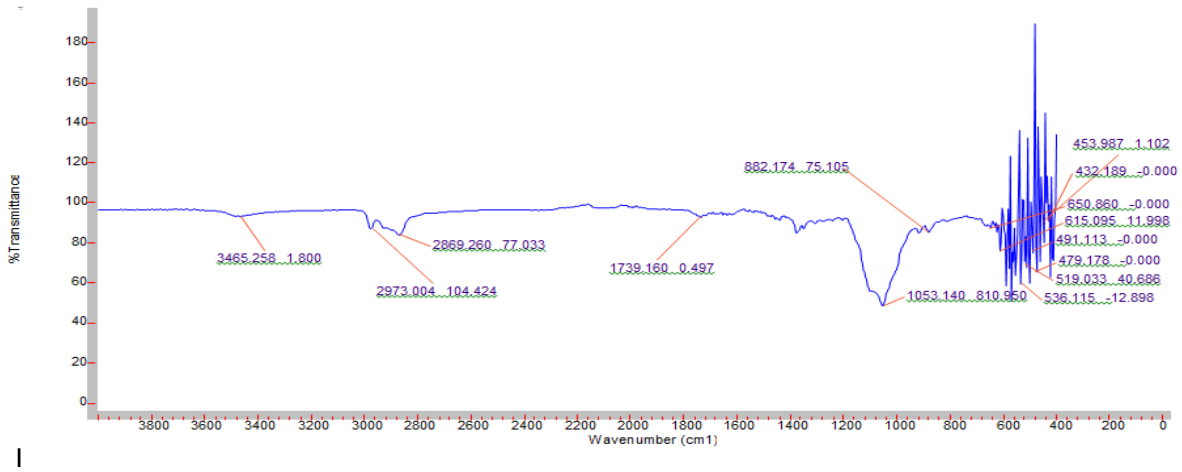

Fig. No. 6: IR spectra after Stability study of Formulation F4

CONCLUSION

The prepared floating microsphere prepared by using Emulsion – Solvent evaporation method showed acceptable drug entrapment and floating behavior with drug release up to 8h. The microspheres can be prepared by using this method. The prepared microspheres were able to pass all the evaluation parameters which are necessary for the ideal properties of microspheres.

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