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DESIGN, DEVELOPMENT AND EVALUATION OF LOPINAVIR FLOATING MICROSPHERES BY IONIC GELATION TECHNIQUE

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ABSTRACT

Aim: The aim of the present study is to prepare and characterize floating microspheres containing Lopinavir using Sodium alginate as the polymer. **Methods:** The Lopinavir loaded floating microspheres were prepared by Ionic gelation method. Floating Microspheres of different core: coat ratio were prepared and characterize for process yield, loading efficiency, particle size, zeta potential, *in vitro* drug release, kinetic studies and stability studies. **Results:** The prepared floating microspheres were white, free flowing and spherical in shape. The infrared spectra and differential scanning colorimetry thermographs showed stable character of Lopinavir in the drug-loaded floating microspheres and revealed the absence of drug polymer interactions. The floating microspheres have a zeta potential -15.5 mV. The formulation with the initial lopinavir concentration of 0.5 mg/ml provided the highest loading capacity. The *in vitro* release behavior from all the drug loaded formulation batches were provided sustained release and follow first order over a period of 12 h. No appreciable difference was observed in the extent of degradation of product during 90 days in which floating microspheres were stored at various temperatures. **Conclusion:** The best-fit release kinetics was achieved with First order followed by Higuchi plot. The release of Lopinavir was influenced by the drug to polymer ratio and particle size and was found to be diffusion controlled. According to the data obtained, this Sodium alginate-based floating microspheres opens new and interesting perspectives as drug carriers for treating the AIDS.

KEYWORDS

Floating Microsphere, Sodium alginate, Lopinavir and Ionic gelation.

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INTRODUCTON

The delivery of therapeutic agents is increasingly being the oral route used because the low cost of the therapy and ease of administration lead to high levels of patient compliance. Oral drug delivery systems are available in the market should be more than 50% of the drug delivery systems[1]. The floating drug delivery system was first described by Davis (1968). Several approaches are presently used to extend gastric retention time. These consist of

floating drug delivery systems. FDDS are known as Hydro dynamically balanced systems or low density system that has been made established in order to rise the gastric transport time of drug. These microspheres are characteristically free flowing powders consisting of natural or synthetic polymers and ideally having a particle size not more than 200µm. Microspheres including a drug spread or dissolved all over particle matrix have the potential for the controlled release of drug[2].

Microspheres are one of the multiparticulate delivery system and are prepared to obtain controlled release from the dosage form to improve bioavailability, reduce the adverse effect and prolong the action of drug, decrease absorption change in patients, decrease the dosing frequency and adverse effects in prolong treatment. It is needed to formulate in long acting dosage form, reaching to effective biological site rapidly.

MECHANISM

Mechanism of flotation of microspheres: When microspheres come in contact with gastric fluid, the gel formers, polysaccharides, and polymers hydrate to custom a colloidal gel barrier that switches the rate of fluid saturation into the device and consequent drug release. As the exterior surface of the dosage form dissolves, the gel layer is maintained by the hydration of the adjacent hydrocolloid layer. The air trapped by the swollen polymer lowers the density and confers buoyancy to the microspheres. However a minimal gastric content is needed to allow proper achievement of buoyancy[3].

Advantages of Floating Microspheres

1. It enhance the bioavailability
2. It sustains the drug delivery and reduces the regularity of dosing.
3. It is directed therapy for local disorder in the upper gastro-intestinal tract.
4. Reduce fluctuation of drug.
5. Minimize adverse activity at the colon.
6. The gastric irritation is reduce affected by acidic drug.
7. When enthusiastic intestinal effort arises in diarrhea, the biological half-life of the drug get decreased. In that condition the floating microspheres float in the gastric content and enhance the absorption[4].

Limitations of Floating Drug Delivery Systems

1. A high level of fluid in the stomach is required for drug delivery to float and work well.
2. Stability and solubility of the drugs having complications in GIT are not suitable candidates for these types of system.1(g)
3. Drugs such as Nifedipine, which under go first pass metabolic rate may not be required for the preparation of these types of systems[5].

Lopinavir is an antiretroviral of the protease inhibitor class. Inhibiting HIV-1 protease, effects in selectively preventing the cleavage of HIV gag and gag-pol polyproteins, thereby preventing viral maturation. Lopinavir inhibits the HIV viral protease enzyme. This stops cleavage of the gag-pol polyprotein and, for that reason, improper viral assembly results. This subsequently results in non-infectious, immature viral particles. Administered alone, has insufficient bioavailability; however, like several HIV protease inhibitors, its blood levels are greatly increased by low doses of ritonavir, a potent inhibitor of cytochrome the recommended therapeutic range of oral Loinavir is 100 to 100mg/day. Lopinavir can be administered either in a thrice daily. It is currently supplied in 100 and 400mg delayed release capsules and Tablet No. 2(a, b). The oral bioavailability of Lopinavir is approximately 98-99% bound to human plasma proteins. Lopinavir binds to both albumin and alpha 1-acid glycoprotein. Protein binding is not affected by renal or hepatic impairment. In the present study, efforts were made to incorporate lopinavir, HPMC and ethyl cellulose polymers by applying solvent evaporation method[6].

MATERIAL AND METHODS

Materials

LOPINAVIR is a gift sample from the Hetero drugs ltd, Hyderabad (India), Sodium alginate was obtained from Rolex chemical industries Mumbai (India), Acetic acid was obtained from Spectrum

reagents and chemical Pvt ltd, Calcium chloride was obtained from SD fine chemicals Ltd. Mumbai (India), Calcium chloride was obtained from Thermo fisher Scientific India Pvt. Ltd. All other chemical and reagent used in this study were of analytical grade.

METHOD OF PREPARATION8

Ionic Gelation Technique[7]

Sodium alginate solutions of different concentrations were prepared by dissolving required amount of alginate in 100 ml of deionized water under gentle agitation. Lopinavir and calcium carbonate (as gas forming agent) were dispersed in sodium alginate solution to form uniform mixing with constant stirring. The dispersion was sonicated for 30 minutes to remove any air bubbles. The resultant dispersion was dropped through a 22 gauge syringe needle into 100 ml of 10% (v/v) acetic acid solution containing 1% (w/v) calcium chloride at room temperature. Then the beads formed were allowed to remain for 10 min in the stirred solution. The beads are filtered and oven-dried at 50ºC for 4 hours. The composition and the formulation design of this microsphere is given in Table No.1.

CHARACTERIZATION OF PREPARED FLOATING MICROSPHERES

Fourier Transforms Infra-Red Spectroscope (FTIR) Analysis[8]

The FT-IR spectra of pure Lopinavir and Sodium alginate floating microspheres loaded Lopinavir were recorded to check drug polymer interaction (Figure No.1).

Differential Scanning Colorimetry (DSC)

The DSC analysis of pure drug and drug loaded floating microspheres were carried out using a Diamond DSC to determine some probable drug polymer interaction. The analysis was performed at a rate 5.00℃ min^{-1} from 10℃ to 400℃ temperature range under nitrogen flow of 25 ml min-1 (Figure No.2).

Bulk Density[9]

Bulk density of a compound varies substantially with the method of crystallization, milling or formulation. Bulk density is determined by pouring sieved granules into a graduated cylinder and measure the volume and weight.

$$\text{Bulk density} = \frac{\text{weight of granules}}{\text{Bulk volume of granules}}$$

Bulk density was expressed in g/cc.

Tapped Density

Tapped density is determined by a graduated cylinder containing a well-known mass of granules and mechanical tapper apparatus, which is operated for a fixed number of taps up to the powder bed volume has stretch to a minimum volume. By means of the weight of the drug in the cylinder and this minimum volume, the tapped density may be computed.

$$\text{Tapped density} = \frac{\text{weight of granules}}{\text{Tapped volume of granules}}$$

Carr's Index (Ci)

Carr's index is measured using the values of bulk density and tapped density. The following equation is used to find the Carr's index.

$$CI = \frac{(TD-BD)\ x100}{TD}$$

Where TD = Tapped density

BD = Bulk density

Hauser's Ratio

It indicates the flow properties of the powder and ratio of Tapped density to the Bulk density of the powder or granules.

Hauser's Ratio = Tapped density / Bulk density

Angle of Repose

The manner in which stresses are transmitted through a bead and the beads response to applied stress are reflected in the various angles of friction and response. The method used to find the angle of repose is to pour the powder ion a conical heat on a level, flat surface and measure the included angle with the horizontal.

$$\mathbf{Tan\theta = h/r}$$

Where, h= height of the heap

r= Radius of the heap

Percentage Yield

The dried microspheres were weighed and percentage yield of the prepared microspheres was calculated by using the following formula.

$$\text{Percentage yield} = \frac{\text{Practical yield} \times 100}{\text{Theoretical yield}}$$

Drug Entrapment Efficiency[10]

The floating microspheres equivalent to 10 mg of Lopinavir were accurately weighed and crushed. The powdered of microspheres were dissolved in methanol (5 ml) in volumetric flask (100ml) and made the volume with 0.1 N HCl. This solution was then filtered through Whatsman filter paper No. Later proper dilution the absorbance was measured at 257 nm by UV spectrophotometer using 0.1N HCL as a blank and corresponding drug concentrations in the sample were calculated from percentage drug encapsulated and the calibration plot was calculated by following formula:

$$\%\ \text{Drug content} = \frac{\text{Calculated amount of lopinavir} \times 100}{\text{Total weight of hollow microspheres}}$$

$$\%\text{Theoretical content} = \frac{\text{Total weight of lopinavir} \times 100}{\text{Total weight of lopinavir and polymer}}$$

$$\%\text{Drug encapsulation} = \frac{\%\ \text{Drug content} \times 100}{\%\text{Theoretical content}}$$

Floating Behaviour (Buoyancy)

50 mg of the floating microspheres were placed in 100 ml of replicated gastrointestinal fluid (pH 1.2) containing 0.02% w/v Tween 20. The mixture was stirred at 100 rpm on a magnetic stirrer. Later 4 h, the layer of buoyant microspheres was pipetted and separated; particles in the sinking particulate layer were also separated by filtration. Particles of both types were dry in desiccators. The microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

$$\%\ \text{Buoyancy} = \frac{\text{Microsphere remained floating} \times 100}{\text{Total mass of microspheres}}$$

Surface Morphology Study[11]

Scanning electron microscopy (SEM) was performed to characterize the surface of formed microspheres. Microspheres were fixed straight on the sample stub and coated by gold film under reduced pressure. This film acts as a conducting medium on which a stream of electron was allowed to flow and then photograph was taken with SEM.

Particle Size Distribution[12]

Microspheres were separated into different size fractions by sieving for 10 minutes using a mechanical shaker containing standard sieves and mean particle sizes of microspheres were calculated.

Zeta Potential[13]

The Zeta-potential of floating microsphere was measured by Zeta sizer (Malvern Zetasizer 3000HS, UK). To decide the zeta potential, floating microspheres samples were diluted with KCl (0.1 mM) and placed in electrophoretic cell where an electrical field of 15.2 V/cm was applied. Each sample was analyzed in triplicate.

***In-Vitro* Drug Release Studies**[14]

A modified USP XXIV dissolution apparatus type I (basket) was used to study *in vitro* drug release from the microspheres. The dissolution test was carried out individually at 100 rpm in distilled water and 1M HCl (pH 1.2) as dissolution media (900 ml) maintained at 37 ± 1 0C. Samples (2ml each) were withdrawn at intervals and analysed spectrophotometric ally at 234 nm. The release medium was replenished with the same amount of fresh medium to maintain sink conditions. All experiments were performed in triplicate. Cumulative drug release (%) was calculated from a standard curve.

KINETIC MODELING[13]

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of floating microspheres were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), Peppas plot (log of cumulative % drug release vs. log time). R2 and 'n' values were calculated for the linear curve obtained by regression analysis of the above plots (Table No.4).

Stability Study[8]

The stability study was carried out using the batch FI-4. Formulation FI-4 was divided into 3 sets of samples and stored at 5 } 3℃in refrigerator, room temperature and 45 ℃ } 2℃, 75% RH in humidity

control ovens. After 90 days drug content of all samples were determined by the method as in drug content (Figure No.5). *In vitro* release study of formulation FI-4 was also carried out after 90 days of storage (Table No.5 and Figure No.6).

RESULTS AND DISCUSSION

Floating Microspheres prepared by Ionic gelation technique were found to be discrete and through SEM analysis. These microspheres were characterized for flow properties and the results are given in (Table No.2). All the formulations offered good flow property. Floating microspheres prepared by Ionic gelation technique were found to be discrete and through SEM analysis (Figure No.3), their mean size distribution was found to be 161 nm. Since the particle size is less than 200 μm, this drug delivery system can be used for parenteral formulations, drugs administered which avoiding first pass hepatic metabolism and reaching a reduction in the dose delivered. The drug entrapment efficiency of floating microspheres containing drug: polymer in various ratios of 1:2, 1:2.5, 1:3, 1:3.5 and 1:4 were found to be 53.3%, 54.04%, 56.5%, 81.56% and 60.01%. Thus there was a steady increase in the entrapment efficiency by increasing the concentration of the polymer in the formulation. The interaction study between the drug and polymer was evaluated using FT-IR spectrophotometer. There was no significant difference in the IR spectra of drug loaded floating microspheres. Differential scanning calorimetry study thermo gram of pure Lopinavir showed a sharp endothermic peak at 170□. The thermo grams of formulations FI-4 of (Figure No.2), showed the same endothermic peak at the similar temperature. This further confirmed that there is no drug to polymer interaction. This indicates that they are stable. Cumulative percentage drug released for FI-1, FI-2, FI-3, FI-4 and FI-5 after 12 h were found to be 92.47%, 80.94%, 80.94%, 78.94% and 88.60%respectively. Zeta potential for FI-4 was found to be -15.9mV and it shows good stability. It was apparent that *in vitro* release of Lopinavir showed a very rapid initial burst and then followed by a very slow release of drug. In order to describe the release kinetics of all five formulations were fitted in various kinetic dissolution models like zero order, first order, and Higuchi respectively. As indicated by higher R2 values, the drug release from all formulations should follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The Peppas model is used to confirm whether the release mechanism is Fickian diffusion, Non-fickian diffusion or zero order. 'n' value could be used to characterize different release mechanisms. The 'n' values for all formulations were found to be greater than 0.50. This indicates that there lease approximates Non-fickian diffusion mechanism.

Table No.1: Formulation Table of Floating Microspheres of Lopinavir

S.No	Ingredients	FI-1	FI-2	FI-3	FI-4	FI-5
1	Sodium Alginate (mg)	200	250	300	350	400
2	Drug (mg)	100	100	100	100	100
3	Calcium Carbonate (mg)	150	150	150	150	150
4	Calcium Chloride (W/V)	1%	1%	1%	1%	1%
5	ACETIC ACID(V/V)	10%	10%	10%	10%	10%

Table No.1 (a): Flow properties and corresponding Carr's index values

Excellent	<10
Good	11 – 15
Fair	16 – 20
Possible	21 – 25
Poor	26 – 31
Very poor	32 – 37
Very very poor	>38

Table No.1 (b): Flow properties and Corresponding Hauser's ratio

Excellent	1.00 – 1.11
Good	1.1 – 1.18
Fair	1.19 – 1.25
Possible	1.26 -1.34
Very poor	1.35 -1.45
Very very poor	>1.60

Table No.1 (c): Flow properties and Corresponding angle of repose

S.No	Angle of repose	Powder flow
1	< 25	Excellent
2	25 – 30	Good
3	30 – 40	Passable
4	> 40	Very poor

Table No.2: Flow Properties of Lopinavir Floating Microspheres

S.No	Formulation code	Fluff bulk density(gm/ml)	Tapped bulk density(gm/mi)	Angle of repose(Ɵ)	Carr's index (%)	Hauser's ratio
1	FI-1	0.5945	0.6470	16º48'	8.1143	1.0883
2	FI-2	0.5869	0.6279	23º19'	6.5297	1.0561
3	FI-3	0.6923	0.7200	25º01'	3.8472	1.0400
4	FI-4	0.7068	0.7454	24º02'	5.1784	1.0546
5	FI-5	0.6562	0.8076	26º56'	18.7469	1.2307

Table No.3: Formulation and Physicochemical Characterization of Lopinavir Floating Microspheres

S.No	Formulation code	Drug: Polymer Ratio	% drug Entrapment Efficiency	Mean Particle Size(µm)
1	FI-1	1:2	53.3	155.3
2	FI-2	1:2.5	54.04	156.3
3	FI-3	1:3	56.5	152.3
4	FI-4	1:3.5	81.56	162.5
5	FI-5	1:4	60.61	178.6

Table No.4: Correlation Coefficients According To Different Kinetic Equations

S.No	Formulation code	%Cumulative drug release	Zero order	First order	Higuchi plot	Peppas plot	'n' Values
1	FI-1	92.47	0.8953	0.9571	0.9663	0.6722	1.2123
2	FI-2	80.94	0.9295	0.9501	0.9649	0.6390	1.1355
3	FI-3	80.94	0.9459	0.9566	0.9718	0.6506	1.1565
4	FI-4	78.90	0.9459	0.9742	0.9210	0.8232	1.3816
5	FI-5	88.60	0.9474	0.9891	0.9651	0.7204	1.2832

Table No.5: Stability Studies - *In Vitro* Release Study of A Selected Formulation Fi-4 after Three Months Storage at 5 □, Room Temperature, 45□±2□ /75% Rh

S.No	Time in hrs	%Cumulative Drug Release		
		5ºC±3ºC	30ºC±2 ºC/65%RH	40ºC±2ºC/75%RH
1	0	0	0	0
2	1	7.62	7.11	5.09
3	2	15.30	14.79	12.78
4	3	22.97	22.46	20.45
5	4	28.72	28.21	26.20
6	5	51.77	51.26	49.25
7	6	55.62	55.11	53.09
8	8	63.33	62.82	60.81
9	10	76.82	76.31	74.30
10	12	78.84	78.33	76.32

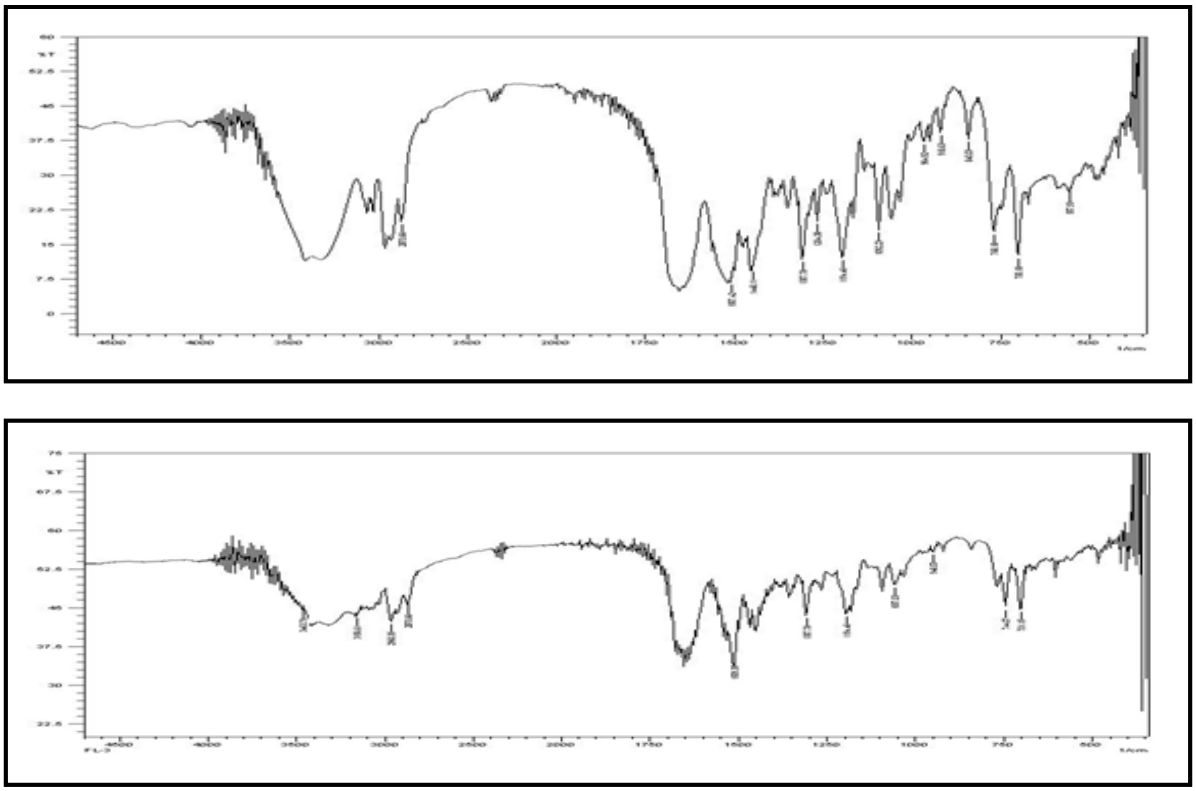

Figure No.1: FT-IR Spectra of (A) Pure Lopinavir, (B) Lopinavir Floating Microspheres

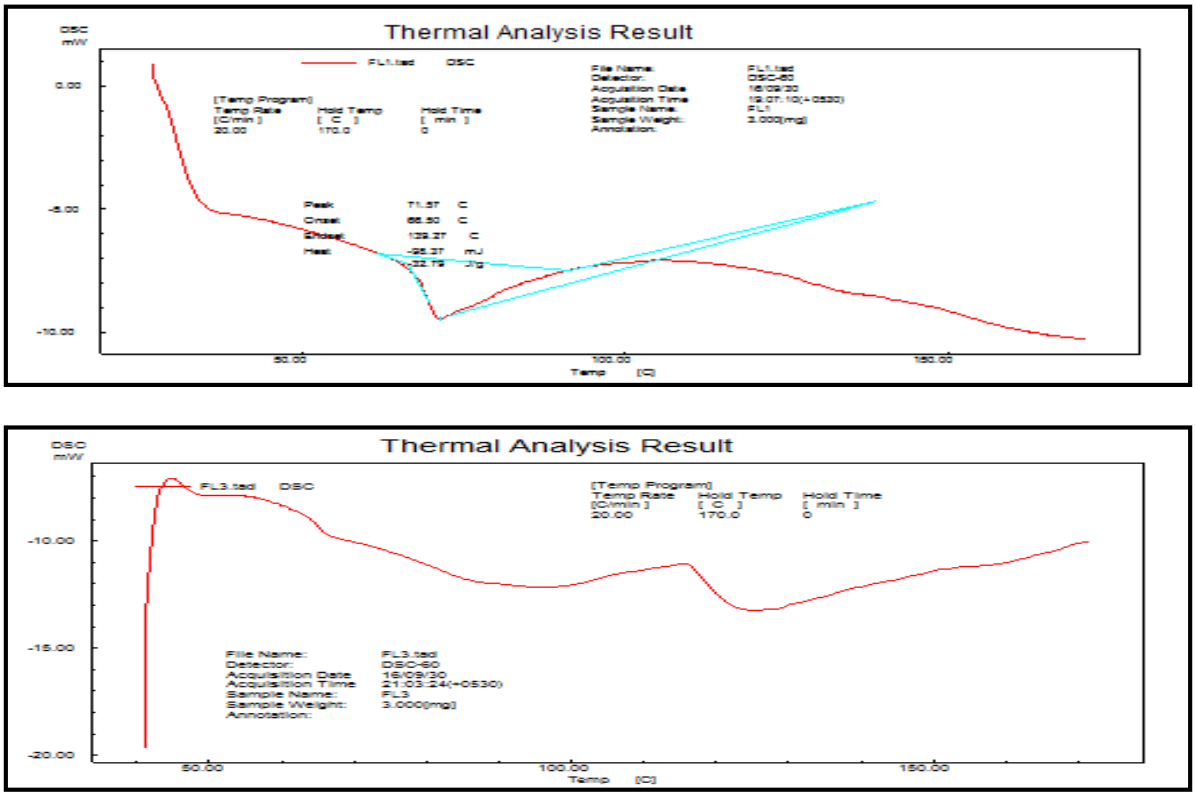

Figure No.2: DSC Thermo Grams of Pure Lopinavir and Lopinavir Loaded Sodium Alginate Microspheres

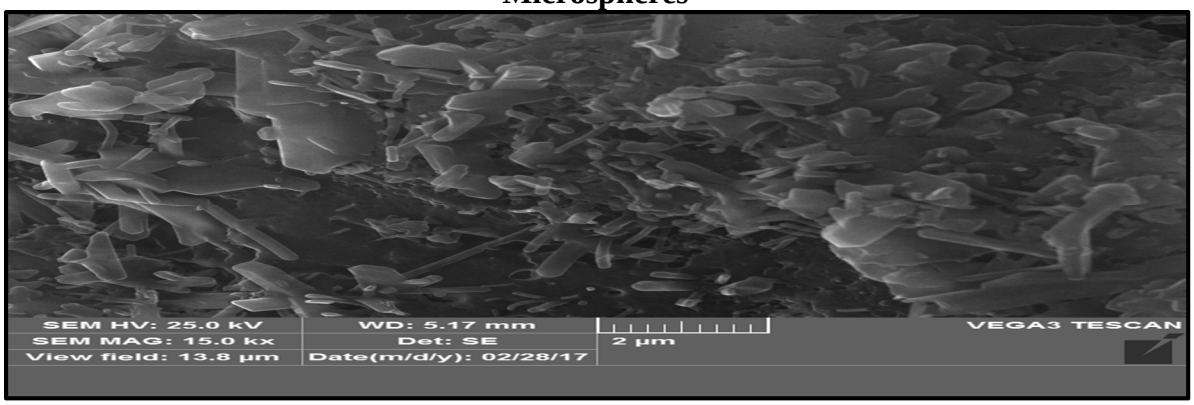

Figure No.3: SEM of Formulation Fi-4

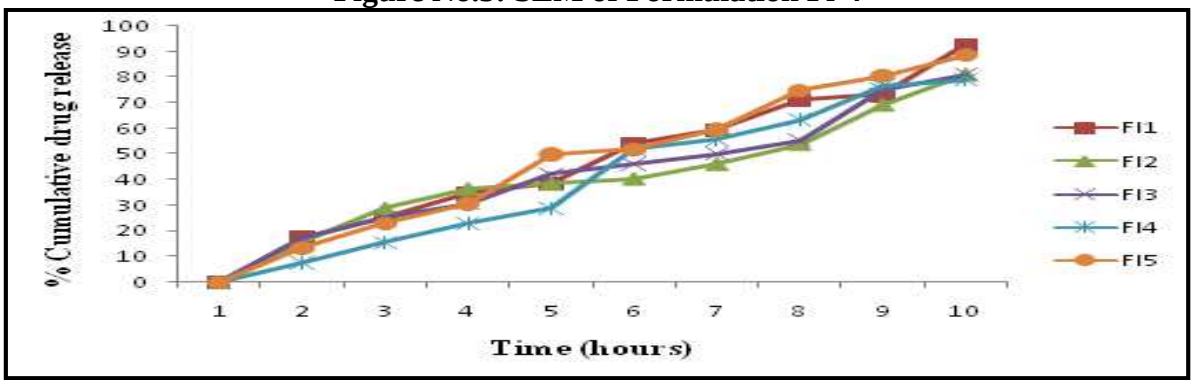

Figure No.4: % Cumulative Drug Release of Lopinavir Microspheres

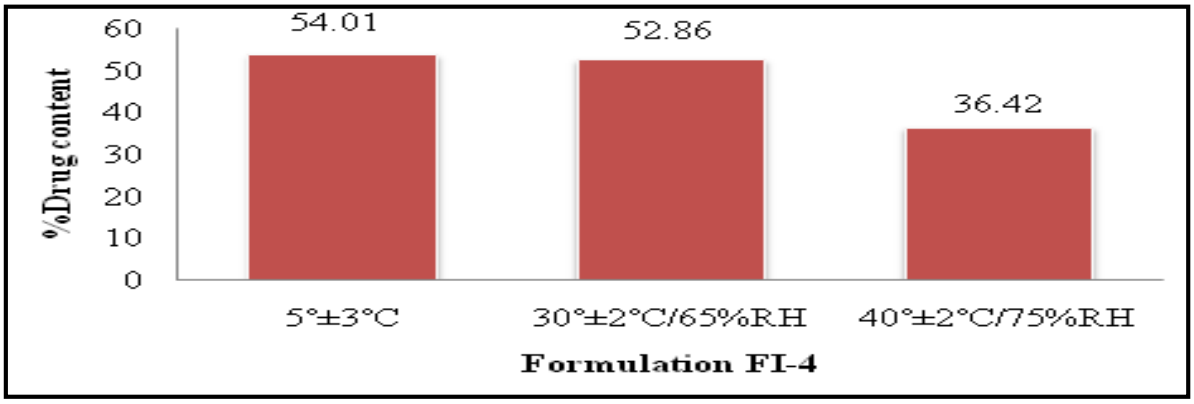

Figure No.5: Stability Study: Comparison of % Drug Content of Formulation Fi-4 At 5 } □, Room Temperature And 45□± 2□/75% Rh

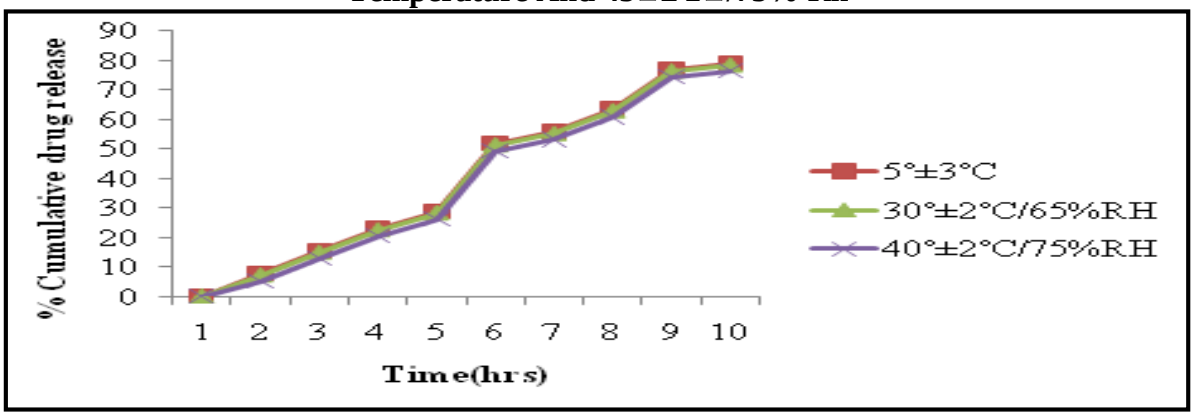

Figure No.6: Stability Study: Comparison of *In Vitro* Drug Release Profile for Formulation Fi-4 at 5 □, Room Temperature and 45□± 2□/75% Rh after Three Months Storage

CONCLUSION

Lopinavir floating microspheres were prepared by ionic gelation technique were found to be suitable for controlled release. The floating microspheres prepared by using Sodium alginate as a polymer show prolonged release rate when compared with other formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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