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IMPACT OF PROCESSING VARIABLES ON THE PREPARATION OF EUDRAJIT L100 & EUDRAGIT RSPO LOADED ACYCLOVIR MICROBEADS BY IONOTROPIC GELATION METHOD

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ABSTRACT

The purpose of the research was to develop sustained release microbeads of acyclovir using Eudragit RSPO, EudragitL100 and sodium alginate as polymers following ionotropic gelation technique. Calcium chloride and gluteraldehyde were used as the ionotropic gelling agent. Sodium alginate was crosslinked by calcium chloride leading to a slower release of the drug. The percentage yield of all the formulations (F1-F5) was found to be within the range of 92.85% to 98.36% which denotes the suitability of the method of formulation and the average particle size was found to be within the range of 1096-1389 µm. The processing variables affected the properties of the microbeads in different ways amongst which the F2 formulation shows high entrapment efficiency, low swelling ratio followed a sustained release profile of the drug. No interaction was found between polymer drug which can be proved by FTIR and DSC analysis**.**

KEYWORDS: Acyclovir, Eudragit RSPO, EudragitL100, Microbeads, Ionotropic gelation technique.

INTRODUCTION

The role of drug delivery today is to select a therapeutically effective molecule with sub-optimal physicochemical and physiological properties and to develop an optimized product that will still be therapeutically effective but with added benefits. This is accomplished using the concepts of bioavailability enhancement and immediate release for predetermined period

of time. The term drug delivery can be defined a technological processing (drug formulation) used to deliver the therapeutic agents inside the human body.

A dosage form is the physical type and amount of a medication, such as a capsule, tablet and injection etc. The route of administration is dependent on the dosage form of a given drug. In pharmacology and toxicology, a route of administration is the path by which a drug, fluid, poison, or other substance is brought into contact with the body. The most frequently used dosage forms include capsules, tablets, injections, liquids, patches, creams, and suppositories etc. As new drugs and medical therapies are created, new dosages forms are constantly under development.[1,2,3]

Microbeads can also be defined as small spherical, with diameters in the micrometers range 1µm to100µm (1mm). Microbeads are sometimes referred to as micro particles. Microbeads are the colloidal drug delivery system. These are characteristically free flowing powders consisting of proteins or synthetic polymers that are biodegradable in nature. Glass microbeads, polymers microbeads, and ceramic microbeads are commercially available. Solid and hollow microbeads vary widely in density and therefore are used for different applications. Hollow microbeads are typically used as additives to lower the density of a material. Solid microbeads have numerous applications depending on what materials they are constructed of and what size they are. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery system. Microbeads form an important part such novel drug delivery system. The success of these microbeads is limited owing to their short residence time at the site of absorption. However, different combination of polymers may give rise to a sustained release microbead delivery system. Microbeads preparation is carried out by following many methods such as single emulsion technique. Double emulsion technique, Polymerization techniques, phase separation coacervation technique, spray drying technique, ionotropic gelation method, solvent evaporation technique.

Acyclovir is an antiviral drug which is primarily used for the treatment of herpes simplex virus infection, chickenpox and shingles. It may be given orally, topically or intravenously. For oral administration, it is given as tablets of doses like 200, 400 and 800 mg depending upon the severity of the infection. It falls under class III category of BCS i.e. having high solubility and less permeability. When orally administered, peak plasma concentration

occurs after 1-2 hours,so frequency of administration is very high of minimum 5 times a day which comes very tough for the patient to take each time. Even its absorption in GIT is slow, variable and incomplete. The oral bioavailability also varies from 10-30% due to its high dose frequency; the patient becomes susceptible to high chance of adverse effect of the drug which is nausea, vomiting, diarrhoea. In order to make oral therapy more patient compliance there is an urge to formulate sustain release drug delivery dosage form. Various works have been carried out in order to sustaining the drug, acyclovir till date using various polymers. Most works were done taking polymers like ethyl cellulose, sodium carboxy methyl cellulose, sodium alginate, HPMC, chitosan and even Eudragit RS 100, RL, S100. But these polymers showed low entrapment efficiency of drug and variable drug release profile. Yet no works have been carried out taking Eudragit RSPO, EudragitL100 and sodium alginate in combination to develop microspheres. So my present objective is to develop sustained release microbeads of acyclovir using sodium alginate, Eudragit L100, Eudragit RSPO as polymers following ionotropic gelation technique. Further investigation will carry out about the effect of formulation processing variables on the preparation of microbeads.[3,4,5,6,7]

MATERIALS AND METHODS

Materials

Acyclovir was supplied from ACE Pharma, Mumbai. Eudragit L100 were purchased from B.S trading, Howrah and Eudragit RSPO was gifted by Evonik Lab, Mumbai. $Cacl_2$ & sodium alginate were supplied by SDFCL, Mumbai. Acetone, Ethyl acetate were supplied by B.S Trading lab. Tween 80 also supplied by SDFCL, Mumbai. Gluteraldehyde was supplied by Merck lab, Mumbai.

Formulation of acyclovir loaded microbeads

20ml of ethyl acetate & 30ml acetone were mixed with 50ml of water containing 1% tween 80 in a homogenizer at about 1300 – 1500 rpm for 15min. Then the drug acyclovir, eudragit L100 & eudragit RSPO were added in the above emulsion, with continuous stirring again for 15 mins, at the above rpm. To this dispersion 2% solution of sodium alginate was added and again homogenized for again 20mins. The final dispersion was taken into a glass syringe and added drop by drop to a combination of cross linking agent solution containing $CaCl_2$ and gluteraldehyde. Due to the presence crosslinking agent microbeads were formed which were separated by using moslin cloth and washed with water. Further they were dried in hot air oven at a temperature about 42°C-45°C over night. The blank microbeads were prepared on

the same way without inclusion of the drug acyclovir as in **Table 1**.[8,9,10] Composition of various formulations was mentioned as in **Table2**.

Percentage yield value

The percentage yield value is defined as the quantity of microspheres produced as a function of loaded drug and polymer.

%Yield= (Actual weight of product / Total weight of excipient and drug)×100 [9,10,11]

Determination of drug entrapment efficiency (DEE)

About 20mg of accurately weighed acyclovir loaded microspheres were dissolved in 100ml mixed phosphate buffer solution (p^H6.8) and kept on for whole night. Then the solution was filtered and analyzed spectrophotometrically at 252nm each experiment was carried out in triplicate. Reliability of the method was judged by conducting recovery analysis using known amounts of acyclovir with or without polymer and recovery average is 98.37±0.33.[10,11]

Particle size analysis

Particle size analysis of the acyclovir-loaded microspheres was done by sieving method. Several british standard sieves ranging 25 mesh to 150 mesh were kept orderly with decreasing size of mesh aperture to form a nest of sieves. A weighed amount of samples were placed on the top and the nest of the sieves was shaken with a mechanical sieve shaker (EGG80432, Geologists' Syndicate Pvt. Ltd, Kolkata). The samples retained on each sieve were collected and weighed. The arithmetic mean diameter was calculated from the method reported elsewhere (parrott 1991) and weight-size distribution has been represented after plotting the cumulative percentage passing the smaller sieve against the smaller sieve aperture.[12]

Swelling study

The swelling behaviors of blank beads cross-linked with different concentrations of $CaCl_2$ were studied separately in acid buffer and phosphate buffer solutions for 2 hr and 4hr respectively. Samples of known weight (10 mg) were placed to 100 ml of the medium and allowed to swell. The swollen beads were periodically removed, blotted with tissue paper, and weighed (Metler Toledo, AB 204-S, Switzerland). Each sample was evaluated thrice. The swelling ratios were calculated following the equation.

$$\text{Swelling Ratio} = (w_2\text{-}w_1)/w_1 \text{ X } 100$$

Where w_2 is the weight of swollen beads and w_1 is the weight of dry beads at the beginning of

the study.[9,10]

***In vitro* release studies**

The *in vitro* release of acyclovir from combination of eudragit polymer & sodium alginate microsphere was carried out in enzyme free, gastric, and intestinal fluids using USP type II dissolution rate test apparatus (model DA-6D,Veego digital dissolution rate test apparatus, Mumbai, India). About 20 mg of dried microspheres, accurately weighed, were suspended in 900ml of 0.1(N) HCl of pH 1.2 and mixed PBS solution of pH – 6.8. The paddle was rotated at 100 rpm and the temperature was set at 37±0.5^{o}c, At predetermined times 5ml sample was withdrawn and replenished with fresh buffer solution. The aliquots were analyzed using a double beam spectrophotometer (Thermo- spectronic UV-Vis spectrophotometer, Great Britain) at 269 nm. Cumulative percentage ofacyclovir release plotted as a function of time. Each formulation was run in triplicate.

Scanning electron microscopy

The shape and surface morphologies of the dried microsphere was investigated using scanning electron microscope (Jeol, JSM-5200, Japan). Prior to examinations samples were mounted onto stubs using double sided dried carbon type and vacuum coated with gold palladium film (thickness2nm) to render them elecctrically conductive using sputter coater (Edward-S-150, UK).

Fourier transforms infrared (FTIR) Spectroscopy

FTIR Spectra of finely powdered dried pure acyclovir, acyclovir loaded microspheres were recorded using Perkin Elmer FTIR Spectrometer (spectrux RX 1, UK) Using KBr disc. Each sample was gently triturated with KBr powder and then pressed by a hydraulic pellet press (TYPE-KP) applying 10 ton pressure for 15 min. The disc was placed in the sample holder and scanned from 4000 to 400cm^{-1}at a resolution of cm^{-1}.[12]

Differential scanning calorimetry (DSC)

DSC thermograms of pure acyclovir, acyclovir loaded microspheres were recorded using Perkin- Elmer on instrument (pyris-diamond TG/DTA, Singapore).Each sample was accurately weighed into 40µl aluminium pan in a hermetically sealed condition. The measurements were performed in an atmosphere of nitrogen between 30 and 500^{0}C at a healing rate of 10^{0}C/min.[12]

RESULTS AND DISCUSSION

In the present research work acyclovir drug was prepared into microspheres with sodium alginate, eudragit L100, eudragit RSPO to made as a controlled release formulations using calcium chloride and gluteraldehyde as a cross linking agents.

Percentage particle yield and average particle size

The percentage yield of all the formulations (F1-F5) was found to be within the range of 92.85% to 98.36% which denotes the suitability of the method of formulation. The percentage practical yield of all the formulations isshown in **Table 3**.

Prepared microbeads were evaluated for average particle size. The average particle size was found to be within the range of 1096-1389 μm. So the average particle size for all the formulations was within the range as mentioned in **Table 3.**

Entrapment efficiency

Prepared microbeads were evaluated for entrapment efficiency. The entrapment efficiency was found to be with the range of 85.39% to 98.37%. Amongst formulations (F1-F5), F2, F3 showed good entrapment efficiency. Based on good entrapment efficiency F2, F3 formulations were subjected to *in vitro* dissolution studies. Entrapment efficiency of all the formulations is shown in **Table 3.**

Swelling study

When the amount of eudragit L100 was increased from 10mg to 20mg the swelling ratio falls abruptly from 13 to 2.5 in phosphate buffer pH6.8. Similarly, the swelling ratio was again increased when 30 mg of Eudragit L100 was taken instead of decreasing. The same case also happens in the swelling behavior of the beads in acid solution pH1.2. Since we know that eudragit L100 dissolves in the range 6-7 so, maximum swelling ratio was found in phosphate buffer compared to that of acid buffer solution. On the other hand, when the amount of eudragit L100 was 20 mg it shows minimum swelling ratio both in the acid & buffer solution. This may be explained by the fact 20mg of eudragit L100 along with eudragit RSPO gives the most stable polymeric hydrogel network system, where the water cannot hydrate them easily. But when 30mg of eudragit L100 was taken instead of decreasing the swelling ratio it increases rapidly providing that no stable hydrogel network system formed along with eudragit RSPO.[4,13] Results of swelling study shown in **fig.1A-1B**.

***Invitro* release study**

The effect of concentration of Eudragit L 100 in the *in vitro* release of Acyclovir in both acidic and alkaline dissolution media, is shown in this **fig. 2A- 2B**. So only 10% of the drug is released within 1 hour, in when, 20mg of Eudragit L100 was taken.

Similar result are also found when, the dissolution study was carried out in alkaline medium, near about 60% of the drug was released up to 8 hrs, and 90% was released when 10mg of eudragit L100 was taken.

The release study was also found following similar fashion when Eudragit RSPO was taken of amount 1mg, that is only 15% of drug is released in 2 hours in acidic medium as mentioned in **fig. 3A**. Whereas 35% was released when 3 mg Eudragit RSPO was taken. In the alkaline medium the microbeads showed similar drug release profile with the increase Eudragit RSPO, there is an increase of amount of drug release in the alkaline medium as shown in **fig. 3B**. F2 containing 2mg of Eudragit RSPO was found to release only 60% of drug in first 8 hours as much as 90% for F5 containing 3mg Eudragit RSPO

Thus examining all the release profiles of the microbeads we can say that the formulation containing EudragitL100 20 mg, Eudragit RSPO1mg is the best one. The maximum stable hydrogel network was found in this formulation (F2).

Scanning electron microscopy

The SEM micrographs revealed that the resulting microbeads were more on less spherical in nature with rough surfaces containing cracks and holes over its surface as shown in **fig. 4-9**. The micrographs showed almost spherical but the morphology appeared to be rough in some cases. The reason behind this morphology change can be attributed to the faster evaporation of water forming a pore like structure.[14,15]

FTIR study

Drug polymer compatibility studies were carried out using Fourier Transform Infrared Spectroscopy to establish any possible interaction of model drug with polymers used in formulation as shown in **fig.10A -10B**. The FTIR spectra of the formulation were compared with the FTIR spectra of the pure drug. The results indicated that the characteristic absorption peaks of the pure model drug have appeared in the formulated microbeads, without any significant change in their position after successful encapsulation, indicating no chemical

interaction between model drug and polymers used. Pure acyclovir showed prominent peaks at 3441cm^{-1}, 1731cm^{-1}, and 2928cm^{-1} because of hydroxyl, carboxy and aromatic amine respectively. These peaks were retained in acyclovir during processing of microbeads.[16,17]

DSC study

Acyclovir showed a sharp endothermic peak that corresponds to melting point in therange of 250-300°C. Acyclovir in the eudragit microbeads also showed a similar characteristic peak with decreased intensity showing its stability during the encapsulation.[15] The results are shown in fig. **11A-11B**.

Table 1: Composition of Blank Microbeads.

Blank Formulation code	Euragit L100 (mg)	Eudragit RSPO (mg)	Soduim alginate (% w/v)	Ethyl acetate (ml)	Acetone (ml)	Calcium chloride (% w/v)	Glutaral-dehyde (% w/v)
B1	10	1	2	20	30	5	2
B2	20	1	2	20	30	5	2
B3	30	1	2	20	30	5	2

Table 2: Composition of Acyclovir Loaded Microbeads.

Formulation code	Amount of drug (mg)	Euragit L100 (mg)	Eudragit RSPO (mg)	Soduim alginate (% w/v)	Ethyl acetate (ml)	Acetone (ml)	Glutaral-dehyde (% w/v)
F1	10	10	1	2	20	30	2
F2	10	20	1	2	20	30	2
F3	10	30	1	2	20	30	2
F4	10	20	2	2	20	30	2
F5	10	20	3	2	20	30	2

Table 3: Effect of Different Concentration of Eudragit L100, Eudragit Rspo On % Yield, Particle Size & Entrapment Efficiency.

Formulation Code	% Particle Yield (± SD,n=4)	Average Particle size (µm)±SD,n=3	Entrapment Efficiency(%)±SD, n=4
F1	96.25±0.248	1389 ± 2.78	85.39± 0. ±035
F2	98.36±0.233	1096±8.45	98.37±0.33
F3	93.45±0.631	1152±5.39	94.40±0.35
F4	92.85±0.789	1100±5.89	91.72±1.21
F5	95.45±0.968	1280±6.25	89.52 ± 1.97

GRAPHS and IMAGES

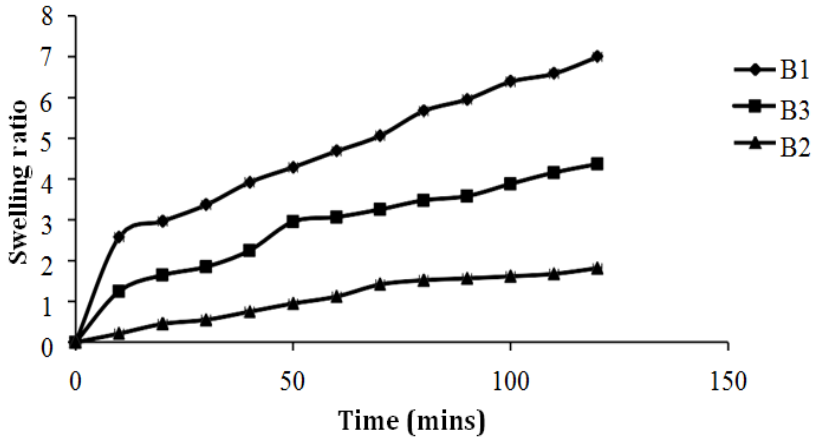

Fig. 1a: Swelling Behavior of The Beads In Acid Buffer Solution.

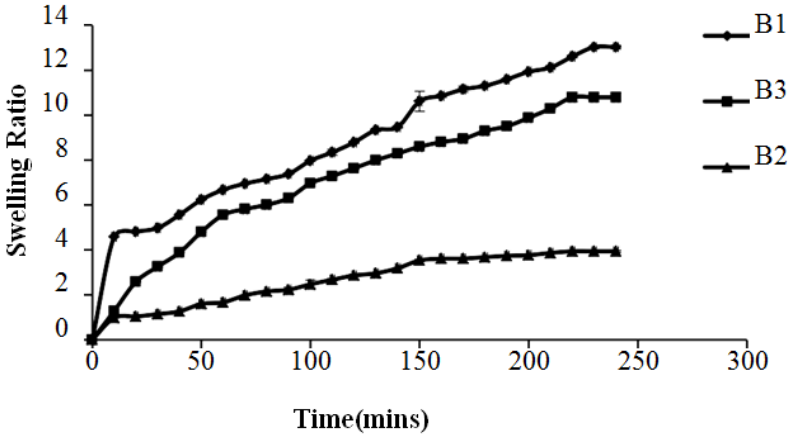

Fig.1b: Swelling Behavior of The Beads In Mixed Phosphate Buffer Solution.

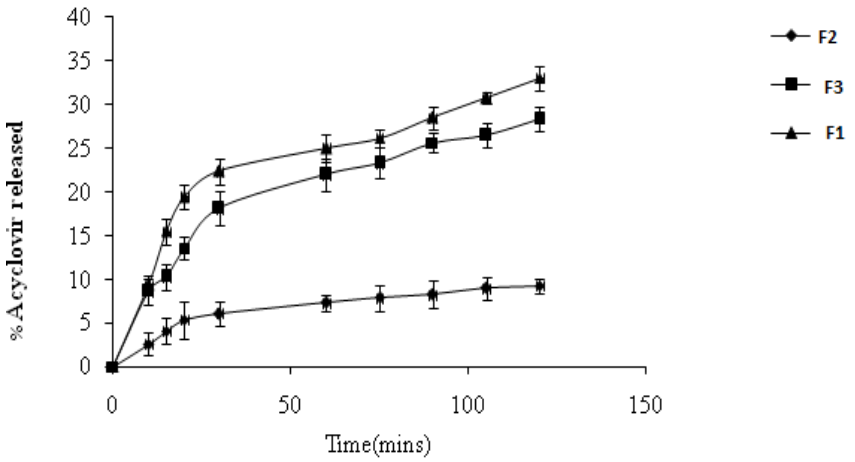

Fig.2a: Release Profile of Acyclovir Loaded Microbeads In 0.1(N) Hcl (Ph 1.2) Prepared By Varying The Concentration of Eudragit L100.

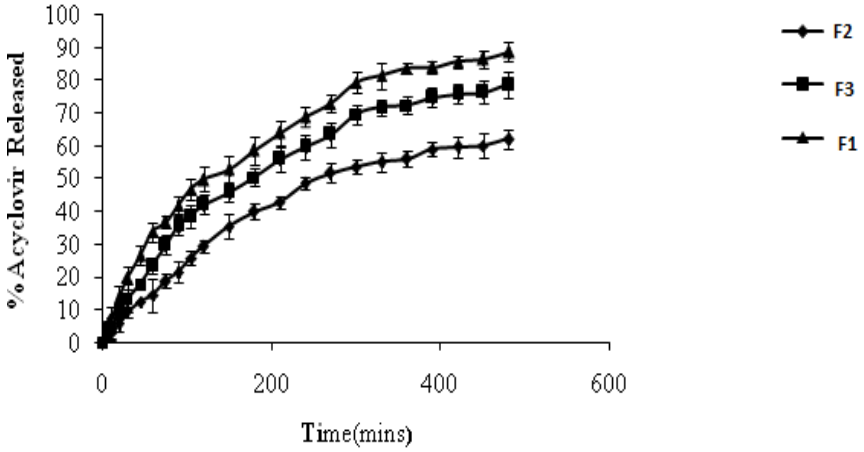

FIG. 2B: Release Profiles of Acyclovir Loaded Microbeads in Mixed Phosphate Buffer (Ph6.8) Prepared By Varying The Concentration Of Eudragit L100.

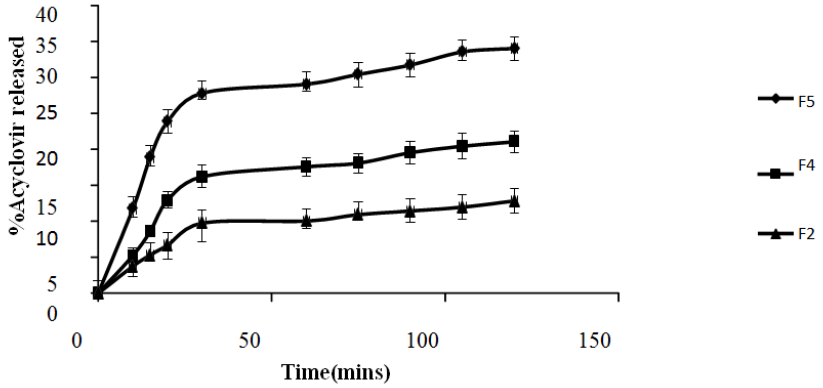

Fig. 3A: Release Profile of Acyclovir Loaded Microbeads In 0.1(N) Hcl (Ph 1.2) Prepared ByVarying The Concentration Of Eudragit RSPO.

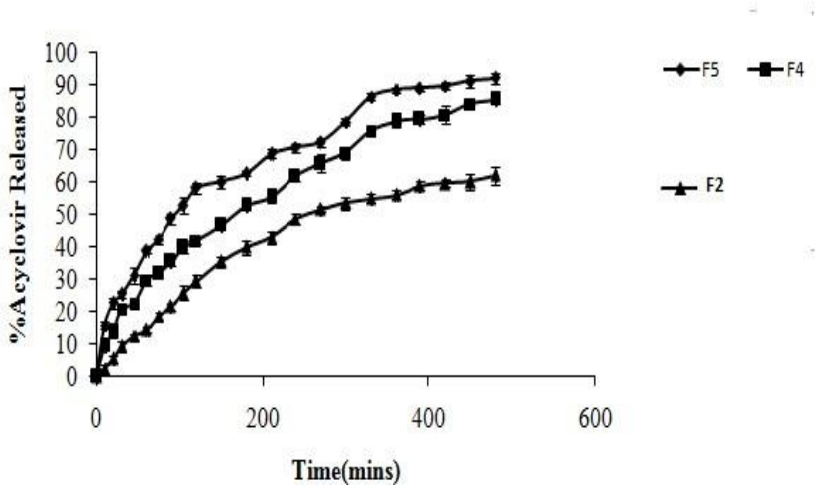

Fig. 3B: Release Profile of Acyclovir Loaded Microbeads In Mixed Phosphate (Ph 6.8) Prepared By Varying The Concentration of Eudragit RSPO.

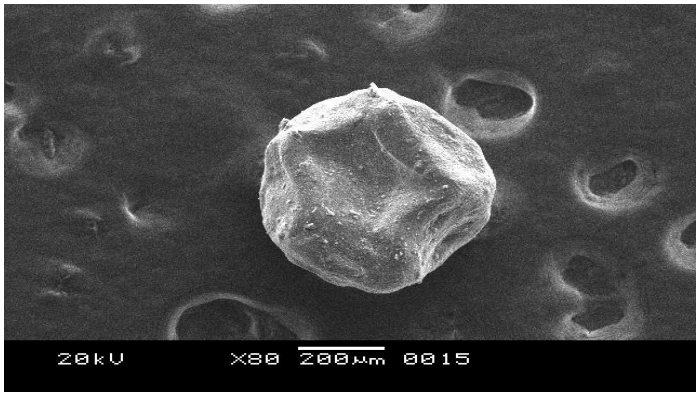

FIG. 4: Scanning Electron Micrograph of Blank Microbeads.

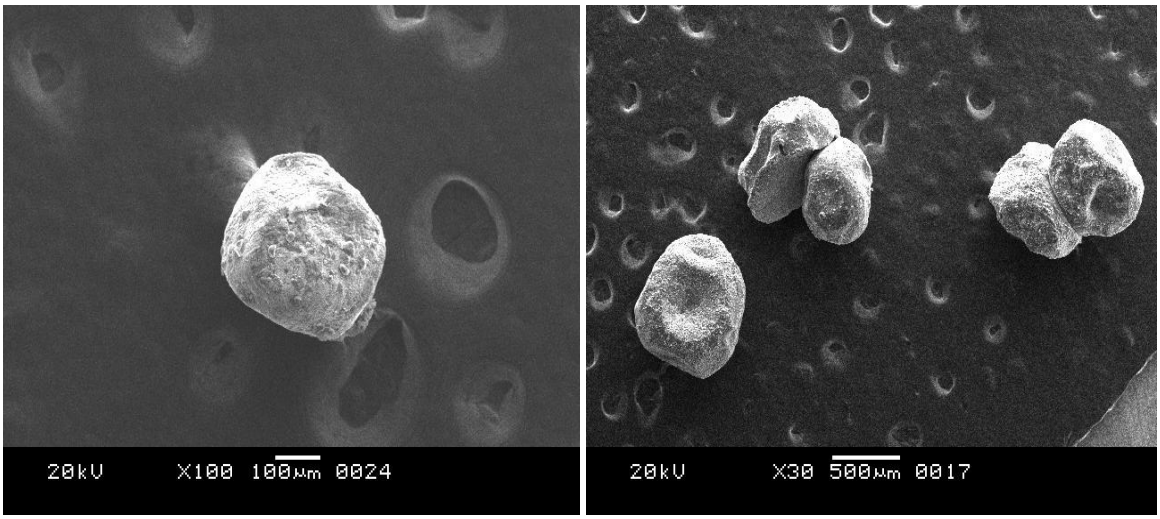

FIG.5: (F1) Scanning Electron Micrographs For The Acyclovir – Loaded Eudragit And Sodium Alginate Combination Microbeads Prepared By Varying Concentration Of Eudragit L100.

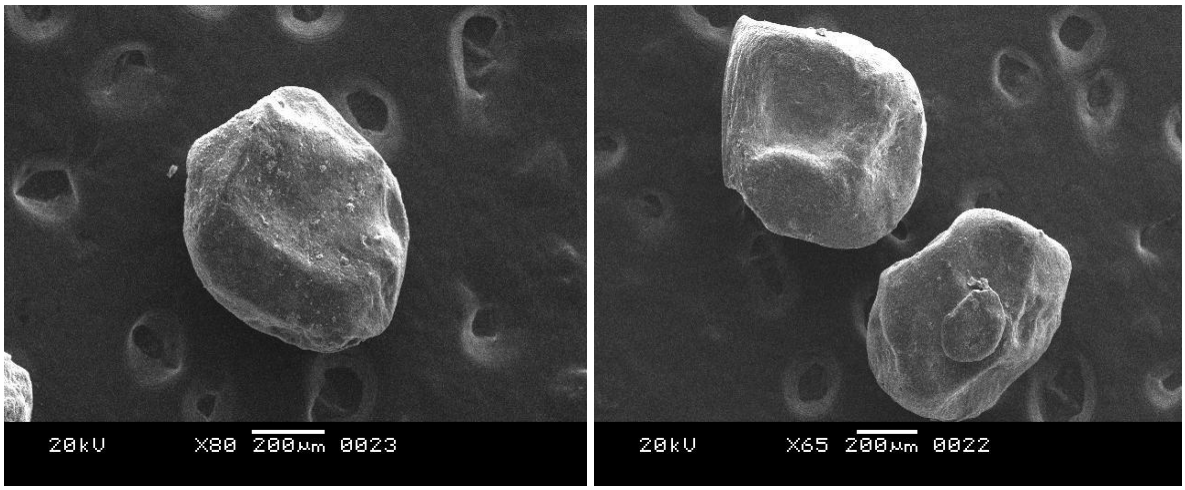

Fig.6: (f2) Scanning Electron Micrographs For he Acyclovir – Loaded Eudragit And Sodium Alginate Combination Microbeads Prepared By Varying Concentration Eudragit L100.

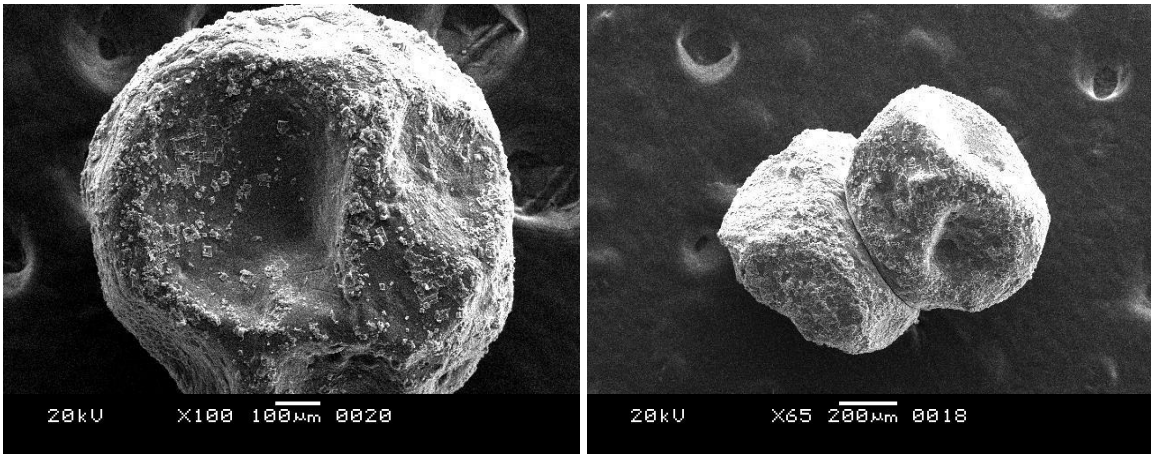

Fig.7: (f3) Scanning Electron Micrographs For The Acyclovir – Loaded Eudragit And Sodium Alginate Combination Microbeads Prepared By Varying Concentration Eudragit L100.

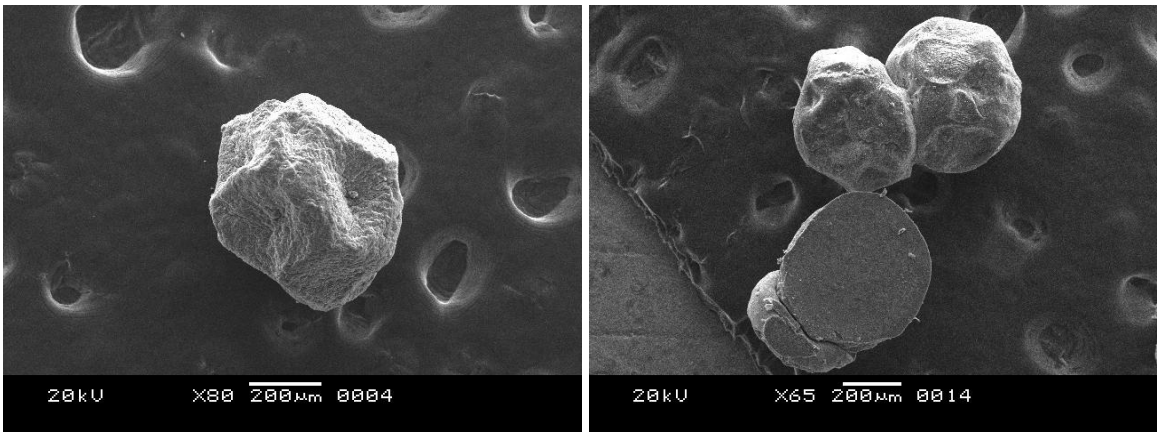

FIG.8: (F4) Scanning Electron Micrographs For The Acyclovir – Loaded Eudragit And Sodium Alginate Combination Microspheres Prepared By Varying Concentration Eudragit Rspo.

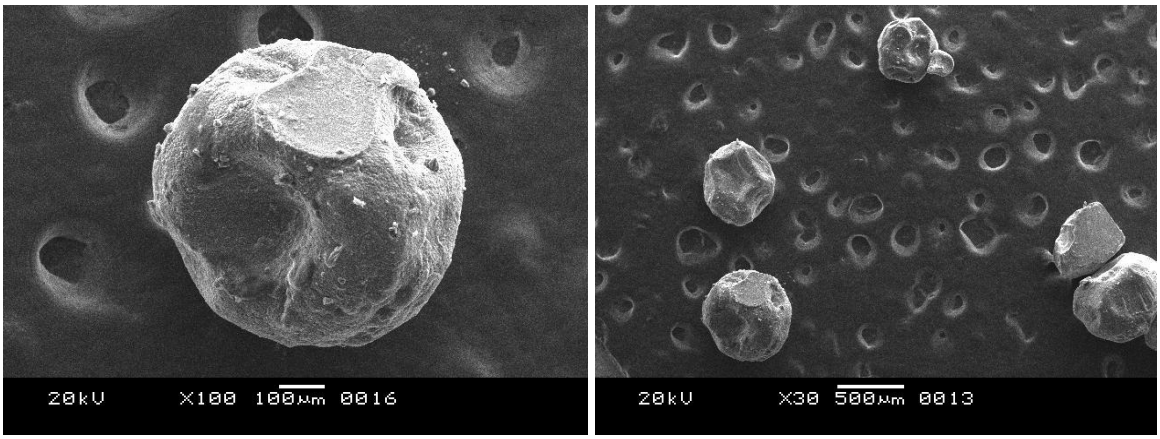

Fig.9: (F5) Scanning Electron Micrographs For The Acyclovir – Loaded Eudragit Sodium Alginate Combination Microspheres Prepared By Varying Concentration Of

Eudragit Rspo.

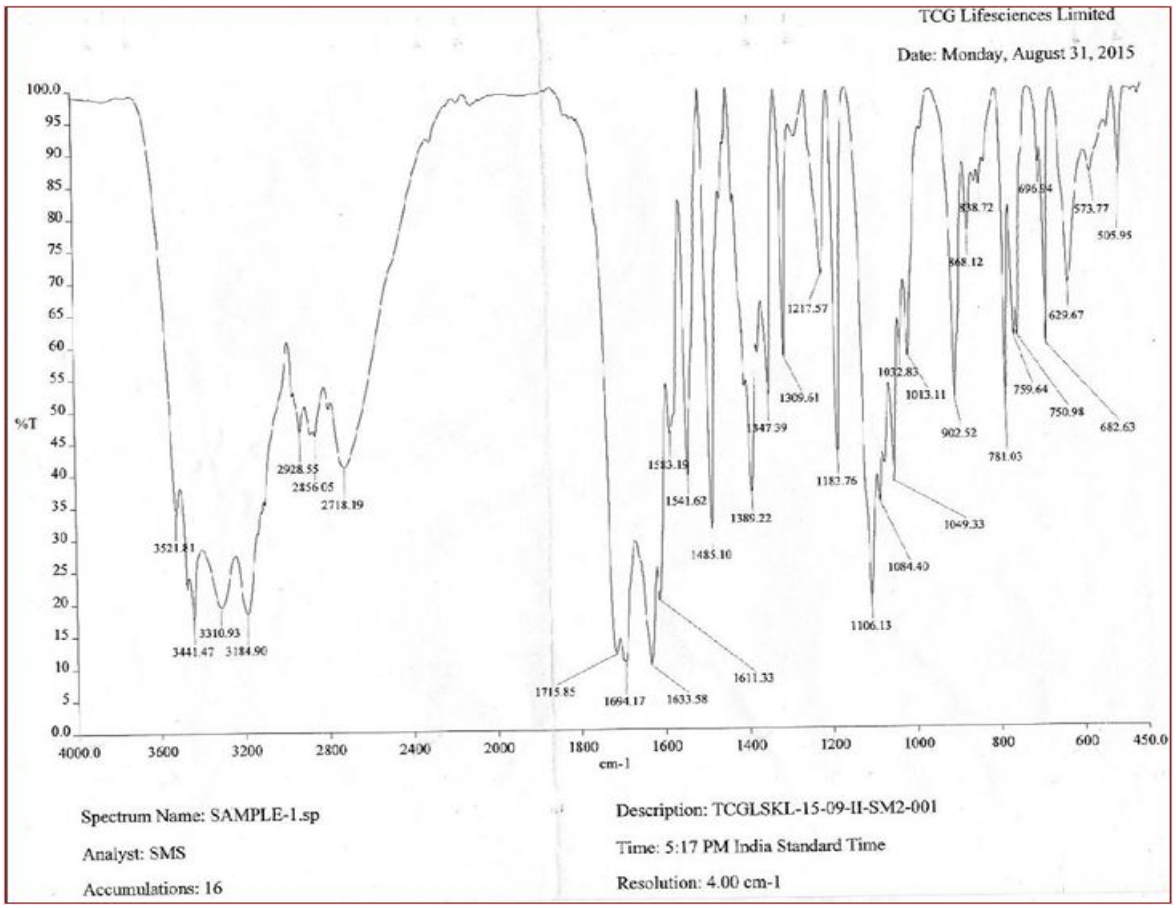

Fig. 10A: FTIR Spectra of Pure Drug, Acyclovir.

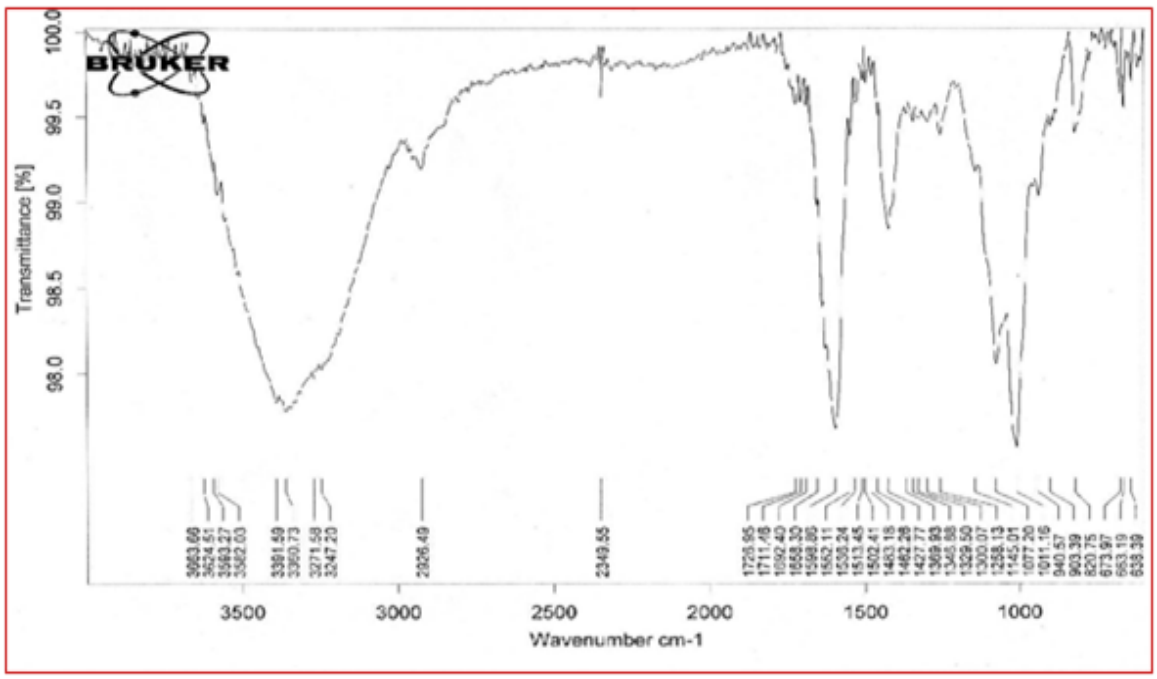

Fig. 10B: FTIR Spectra of Acyclovir Loaded Eudragit-Alginate Microbeads.

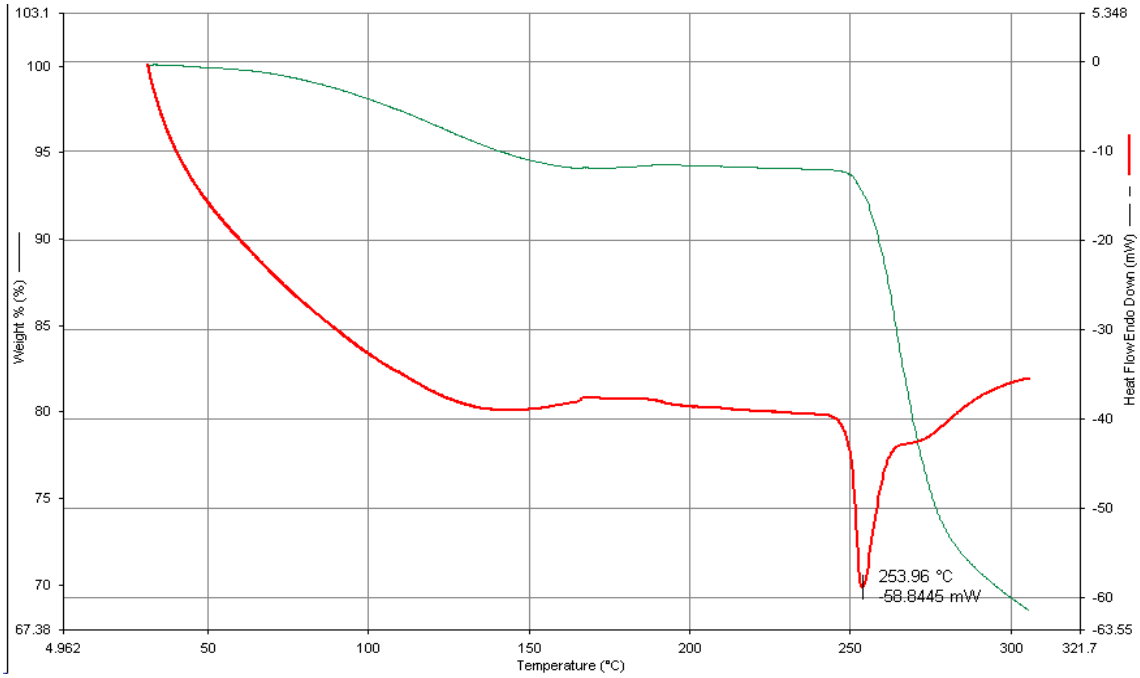

Fig. 11A: DSC Study of Drug Acyclovir.

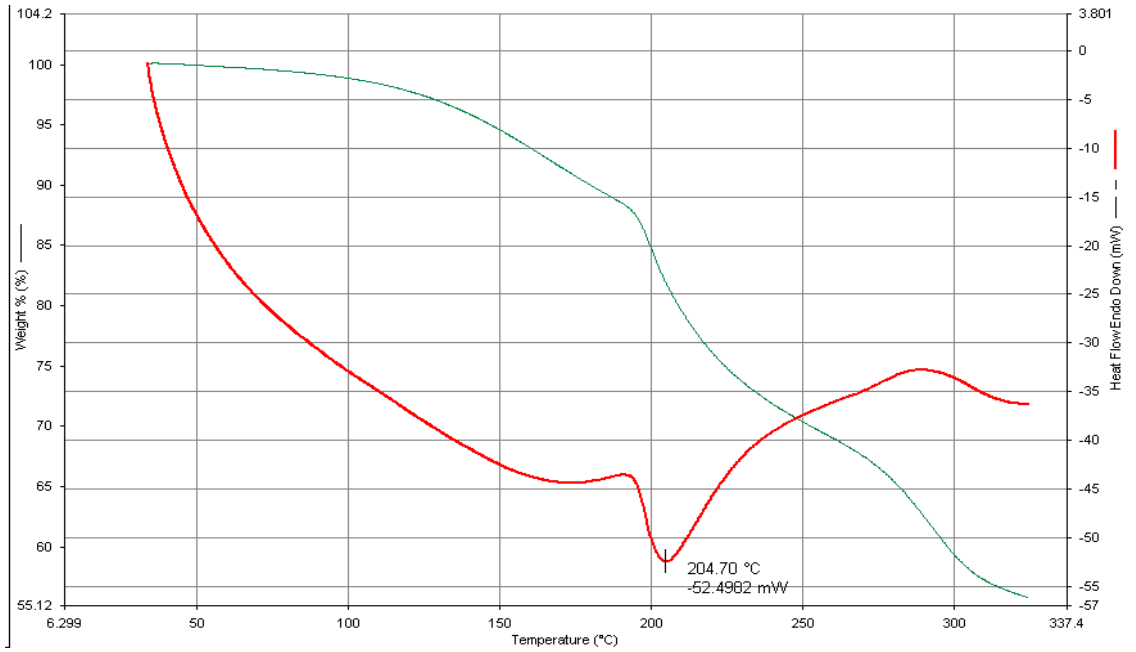

FIG. 11B: DSC Study of Acyclovir Loaded Eudragit-Alginate Microbeads.

CONCLUSION

Acyclovir could be incorporated effectively into combination of Eudragit and sodium alginate microbeads by ionotropic gelation method. Several formulation variables were studied to establish the optimum condition for the preparation of almost spherical eudragit microspheres having reasonably high acyclovir entrapment. The processing variables affected the properties ofthe microspheres in different ways amongst which the F2 formulation shows

high entrapment efficiency, low swelling ratio followed a sustained release profile of the drug. No interaction was found between polymer drug which can be proved by FTIR and DSC analysis.[18,19]

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