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Research Article

Formulation and Evaluation of Floating Microspheres by Using Eudragit RSPO

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ABSTRACT
Acyclovir floating microspheres using Eudragit RSPO alone or in combination with different viscosity grades of hydroxypropyl methylcellulose were prepared for the purpose of improving the oral bioavailability of acyclovir. Floating microspheres were prepared by using an emulsion solvent diffusion technique. During process optimization various parameters were studied such as: drug: polymer ratio, polymer ratio, concentration of emulsifier and stirring speed. Prepared microspheres were analyzed for particle size, surface morphology, entrapment efficiency, buoyancy, differential scanning calorimetry and *in vitro* drug release. Microspheres prepared were spherical shaped with smooth surface. Size of microspheres was in the range of 621 to 784µm. The said procedure resulted in formulations with good entrapment efficiency and % buoyancy and controlled release characteristics. The drug polymer ratio and viscosity of HPMC used had a significant effect of the various characteristics of formulation devised.
Keywords: Acyclovir, Floating microspheres, Buoyancy

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INTRODUCTION
Acyclovir [9-(2-hydroxyethoxymethyl) guanine] (ACV), a synthetic purine nucleoside analog derived from guanine, is one of the most effective and selective antiviral drugs. The drug has short half-life[1] (about 2.5 h). ACV shows an antiviral effect on Herpes simplex virus HSV-1, HSV-2 and Varicella Zoster (VZV) virus through interfering with DNA synthesis and inhibiting viral replication[2]. Unfortunately, its poor oral bioavailability (about 15–30%) due to its low water-solubility (about 0.2%, 25 C) limits the use of the drug as such. To overcome the oral absorption barrier, several approaches like design of prodrugs with enhanced solubility (such as valacyclovir) and different delivery systems containing ACV have been developed, including poly (N-2-hydroxyethyl)-DL-aspartamide conjugate[3], malonylchitosan microspheres[4], liposomes[5], cyclodextrin complex, etc [6] .It was also reported that the poor bioavailability of acyclovir is attributed to the short retention of its dosage forms at the absorption sites (in upper gastrointestinal tract to duodenum and jejunum)[7]. Floating microspheres could contribute to improve absorption and enhance bioavailability of the drugs due to a withholding drawn out in stomach, prolonged retention in the gastrointestinal tract, or specific targeting of drugs to the absorption site, etc[8]. Over the last two decades, there has been considerable interest in floating drug delivery systems for its potential to optimize localized drug delivery, by retaining a dosage form at the site of action, or systemic delivery, by retaining a formulation in intimate contact with the absorption site [9]. Despite the floating, the advantage of using microspheres as floating drug delivery system is that the small size microspheres can be trapped in the reductus of stomach and stay there longer. Besides, when

the poor soluble drugs were loaded in the floating microspheres, they were either adsorbed at the surface of the microspheres or highly dispersed in the inner part of the microspheres, which may help enhance the solubility of the drugs.

Floating microspheres are gastroretentive drug delivery systems based on nonefferevescent approach[10]. This gastrointestinal transit controlled preparation is designed to float on gastric juice with a specific density of less than 1[11]. This property results in delayed transit through the stomach. The drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration.

Eudragit RSPO and various types of hydroxypropyl methylcellulose (HPMC) are the most commonly used polymers for the preparation of the floating drug delivery system. The drug release from the microspheres consists of only Eudragit RSPO is very less[12-13]. According to Lee *et al.*[14], many drugs are not released in significant amount from these microparticles at the pH of gastric fluids. So there is a need of some hydrophilic polymers to be added into the formulation. These polymers cause rapid ingress of the dissolution medium into the microspheres facilitating more drug releases.

The objective of the present investigation was to formulate floating microspheres of ACV using Eudragit RSPO alone or in combination with HPMC K15M and K4M and to study the effect of the various polymers on drug release profile and other physicochemical properties prepared by emulsion solvent diffusion technique.

MATERIALS AND METHODS

Acyclovir was obtained from Zydus Cadilla as gift sample. Eudragit RSPO gift sample Glenmark Pharmaceuticals, Mumbai , Hydroxy propyl methyl cellulose (HPMC) K15M and K4M gift sample from Micro Lab Ltd., Bengaluru, Karnataka, Sodium CMC purchased from Central drug house(P)Ltd., New Delhi, Methanol and Dichloromethane were purchased from Merck Specialities Pvt. Ltd., Mumbai, India.

All chemicals were of analytical reagent grade.

Preparation of drug loaded floating microspheres

The floating microspheres were prepared by emulsion solvent diffusion technique.[10] Weighed amount of polymers (Table1) were dissolved in 10 ml of dichloromethane: methanol mixture (6:4). Drug was then added to polymer solution and mixed completely in magnetic stirring. This resulting solution was poured slowly in deionised water containing sodium CMC while stirring with a mechanical stirrer at 600 RPM for 2.5 h. The resulting microspheres formed were washed with de-ionized water and subsequently dried at room temperature.

Surface morphology

The external morphology of the microspheres was studied by scanning electron microscope (JEOL,JXA-8100, Japan). The microspheres were attached to solid metal specimen stubs, which had a circular face. The upper surface of microspheres was then coated under vacuum with a platinum film. The metal stubs with their coated microspheres were placed in the specimen chamber. The field was scanned at various magnifications (×40, ×100 and ×200) for examination of microspheres.

Particle size analysis

Particle size analysis[15] of the microcapsules was done by sieving method using Indian Standard Sieves #22, #30, #44 and #60.

Micromeritic properties

The microspheres were characterised for micromeritics such as tapped density and compressibility index[16] .To determine tapped bulk density 1 g of Acyclovir microspheres was introduced into a 10 ml measuring cylinder. Initial volume was measured as bulk volume; the cylinder was placed on bulk density apparatus. The tapping was continued until no further change in volume was noted. Tapped densities were calculated by using the equation, Tapped density= (Mass of microspheres/Volume of microspheres after tapping).

Compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content and cohesiveness of materials. The following formula was used to calculate compressibility index:

% Compressibility index = $(1(V/V_0) \times 100$, where V and V_0 are the volumes of the sample after and before the standard tapping, respectively.

% Yields

The practical yield of microspheres of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres [17] .Yields were calculated as per the formula, %Yield= (Actual weight of product)/ (Total weight of drug and excipient) X 100.

Drug content and encapsulation efficiency of microspheres

Predetermined amount of microspheres (50 mg) containing drug was dissolved in 0.1(N) HCl (25 ml) by ultrasonication (Model USB 40,

Spectralab, India). The solution was filtered through 0.45 µm Whatman filter paper. The drug content was estimated after appropriate dilution with 0.1(N) HCl..Absorbance was determined by UV spectrophotometer at 252 nm (L-25, PerkinElmer) and the drug content was calculated according to the equation[17].

%EE= (calculated drug concent /theoretical drug content) ×100.

Floating behavior

The floating test on the microspheres was carried out using the dissolution type II apparatus specified in the USP XXII (Electrolab, TDT08L,USA). The microspheres were spread over the surface of the dispersing medium (900 ml) which is agitated by a paddle rotated at 100 rpm. Dissolution medium 0.1N HCl (pH 1.2) containing Tween 20 (0.02%, w/v) was used as dispersing medium to simulate gastric fluid. After agitation for a previously determined interval, the hollow microspheres that floated over the surface of medium and those that settled to the bottom of the flask were recovered separately. After drying, each fraction of the hollow microspheres was weighed. The buoyancy of the hollow microspheres was represented by the equation[16].

Buoyancy(%)=(Q_f /(Q_f +Q_s))×100, where, Q_f and Q_s are the weights of the floating and the settled hollow microspheres, respectively.

Thermal analysis

Thermal analysis has rapidly gained importance as a routine instrumental method for obtaining qualitative predictions on the stability of drugs, excipients or their mixtures. The melting range of a substance is defined as those points of temperature where the solid coalesces and is completely melted. Because of this a melting temperature range must be reported unless the melting of the compound takes place instantaneously. Differential scanning calorimetry (DSC) is particularly valuable in studying the beginning of melting of a compound [19].

Thermal analysis using DSC method was performed using an automatic thermal analyzer system (Mettler FP80HT Central Processor and FP85 TA Cell). The data processing system Mettler FP89HT was connected to the thermal analyzer. Sealed and holed aluminum pans were used for all the experiences. Temperature calibrations were made using indium as a standard. An empty pan, sealed in the same way as the sample, was used as reference. All the samples were run at a rate of 10 °C/min, from 50 to 310 °C.

Fourier Transforms Infrared Spectroscopy (FT-IR) [20]

The FT-IR spectra acquired were taken from dried samples. An FT-IR (PerkinElmer, model Spectrum 65, United States) spectrometer was used for the analysis in the frequency range between 4000 and 400 cm^{-1}, and 4 cm^{-1} resolution. The results are the means of 16 determinations. A quantity equivalent to 2 mg of pure drug and drug loaded microcapsules were used.

X ray diffractometer (XRD) study

X ray diffractometry was used for diffraction studies. XRD studies were performed on the samples by exposing them to cupper (cu kα) radiation (40kv,30mA) and scanned from 2°C to 80°C, 2 theta(θ) at a step size of 0.045° and step time of 0.5 sec. XRD analysis was performed on the pure drug and for the prepared formulation of microspheres with various polymers.

***In vitro* drug release study**

In vitro dissolution studies can be carried out in a USP XXII paddle type dissolution apparatus. Microspheres equivalent to the drug dose are introduced into 500 ml of the dissolution medium stirred at 100 rpm at 37±0.5°. The dissolution medium was 0.1 N HCl (pH 1.2) containing 0.2% Tween 20 to maintain the sink condition. Aliquots of 2 ml were withdrawn at an interval of 1h. The equivalent volume was replaced with dissolution medium to maintain the sink condition. The withdrawn samples were filtered through 0.45 µm syringe filter. The samples were analysed spectrophotometrically at 252 nm to determine the drug concentration.

RESULTS AND DISCUSSION

The floating microsphere was successfully prepared by using above mentioned polymers by emulsion solvent diffusion method. Water insoluble polymers show higher solubility in dichloromethane than methanol. However, methanol has higher solubility in water. As soon as the polymer solution was added to the aqueous medium, the methanol diffuses swiftly from the droplets of the polymer solution.

Simultaneous diffusion of water inside the sphere further decreased the methanol concentration, and hence the polymer precipitated resulting in the formation of microspheres. Dichloromethane remaining as the central core diffused slowly due to its low water solubility. Due to the poor miscibility, water could not effectively invade the dichloromethane rich core. Therefore, the diffusion of dichloromethane began late, after the initial solidification, and formed a central hollow structure. During the diffusion of the solvents,

the polymer was pulled outward as a result of the dragging force of the solvents and thus the central void space emerged.

Formulation Code	Acyclovir (mg)	Eudragit RSPO (mg)	Sodium CMC(g)	Dichloromethane (ml)	Methanol (ml)	HPMC K_{15}m (mg)	HPMC K_4m (mg)
F1	250	750	1	6	4	-	-
F2	250	500	1	6	4	-	-
F3	250	250	1	6	4	-	-
F4	250	700	1	6	4	50	-
F5	250	700	1	6	4	-	50

Table 1: Formulation codes with quantities

Formulations	Yield (%)	EE (%)	MPS (µm)	Carr's Index	% Bouyancy	n value Korsemeyer-Peppas
F1	91.5± 1.56	52.3± 2.33	781± 2.31	12.33 ±1.18	90.12 ±1.1	0.014
F2	90.2± 2.71	46.5± 4.52	664± 4.75	11.14 ±2.89	84.03 ±2.3	0.258
F3	91.2± 2.41	43.4± 5.21	621± 5.94	11.85 ±1.76	86.56 ±3.3	0.156
F4	91.7± 2.49	55.4± 3.52	784± 3.14	11.36 ±1.54	82.35 ±2.6	0.698
F5	92.5± 2.40	58.2± 2.65	783± 3.28	10.63 ±1.80	95.80 ±5.8	0.656

Table 2: Effect of formulations on percentage yield, percentage entrapment efficiency (EE), mean particle size (MPS) , Carr's Index , % Buoyancy and n value Korsemeyer-Peppas of Acyclovir floating microspheres
[a]: Mean±SD, n=3.

The central cavity produced by the solvents was gradually filled with water due to the reduced internal pressure. Water escaped out of the cavity during the drying process ultimately forming hollow microspheres.

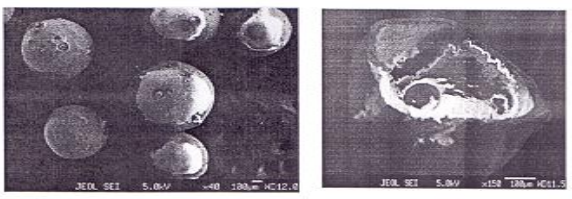

Figure 1: SEM Photographs indicate Formulation F1 [A], Formulation F1 cross section [B]

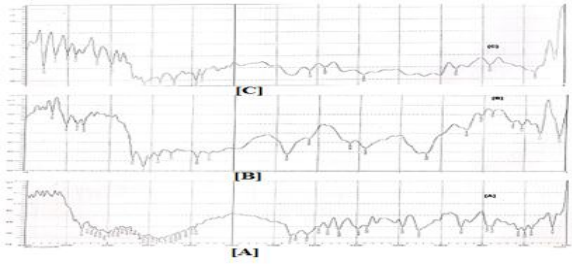

Figure 2: FTIR [A] Pristine of Acyclovir [B] Formulation F1 [C] Formulation F4

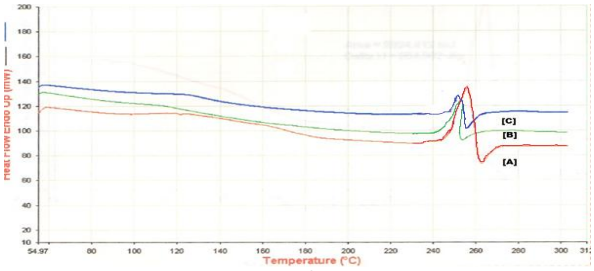

Figure 3: DSC [A] Pristine of Acyclovir [B] Formulation F4 [C] Formulation F1

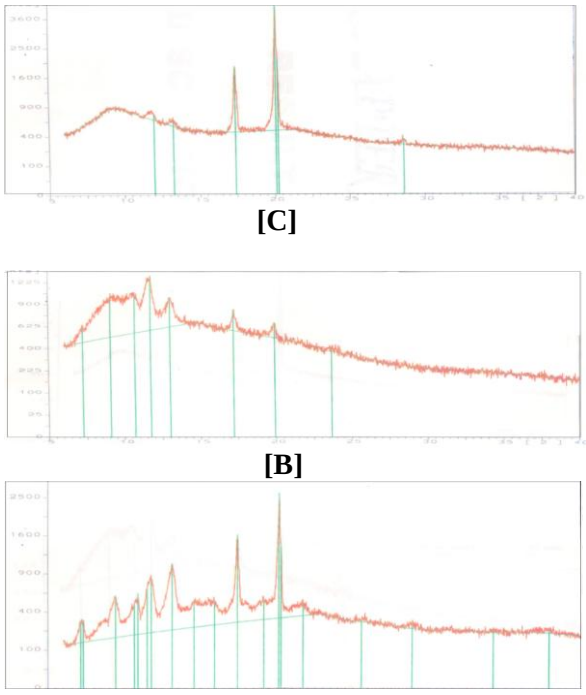

Figure 4: XRD [A] Pristine of Acyclovir [B] Formulation F1 [C] Formulation F4

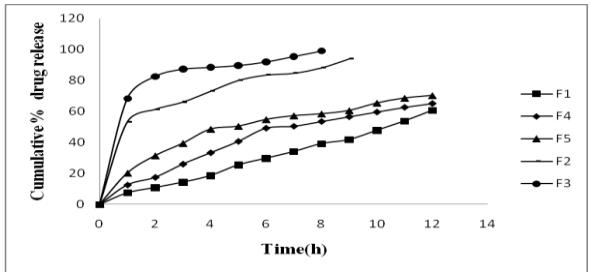

Figure 5: Comparative studies of % CDR of various formulations in 0.1(N) HCl

The percentage yield of formed microspheres is shown in Table 2. The product yield for microspheres was found to be in the range of 90.2±2.71to 92.5±2.40 %. The product yield depended upon the agglomeration and sticking of polymer to magnetic stirrer and to the wall of the beaker during microsphere formation. The product yield was also found to be dependent on the choice of the polymer.

The particle size analysis revealed that the mean diameter of the microspheres with higher polymer ratio was high .inclusion of HPMC

K_{15}m; HPMC K_4m polymers (Table 2) also increased the size of the resulting microspheres. The formed beads of all formulation were more or less spherical in nature.

The compressibility index of all formulation was below 15 indicating the formulationsexhibited better free flow property.

The percentage drug content of floating microspheres were shown in Table 2. Formulation F5 showed highest percentage of EE and F1 showed lowest percentage of EE. The ratio of polymer and type of polymer used to formulation had a significant effect on % EE.

Formulation F5 gave the best floating ability in 0.1 N HCl containing Tween 20 (0.02%, w/v) as evidence by % of particles floated (Table 2). Similarly formulation F4 exhibit lower ability of floating (Table 2).

SEM was used to investigate the physical appearance of beads before dissolution study. The SEM micrographs a shown in Fig. 1A revealed that the resulting microspheres were almost spherical in nature with rough surface containing cracks and holes over its surface. The cross section of microspheres was show in (Fig. 1B) which gives on idea that the microspheres are highly porous in nature.

Pure acyclovir (Fig. 2) showed peaks at 3522.02cm-^{1}(O-H stretching),1608.63cm^{-1} (O-H deformation),3471.87 cm^{-1} (10 N-H stretching) , 2927.94 cm^{-1} (aliphatic C-H stretching anti symmetric), 2854.65 cm^{-1} (aliphatic C-H stretching symmetric), 1485.19 cm^{-1} (aliphatic C-H deformation), 1712.79 cm^{-1} (C=O stretching) and 1105.21 cm-^{1} (C-O stretching).From IR spectrum it was found that there is no hydrogen bonding in between the drug and polymer and there was no significant difference in the IR spectrum of formulation. So it may be concluded that the drug is in intact form in the formulation.

Differential scanning calorimetry (DSC) scans of Acylovir and formulation (Fig. 3) Acyclovir showed a sharp endothermic peak that corresponding to melting in the range of 246.95^{0}C to 261.36^{o}C. The melting temp or the peak of the drug Acyclovir was found to be 255.87^{0}C.The peak of formulation F1 is (253.86^{o}C) and the peak of formulation F4 is (254. 13°C).All the peak of formulations is near about the peak of the drug. So there is a significant preservation of drug crystallinity in the polymer matrix. Thus Acyclovir showed stability during encapsulation process and was found to be no interaction.

The X-ray powder diffraction patterns of pure drug (Acyclovir), Formulation. F1 and formulation F4 are shown in Fig. 4 X-RD studies suggest that when pure Acyclovir drug incorporated in the Eudragit RSPO, HPMC K15M the intensity of the drug peak decreases due to decreasing in crystallinity of drug.

In all formulation the rate of release (Fig. 5) of drug from formulation was different due to their differences in composition. The drug release was more sustained in F1as compared to F2 and F3. This may be due to the higher polymer ration in F1 which inhibited the rate of release of the drug. If we compare the formulation F1 with that of F4 and F5 it was observed that introduction of hydrophilic polymer increased the release rate to some more extent. In between F4 and F5 the F4 released the drug in a more sustained manner than F5 which, may be due to the higher viscosity of the gel formed in case of HPMC K15 M the more resistant the gel is to dilution or erosion; thus, the viscosity of a gel also is a rate-controlling factor in drug dissolution.

The results of the analysis of drug release kinetics were based on Korsemeyer-Peppas model (shown in Table 2). It is evident from the results that in case of formulations F1,F2 and F3 the value of n was below 0.5 indicating the mechanism of drug release predominantly by diffusion method where as in F4 and F5 the value lies between 0.5 and 1.This indicates that the release may be due to both diffusion and dissolution controlled.

Form the above results, we concluded that formulation F5 is better in terms of % EE, % Floating and % yield as compared to other formulation and also has ability of releasing the drug in a controlled manner .So the formulation F5 may be chosen as the best floating microsphere among the others formulations.

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