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Research Article

FORMULATION AND IN VITRO EVALUATION OF FLOATING MICROSPHERES OF ANTI-RETRO VIRAL DRUG AS A GASTRO RETENTIVE DOSAGE FORM

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ABSTRACT

A reversed-phase liquid chromatographic (RP-HPLC) method was developed for the purpose of the research work was to prepare and evaluate the floating microspheres of stavudine as a model drug for prolongation of gastric retention time for oral delivery. Stavudine is a synthetic analog of reverse transcriptase inhibitor with short half life (0.8 to 1.5hr). The floating microspheres of Stavudine were prepared by emulsion solvent diffusion method using Eudragit RS 100 as a rate controlling polymer. The floating microspheres were evaluated for micromeritic properties, particle size, % yield, *in vitro* buoyancy, incorporation efficiency and drug release. The size or average diameter of prepared microspheres were recognized and characterized by scanning electron microscopic methods. The prepared microspheres were found to be spherical and free flowing and remain buoyant for more than 12 hrs. The drug-loaded microspheres (A1) showed encapsulation efficiencies up to 88% and also showed good micromeritic properties for their suitability as oral dosage forms. The microspheres having lower densities exhibited good buoyancy effect and hence, these could be retained in the gastric environment for more than12 h. Thus, the present formulations would be capable of reducing the frequency of administration and the dose-dependent side effects associated with the repeated administration of conventional stavudine tablets.

Keywords: Eudragit RS100, Stavudine, Floating microspheres.

INTRODUCTION

Oral drug delivery system is the most preferable system because of ease in administration, patient compliance and flexibility[1] .To develop an oral drug delivery system, it is necessary to optimize both the residence time of system within the gastrointestinal tract and release of drugs from the system. Drugs that are easily absorbed from the gastrointestinal tract and have short half life are eliminated quickly from the blood circulation and require frequent dosing. To avoid these problems, the oral controlled release formulations have been developed in attempt to release the drug slowly into the gastrointestinal tract and maintain the constant drug concentration in the serum for longer period of time. Such oral controlled drug delivery device may be complicated by limited gastric residence time (GRT), a physiological limitation. Rapid gastrointestinal transit can prevent complete drug release in the absorption zone and reduce the efficacy of

administered dose since majority of the drugs are absorbed in the stomach or the upper part of the small intestine. So various attempts have been made to prolong the residence time of the dosage form within the stomach[2].

Dosage forms that can be retained in the stomach are called gasroretentive drug delivery system. Gastroretentive floating drug delivery systems have a bulk density less than that of gastric fluids and thus remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system floating on gastric contents, the drug is released slowly at a desired rate from the system, both single and multiple unit systems have been developed. The single unit system have disadvantage like local irritation and rapidly emptied from the stomach since there is high variability of gastrointestinal transit time but multiple unit system as floating microspheres may be better suited because they are claimed to reduce intersubject variability in absorption and also lower the probability of dose dumping[3].

The population of patient with chronic disease like HIV, AIDS or complications of other disease has recently been increasing. Stavudine (D4T - thymidine) is the FDA-approved drug for clinical use for the treatment of HIV infection, AIDS and AIDS-related conditions either alone or in combination with other antiviral agents. Stavudine is typically administered orally as a capsule and an oral solution. The virustatic drug has a very short half-life (1.30 h). Its dose is 40 mg twice or sometime three times a day. However patients receiving stavudine develop neuropathy and lactic acidosis. The side effects of stavudine are dose-dependent and a reduction of the total administered dose reduces the severity of toxicity[4]. The physicochemical properties of short half life of stavudine make it as a suitable candidate for floating drug delivery. On the other hand Eudragits are another class of biocompatible copolymers synthesized from acrylic and methacrylic acid esters. Eudragit RS100 is referred to as ammoniomethacrylate copolymers having 5% functional quaternary ammonium groups. These polymers are well tolerated by the skin and have been used in the formulation of dosage form. Eudragit polymer having good properties like inertness, non toxic, solubility etc, which makes it a suitable carrier for drug [5, 6].

In the present study the floating microspheres of stavudine were prepared by emulsion solvent diffusion method using Eudragit RS100 as a rate controlling polymer.

MATERIALS AND METHOD

Materials

Stavudine was obtained as a gift from Strides acrolabs Ltd. (Bangalore, India). Eudragit RS 100 was obtained from Rohm Pharma, GmbH, Germany. Ethanol, Dichloromethane was obtained from SD Fines chemicals Ltd Mumbai (India). Sodium lauryl sulphate was obtained from Merk. All other reagents and solvents used were of pharmaceutical or analytical grade. USP XXI

Paddle type dissolution apparatus, FT-IR (Shimadzu IR spectrophotometer, Model 840, Japan) and UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) were the instruments employed in the current study.

Method of preparation

Floating microspheres loaded with stavudine were prepared by emulsion solvent diffusion method using Eudragit RS100 as a polymer. Drug and polymer in the proportion of 1:1, 1:2, 1:3, 1:4 and 1:5 were dissolved in 1:1 mixture of solvent system of ethanol and dichloromethane. This clear solution was poured slowly into the aqueous solution of 0.2% SLS (sodium lauryl sulphate). The emulsion was continuously stirred for 1h at a speed of 600 rpm at room temperature to allow volatile solvents to evaporate. The finely developed floating microspheres were then filtered, dried at room temperature and stored in desiccator until further use[7]. Table 1 gives the details of the various formulations.

Evaluation of floating microspheres

Particle size analysis

The particle size of microspheres was determined by optical microscopy method; approximately 100 microspheres were counted for particle size using a calibrated optical microscope. The microspheres were uniformly spread on a slide. The particle size of the microsphere was measured, along the longest axis and the shortest axis (cross shaped measurement). Average of these two readings was given as mean diameter of particles. The diameter of a minimum number of 100 microspheres in each batch was calculated.

Scanning electron microscopy

Scanning electron microscopy was performed to characterize the surface morphology of the formed microspheres and this was done by using a JSM 6100 JEOL Scanning electron microscope at 20 kV. Prior to examination, samples were gold-coated to render them electrically conductive and examined under the microscope[8, 9].

Determination of percentage yield

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres[10].

$$\text{Yield (\%)} = \frac{\text{Weight of microspheres}}{\text{Total expected weight of drug and polymer}} \times 100$$

Micromeritic properties of microspheres

The floating microspheres are characterized by their micromeritic properties such as bulk density, compressibility index, Hausner's ratio and angle of repose[8, 9].

Drug-polymer interaction by FT-IR

Drug polymer interaction was studied by taking FT-IR. Infrared spectra of stavudine, Eudragit RS100 and Stavudine floating microspheres were carried out by using KBR pellet technique and were recorded on a shimadzu FT-IR spectrometer[11].

Determination of drug loading (%) and entrapment efficiency (%)

50 mg of the microspheres were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 1700, Shimadzu, Japan) at 266 nm against appropriate blank[8,9]. The drug loading (%) and entrapment efficiency (%) was calculated according to the following relationship

$$\text{Drug loading (\%)} = \frac{\text{Actual drug content}}{\text{Weight of powdered microspheres}} \times 100$$

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Floating behavior (buoyancy %)

50 mg of the microspheres were placed in 100 ml of simulated gastric fluid (pH 1.2) containing 0.02% w/v tween 80. The mixture was stirred at 100 rpm on a magnetic stirrer. After 12 h, the layer of buoyant microspheres was pipetted and separated by filtration; particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in desiccators. Both the fractions of microspheres were weighed and buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres[12].

$$\text{Buoyancy (\%)} = \frac{\text{Weight of floating microspheres after time t}}{\text{Initial weight of microspheres}}$$

In vitro Drug release studies

The drug release studies were carried out using six basket dissolution apparatus USP type I. The microspheres were placed in a non reacting mesh that had a smaller mesh size than the microspheres. The dissolution medium used was 900ml of 0.1N HCl at 37°C. At specific time intervals, 1 ml aliquots were withdrawn and analyzed by UV spectrophotometer at the respective λmax value 266 nm after suitable dilution against

suitable blank. The withdrawn volume was replaced with an equal volume of fresh 0.1N HCl[10].

Micromeritic properties

The microspheres were evaluated for process particle size, bulk density, compressibility index, Hausner's ratio and angle of repose and the results are shown in Table 2.

TABLE 1

Code	polymer	Drug polymer ratio	Organic solvent ratio (dihloromethane :ethanol)	Surfactants
A 1	Eudragit RS100	1:1	1:1	SLS
A 2	Eudragit RS100	1:2	1:2	SLS
A 3	Eudragit RS100	1:3	1:3	SLS
A 4	Eudragit RS100	1:4	1:4	SLS
A 5	Eudragit RS100	1:5	1:5	SLS

Table 2

Batch Code	Mean Particle size (µm)	Bulk Density (gm/ml)	Carr's Index	Hausner's ratio	Angle of repose (θ)
Stavudine	***	0.167 ±0.01	24.38±0.16	1.43 ±0.07	***
A1	23.87±3.21	0.287±0.01	7.12±0.13	1.07 ±0.06	22.92±0.21
A2	30.46±2.56	0.303±0.02	9.82±0.17	1.10 ±0.03	23.96 ±0.63
A3	36.10±2.23	0.306±0.01	10.0 ±0.09	1.11 ±0.07	24.30 ±0.55
A4	39.97±4.19	0.315 ±0.03	12.5±0.21	1.14 ±0.08	26.56 ±0.41
A5	44.43±1.15	0.323±0.02	13.17±0.19	1.15±0.05	28.29 ±0.37

Table 3: Percentage Yield (%), Drug loading and encapsulation efficiency

Batch code	Percentage Yield (%)	Drug loading (%)	Encapsulation efficiency (%)
A1	82.47	43.3±0.22	71.42±0.29
A2	89.13	27.9±0.67	74.59±0.52
A3	90.57	21.5±0.41	77.89±0.66
A4	92.37	18.31±0.13	84.54±0.43
A5	93.11	15.58±0.36	87.73±0.39

(n=3 ± SD)

Table 4: Floating behaviour (Buoyancy) (%)

Batch code	A1	A2	A3	A4	A5
Buoyancy (%)	71.19 ±0.7	74.69 ±1.2	76.29 ±1.9	77.91 ±2.4	78.67 ±1.6

RESULT AND DISCUSSION

Drug-Polymer compatibility by FT-IR

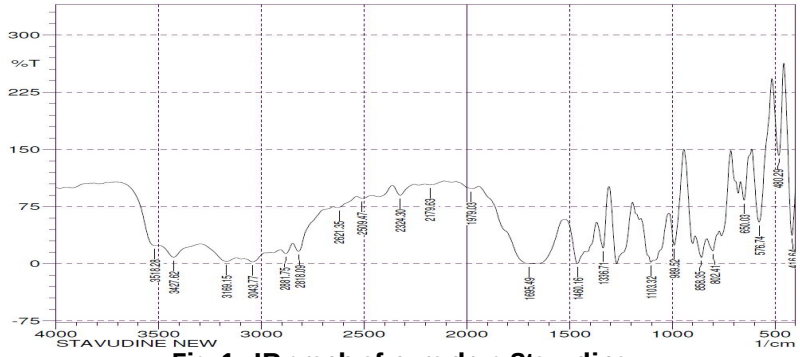


Fig. 1: IR graph of pure drug Stavudine

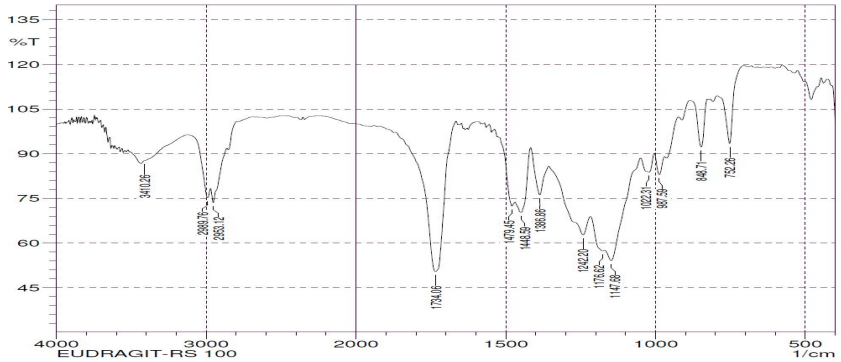


Fig. 2: IR graph of Polymer (Eudragit RS 100)

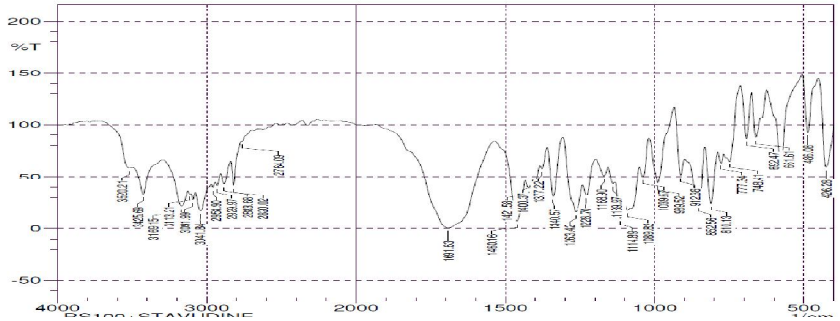


Fig. 3: IR graph of drug and polymer

Drug- polymer compatibility by DSC

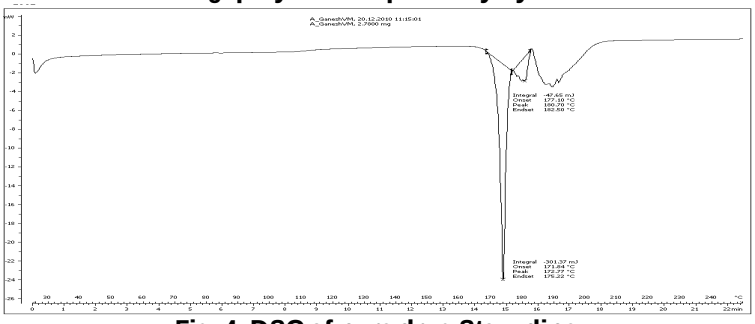


Fig. 4: DSC of pure drug Stavudine

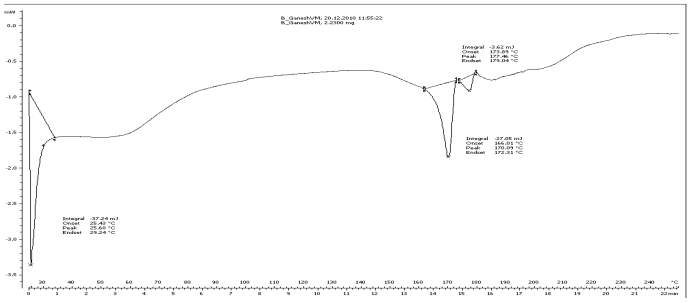
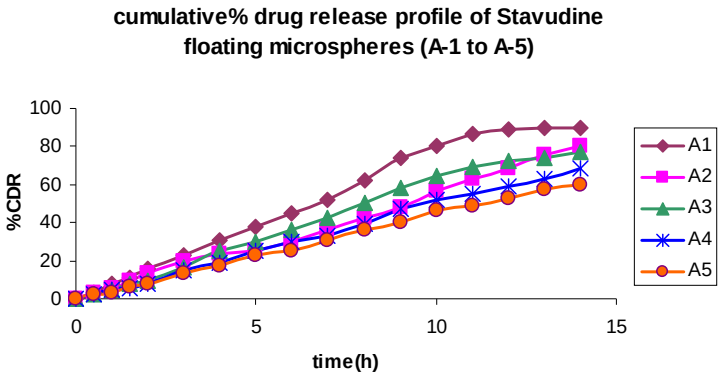


Fig. 5: DSC of floating microspheres of stavudine containing Eudragit RS 100



Surface morphology studies by SEM

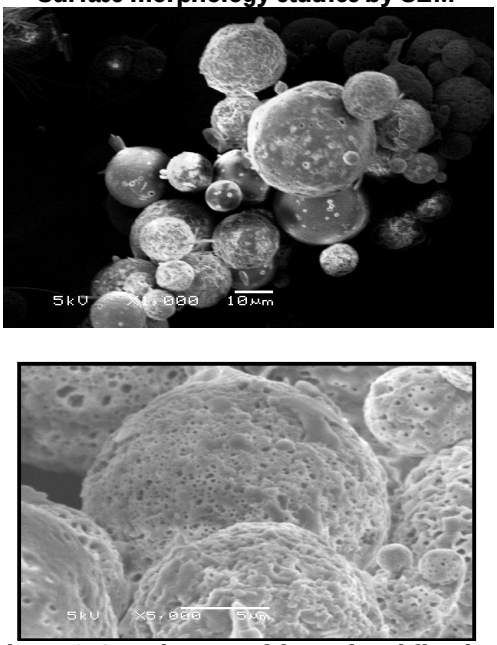


Figure 6: SEM images of formulated floating microspheres of Stavudine

In-vitro drug release studies

Figure 7: Cumulative percentage drug release profile of Stavudine Floatingmicrospheres Release kinetics

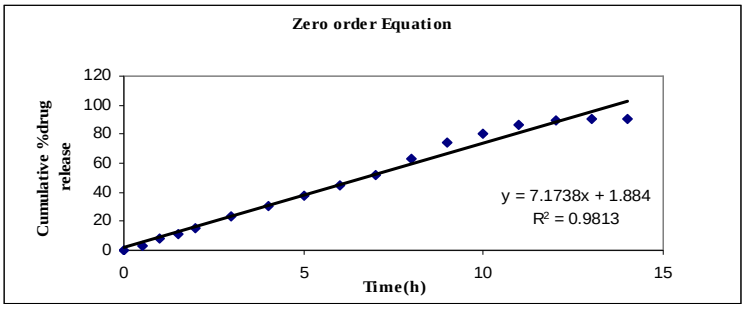
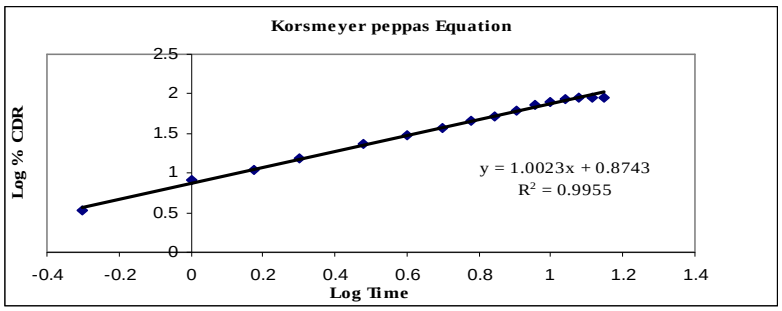


Figure 8: release kinetics graphs for A1



DISCUSSION

The development of floating drug delivery systems would clearly be advantageous. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency, and decrease dose requirements. Thus, an attempt was made in this investigation to use Eudragit RS 100 as polymer to prepare Stavudine (antiviral drug) floating microspheres.

Drug–polymer interaction was studied using FTIR analysis. The results showed that there were no changes in the IR spectra of pure Stavudine in the presence of Eudragit RS 100 (Figures 1 to 3). Thus revealing compatibility of the selected drug with the polymer. DSC thermographs of pure Stavudine showed a sharp endothermic peak at 172 ^{o}C .The thermographs of Stavudine loaded floating microspheres containing Eudragit RS 100 showed a similar endothermic peak at 170 ^{o}C (Figure 4 & 5). This also confirmed that there was no drug polymer interaction.

The Percentage yield of the developed fomulations of Stavudine floating microspheres (A1 to A5) were found to be in the range of 82.47 to 93.11%. The mean particle size of the developed formulations (A1 to A5) of Stavudine floating microspheres were found to be in the range of 23.87-44.43µm.Which indicates the percentage yield and particle size were increased with increasing the polymer concentration.

The drug loading of Stavudine microspheres for formulations (A1-A5) was in the range 43.3 to 15.58%. The drug entrapment efficacy of Stavudine floating microspheres (A1 to A5) was in the range 71.42% to 87.73%. The drug loading of Stavudine floating microspheres decreased with increase in the concentration of polymer and drug entrapment efficiency of Stavudine floating microspheres increased with increase in the concentration of polymer.

The microspheres floated for prolonged time over the surface of the dissolution medium without any apparent gelation. Buoyancy (%) for Stavudine floating microspheres was (A1-A5): 71.19 – 78.67%.

The cumulative percentage Stavudine released from the prepared floating microspheres (A1 to A5) was 90.13-60.14%. The cumulative percentage drug released from floating microspheres decreased with increase in concentration of polymer.

Among the different Stavudine floating microspheres formulations, the formulation A1 was selected as the ideal formulation, based on its micromeritic properties, spherical in shape, floating behavior, drug loading, drug entrapment efficiency and percentage of drug released for a prolonged period over 12h, for further studies such as release kinetics and stability studies.

The in vitro release data obtained from Formulation-A1 was fitted to kinetic models. The zero order plots were found to be fairly linear as indicated by high regression value of 0.981 for A1. The n value 1.0023 obtained from the Korsmeyer-Peppas model showed that the formulation A1 followed the super case-II transport, which indicated that drug release from floating microspheres was diffusion controlled followed by polymer relaxation. The stability studies at room temperature and 45°C/75%RH for selected Stavudine floating microspheres formulations A1 was carried out. There were no significant changes in their physical appearance, average weight of capsule and FTIR pattern. The drug contents of the samples were analyzed after 10, 20 and 30 days of storage and there were no significant changes in the drug content. The drug release profile indicated that there were no significant changes in the physical as well as chemical characteristics of the formulation. Hence, it can be concluded from the results that the developed Stavudine floating microspheres were stable and retained their pharmaceutical properties over a period of 1 month.

CONCLUSION

The present study reported the development of Stavudine loaded floating microspheres using polymer Eudragit RS100 by emulsion solvent diffusion method. Floating microspheres indicated different micromeritic properties, floating behavior, drug content, drug entrapment efficiency and drug release by varying the polymer concentration. The floating microspheres prepared were found to be spherical and free flowing. All the formulated floating microspheres remained buoyant for more than 12h. *In vitro* Stavudine release data showed that all the prepared formulations released Stavudine in a controlled manner for over 12 h. Based on the results obtained it can be concluded that A1 was found to be the ideal formulation considering its micromeritic properties, drug content, drug entrapment efficiency, floating behavior and release profile. The mechanism of drug released was found to be diffusion controlled. They are thus may be reduce frequency of dosing, thereby minimizing the occurrence of side effects, increase residence time in stomach and increase the effectiveness of the drug.

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