Asian J. Pharm. Res. Vol 2, Issue 2, 70-75, 2012.

e-ISSN 2231 – 363X
Print ISSN 2231 – 3621

Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

FORMULATION AND IN-VITRO CHARACTERIZATION OF FLOATING MICROCARRIERS OF STAVUDINE

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ABSTRACT

The objective of the present research work was to develop floating microcarries of Stavudine, to prolong the gastric residence time, bioavailability, to minimize the dose depend side effects and improves patient compliance. Emulsion gelation technique was used to prepare the floating microcarriers using sodium alginate as the polymer. Microcarriers containing oil was prepared by gently mixing and homogenizing oil and water phase containing sodium alginate which was then extruded into calcium chloride solution. The prepared microcarriers were evaluated for drug entrapment efficiency, particle size and shape, micrometric properties, buoyancy and invitro drug release studies. The results of FTIR spectroscopy showed stable character of Stavudine. The mean particle size of microcarriers was in the range of 0.59-1.25mm. Microcarriers were spherical and free flowing. The drug entrapment efficiency was found to be 44.6-69.1%. The microcarriers remained buoyant for more than about 12h. The drug release study showed that Stavudine from the microcarriers was prolonged more than 10hrs. The results demonstrate that the amount of the oil entrapped in each microcarrier is play role in particle size entrapment efficiency and in vitro drug release.

Key words: Microcarriers, Emulsion gelation, Sodium alginate, Stavudine.

INTRODUCTION

Even though various advancement in drug delivery system, oral administration is most convenient and preferred route for the administration of drug in to the systemic circulation because of low cost therapy and ease of administration [1]. Oral controlled release drug delivery has gaining importance over conventional drug delivery because it is having control over drug release and maintains drug levels in the plasma within therapeutic level without offering any fluctuations for longer duration of time. Frequent dosing of drugs are required, if the drugs are having good absorption gastrointestinal tract (GIT) and have short half-lives because those drugs are eliminated rapidly from the systemic circulation. These drugs are suitable candidates for controlled drug delivery system which can release the drug slowly into gastrointestinal tract (GIT) and maintain an effective drug concentration in the systemic circulation for a longer period of time. The drug delivery is in such a way that after oral administration of drug; the drug would be retained in the stomach and release the drug in a controlled manner so that the drug could be available for longer time its absorption sites [2].

Oral controlled drug delivery system has two main draw backs: 1) limited gastric residence time (GRT), 2) varying gastric empting time; leads to incomplete drug release from the dosage form in absorption site and reduce the efficacy of administered dose, so a variety of techniques have been developed to prolong the gastric residence time by retaining the dosage form in the stomach.

Gastroretentive drug delivery is an approach to prolong gastric residence time, by this means targeting site-specific drug release in the upper gastrointestinal tract (GIT) for local or systemic effects. Gastroretentive dosage forms can remain in the gastric region for long periods and hence significantly prolong the gastric retention time

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(GRT) of drugs. Floating drug delivery systems have bulk density less than gastric fluids that have sufficient buoyancy to float over gastric contents and remain in stomach for longer duration of time and release the drug slowly at a desired rate from the system.

Stavudine (D4T, thymidine) is a thymidine analog and chemically known as 1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methyl-1,2,3-tetrahydropyrimidine-2,4dione and it is FDA-approved drug for clinical use for the treatment of HIV infection [3]. Stavudine is administered either alone or in combination with other antiviral agent. Stavudine upon phosphorylated using cellular kinases to reactive metabolite stavudine triphosphate. Stavudine triphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate thymidine triphosphate and by causing DNA chain termination following its incorporation into viral DNA. Stavudine triphosphate inhibits cellular DNA polymerases β and γ and markedly reduces the synthesis of mitochondrial DNA [4].

Stavudine is typically administered orally as a capsule and an oral solution. The drug has a very short half-life (1.30 h) thus necessitating frequent administration to maintain constant therapeutic drug levels. However patients receiving Stavudine develop neuropathy and lacticacidosis. The side effects of Stavudine are dose-dependentand a reduction of the total administered dose reduces the severity of the toxicity [5].

The main objective of present investigation is to develop floating microcarriers of Stavudine by emulsion gelation method, which will retain in stomach and prolong the gastric retention time. The present work also concerned with the drug release modification from the microcarriers in order to maintain the drug levels in the blood for longer duration of time at desired rate.

MATERIALS AND METHODS

Materials

Sodium alginate was purchased from Sigma-Aldrich Chemicals (India), Stavudine was received as a gift sample from Strides Arco Labs, Bangalore, Liquid paraffin was purchased from Sd Fine Chemicals Ltd., Mumbai and all other chemicals used were of analytical grade.

Methods

Preparation of oil-entrapped microcarriers

1. Ionotropic gelation method

Formulation AF1 was prepared without using liquid paraffin by conventional ionotropic gelation method.

2. Emulsion gelation method

Formulations AF2-AF6 was prepared by emulsion gelation method. Sodium Alginate (4%) was dissolved in water with agitation. Stavudine and different concentrations of liquid paraffin were added to polymer solution. The resulting mixture was homogenized for 15 minutes and was extruded via 24 G needle in to 5% calcium chloride. The resultant microcarriers were washed twice with distilled water and kept for drying at room temperature up to 12 hours.

EVALUATION OF FLOATING MICROCARRIERS

FTIR Studies

IR spectroscopic studies were carried out for prepared beads, by using Shimadzu FT IR 8700 model to determine the integrity of the drug in the formulation.

Particle Size

The mean diameter of 100 dried beads was determined by optical microscopy. The optical microscope was fitted with a stage micrometer by which the size of microcarriers could be determined [6].

Micromeritic Properties

The floating microcarriers were characterized by their micrometric properties such as bulk density, angle of repose, Carr's index, Hausner's ratio [7].

Surface Morphology

The shape and surface morphology of prepared microcarriers, were determined by scanning microscopy (model- JSM, Joel, Japan) using gold sputter technique. The particles were vacuum dried, coated to 200 nm thickness with gold palladium using prior to microscopy. A working distance of 20 nm, a tilt of zero- degree and accelerating voltage of 20kv were the operating parameters. Photographs were taken within a range of 50-500 magnification [8].

Drug Loading (%) and Entrapment Efficiency (%)

50 mg of floating microcarries were weighed and ground to fine powder in a pastel mortar and fine powder was dissolved in 2 5ml of 0.1 N HCl. Volume of this solution was made up to 50ml with washings of mortar. The solution was kept for 24 hrs. Then it was filtered. The filtrate was assayed by UV at 266nm (17). The drug loading (%) and entrapment efficiency (%) was calculated according to following relationship [9].

$$\% \text{ Drug loading} = \frac{\text{Actual drug content}}{\text{Weight of powered microcarriers}} X100$$

$$\text{Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{theoretical drug content}} X100$$

Floating Behavior

300 mg of the dried microcarriers were spread over the surface of USP XXIV dissolution apparatus type II. Simulated gastric fluid without enzyme of pH 1.2 was used as medium (900 ml) and the temperature of the

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medium was maintained at 37^0 C ± 0.5^0 C for 12 hrs. The paddle speed was maintained at 100 rpm. The floating and the settled portion of beads were recovered separately. After drying, each fraction of the microspheres was weighed and their buoyancy was calculated by the following equation [10].

Buoyancy (%) = Qf / (Qf + Qs) ×100

In-Vitro Drug Release Study

In vitro release rate studies were carried out using XXIV apparatus type II. Simulated gastric fluid without enzymes of pH 1.2 was used as dissolution medium (900 ml) and was maintained at 37^0 C ± 0.5^0 C. Approximately 0.1 g microcarriers were used for each experiment. The paddle speed was controlled at 50 rpm. Aliquots of 5 ml were withdrawn at different time intervals up to 10 h and a 5 ml of fresh medium was added to replace the sample that was withdrawn. Drug content of the beads was determined by UV/Visible spectroscopy at 266 nm, after suitable dilution of the samples [11].

RESULTS AND DISCUSSION

FTIR Studies

IR spectra were recorded for the pure drug and drug loaded microcarriers and shown in Fig.1. The results of FTIR spectra confirm that there were no interactions between drug and polymer. Four bands characteristic of O-H stretching (3198 cm^{-1}), N-H stretching of secondary amine (3425 cm^{-1}), C-H stretching (2931 cm^{-1}) and C=O stretching (1685 cm^{-1}) of the pure drug were unchanged in the prepared formulation.

Particle Size

Particle size was determined by using optical microscopy. The mean particle size was in range of 0.59 mm to 1.254 mm. The mean particle size of the floating microcarriers was increased as the concentration of oil increases. As the concentration of oil increases the amount of oil entrapped in floating microcarriers was increased as a result the size of the microcarriers increases.

Table 1. Formula of floating microcarriers

Formulation	Stavudine	Sodium alginate	Liquid paraffin	Distilled water	Curing time (min)
F1	500mg	1.5g	0g	50ml	10
F2	500mg	1.5g	5g	50ml	10
F3	500mg	1.5g	7.5g	50ml	10
F4	500mg	1.5g	10g	50ml	15
F5	500mg	1.5g	15g	50ml	15
F6	500mg	1.5g	20g	50ml	15

Fig 1. FTIR spectra of Stavudine, F1 and F2 formulation

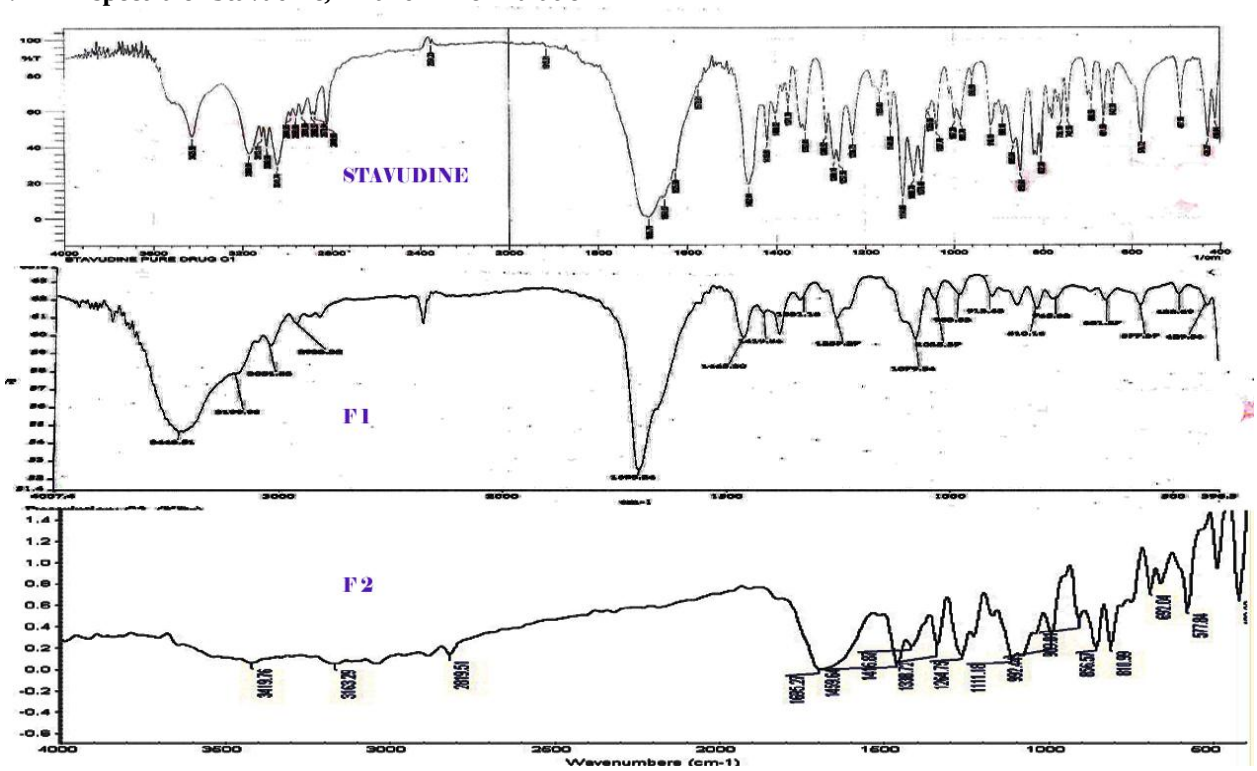

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Fig 2. The mean particle size of Floating microcarriers

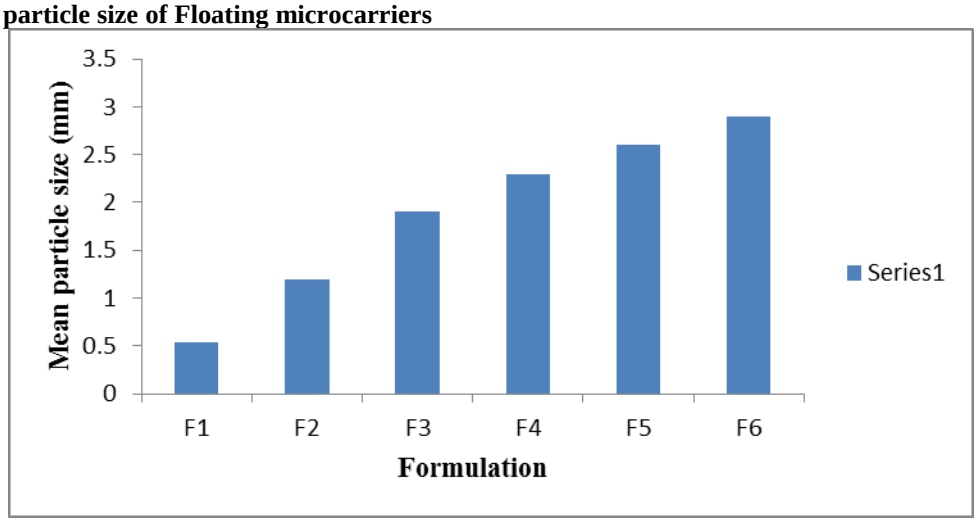

Table 2. Mean flow properties and particle size

S.NO	Formulation	Mean Particle Size (mm)	Angle of repose	Carr's index	Hausner's ratio
1	F1	0.59	20.56 ±0.92	14.46 ± 0.91	1.17 ± 0.03
2	F2	0.825	21.54 ± 1.25	16.42 ± 0.97	1.14 ± 0.03
3	F3	0.9585	21.79 ± 0.72	15.58± 1.29	1.21 ± 0.03
4	F4	1.04	21.96 ± 0.97	13.08 ± 0.29	1.14 ± 0.03
5	F5	1.195	22.46 ± 1.02	15.56 ± 0.97	1.15 ± 0.01
6	F6	1.25	22.86 ± 0.7	13.31 ±0.35	1.16 ± 0.02

Fig 3. Scanning electron photomicrographs of floating microcarriers

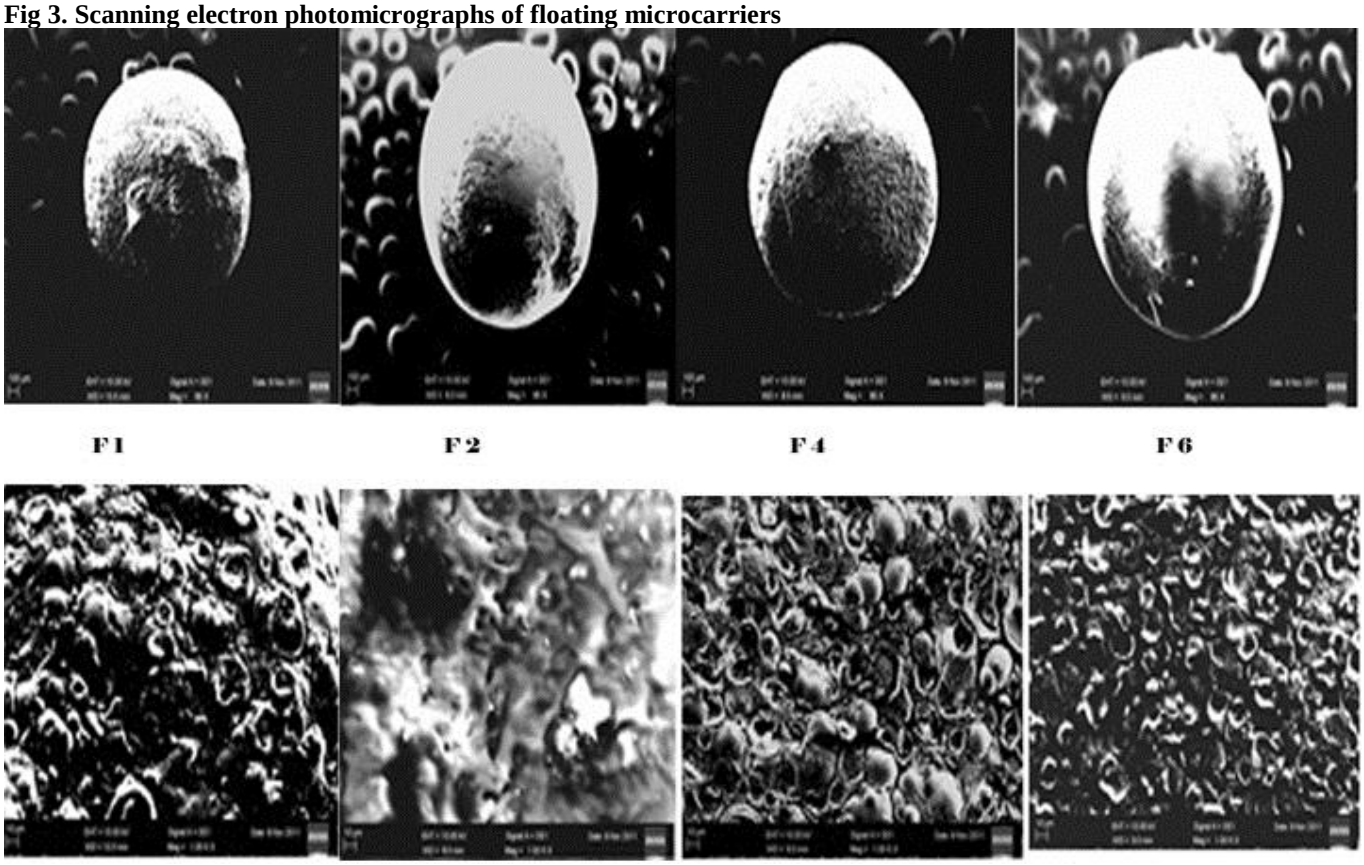

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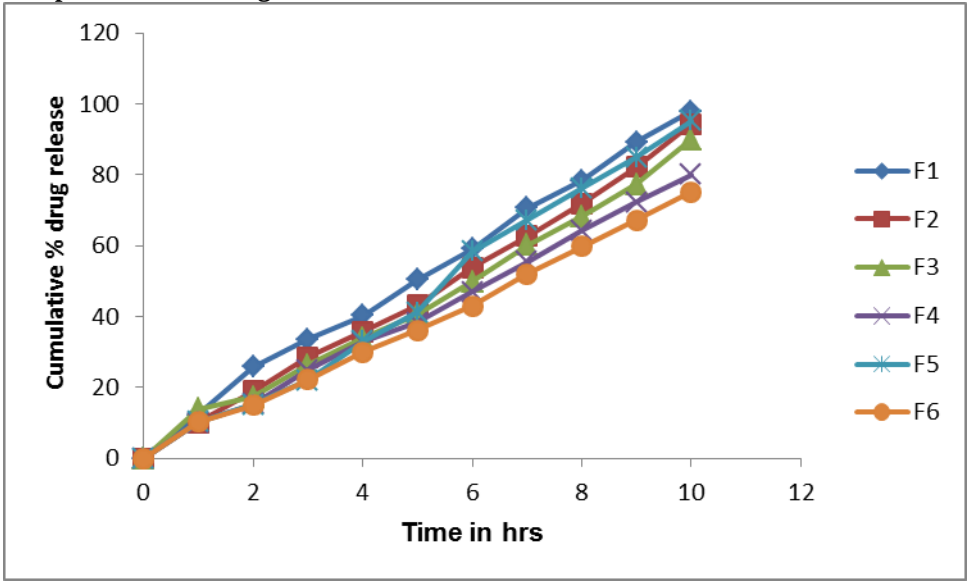
Table 3. % drug loading and % Entrapment efficiency

SNO	Formulation	% Drug loading	% Drug entrapment efficiency
1	1	36.68	69.61
2	2	29.98	64.17
3	3	30.11	58.50
4	4	25.77	45.57
5	5	28.56	55.32
6	6	33.72	44.6

Fig 4. %Drug release profiles of Floating microcarriers



Micrometric Properties

The prepared floating microcarriers were evaluated for micrometric properties. Results of the angle of repose, Carr's index (compressibility index), and Hausner's ratio of all microcarriers confirms better flow properties, values were reported in the Table no.2.

Surface Morphology

F2, F3, F5, F6 of the prepared microcarriers were evaluated for the surface morphology and shown in the Fig.3. Scanning electron microscopy revealed that the prepared microcarriers were spherical and the surface of the microcarriers was porous and rough. The porous nature of the microcarries increases the floating behavior of the microcarriers. The results of the SEM suggest that upon increasing the oil concentration, the shape of the microcarriers is somewhat irregular.

Drug Entrapment Efficiency

The %drug entrapment was found in the range of 44.60 to 69.61. F1 formulation showed highest entrapment efficiency (69.91). Up on addition of oil to the formulation the %drug entrapment efficiency was decreased. As the concentration of the oil increases a gradual decrease in the %drug entrapment efficiency was observed because of the enhanced occupancy of oil results in the decreased entrapment of the drug.

Floating Behavior

F1 formulation contains 0% liquid paraffin so the microcarriers sink uniformly in the medium. 100% buoyancy was observed for the remaining formulations. Liquid paraffin has lower relative density (0.86). It helped the microcarriers to become buoyant. There was no lag time was observed, the microcarriers immediately floated and remained floating for 12hrs. The floating behavior depends on the amount of the liquid paraffin entrapped in the microcarriers.

***In-Vitro* Drug Release**

In-vitro dissolution studies of all prepared microcarriers of Stavudine were carried out in simulated gastric fluid without enzymes of pH 1.2. The cumulative release of Stavudine was deepened upon the amount of liquid paraffin entrapped in each microcarrier. The drug release was extended up to more than 10 hrs by varying the concentration of liquid paraffin. The low concentration of oil containing formulation exhibited greater release of drug. As the concentration of oil increases, the drug release decreased to certain extent, it implies that the use

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of different concentration of permit efficient control of the release of the drug.

CONCLUSION

The present work showed that the emulsion ionotopic gelation technique can be effectively used to prepare floating microcarriers of stavudine. The results of FTIR suggested there were no drug and polymer interactions. SEM photographs of microcarriers showed their spherical nature, porous and rough surface. All prepared microcarriers having good flow properties and remained buoyant for more than 12hrs. *In-vitro* drug release results demonstrate that the drug release can be prolonged more than 10hrs. The drug release was mainly depending upon the amount of oil entrapped in each microcarrier. The drug release can be controlled by varying the concentration of the oil amount. From the results it can be expected to reduce the frequency of dosing and dose depended side effects, increase residence time in stomach and increase the effectiveness of the stavudine.

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